Fibroblast growth factor homologous factors tune arrhythmogenic late Na\textsubscript{V}1.5 current in calmodulin-binding-deficient channels

Jeffrey M. Abrams, …, Manu Ben-Johny, Steven O. Marx

JCI Insight. 2020. https://doi.org/10.1172/jci.insight.141736.

Graphical abstract

Find the latest version:
https://jci.me/141736/pdf
Fibroblast growth factor homologous factors tune arrhythmogenic late Na\textsubscript{v}1.5 current in calmodulin-binding-deficient channels

Jeffrey Abrams \textsuperscript{1,+}, Daniel Roybal \textsuperscript{2,+}, Nourdine Chakouri \textsuperscript{3,+}, Alexander N. Katchman \textsuperscript{1}, Richard Weinberg \textsuperscript{1}, Lin Yang \textsuperscript{1}, Bi-xing Chen \textsuperscript{1}, Sergey I. Zakharov \textsuperscript{1}, Jessica A. Hennessey \textsuperscript{1}, Uma Mahesh R. Avula \textsuperscript{1}, Johanna Diaz \textsuperscript{3}, Chaojian Wang \textsuperscript{4}, Elaine Y. Wan \textsuperscript{1}, Geoffrey S. Pitt \textsuperscript{5}, Manu Ben-Johny \textsuperscript{3,+}, Steven O. Marx \textsuperscript{1,2,*}

\textsuperscript{1}Division of Cardiology, Department of Medicine, \textsuperscript{2}Department of Pharmacology, \textsuperscript{3}Department of Physiology and Cellular Biophysics, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY; \textsuperscript{4}Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, North Carolina; \textsuperscript{5}Cardiovascular Research Institute, Weill Cornell Medical College, New York, NY

+Contributed equally

* Correspondence and requests for materials:
Manu Ben-Johny, PhD
Vagelos College of Physicians and Surgeons
Department of Physiology and Cellular Biophysics
Russ Berrie Medical Science Pavilion
1150 St Nicholas Ave
New York, NY 10032
Email: mbj2124@cumc.columbia.edu
Phone: 212-851-4968

Steven O. Marx, MD
Vagelos College of Physicians and Surgeons
Department of Medicine/Cardiology
622 W168th Street
PH 3-Center
New York, NY 10032
Email: sm460@cumc.columbia.edu
Phone: 212-305-0271

Conflict of interest statement: The authors have declared that no conflict of interest exists
Abstract
The Ca\(^{2+}\)-binding protein calmodulin has emerged as a pivotal player in tuning Na\(^{+}\) channel function, although its impact in vivo remains to be resolved. Here, we identify the role of calmodulin and the Na\(_V\)1.5 interactome in regulating late Na\(^{+}\) current in cardiomyocytes. We created transgenic mice with cardiac-specific expression of human Na\(_V\)1.5 channels with alanine-substitutions for the IQ motif (IQ/AA). The mutations rendered the channels incapable of binding calmodulin to the C-terminus. The IQ/AA transgenic mice exhibited normal ventricular repolarization without arrhythmias, and an absence of increased late Na\(^{+}\) current. In comparison, transgenic mice expressing a lidocaine-resistant (F1759A) human Na\(_V\)1.5 demonstrated increased late Na\(^{+}\) current and prolonged repolarization in cardiomyocytes, with spontaneous arrhythmias. To determine regulatory factors that prevent late Na\(^{+}\) current for IQ/AA mutant, we considered fibroblast growth factor homologous factors (FHF), which are within the Na\(_V\)1.5 proteomic subdomain shown by proximity labeling in transgenic mice expressing Na\(_V\)1.5 conjugated to ascorbate peroxidase. We find FGF13 diminishes late current of the IQ/AA but not F1759A mutant cardiomyocytes, suggesting that endogenous FHF may serve to prevent late Na\(^{+}\) current in mouse cardiomyocytes. Leveraging endogenous mechanisms may furnish an alternative avenue for developing novel pharmacology that selectively blunts late Na\(^{+}\) current.
Voltage-gated Na\textsubscript{v} channels initiate action potentials in excitable tissue and are fundamental to defining cellular excitability. SCN5A-encoded Na\textsubscript{v}1.5 is the major cardiac Na\textsubscript{v} channel, and its dysfunction is linked to multiple cardiac disorders. Voltage-gated Na\textsuperscript{+} channels consist of four transmembrane domains, each containing six transmembrane \(\alpha\)-helices, connected by intracellular linkers, and intracellular N-terminal and C-terminal domains (Figure 1A). Each domain is composed of a voltage sensor (S1-S4) and pore-forming (S5-S6) transmembrane helices that also serve as an intracellular gate. Voltage sensor movement drives channel openings, while ensuing channel inactivation depends on the allosteric blockade of ion conduction triggered by the interaction of the hydrophobic IFM motif in the III-IV linker with a hydrophobic pocket between domains III and IV (1). Impaired inactivation leads to late inward Na\textsuperscript{+} current that can cause cardiac arrhythmias or dilated cardiomyopathy (2, 3). Increased inward late Na\textsuperscript{+} influx also underlies SCN5A-mediated Long QT syndrome type 3 (LQT3) (4). Increased late Na\textsuperscript{+} current is sufficient to cause structural and electrical remodeling in the atria of mice, leading to spontaneous atrial fibrillation (5). In contrast, loss-of-function mutations in Na\textsubscript{v}1.5 or its auxiliary subunits cause the arrhythmogenic Brugada syndrome (6). Determining how changes in Na\textsuperscript{+} channel structure (7) and interactome cause functional alterations in channel properties that beget cardiac disease is critical to devise new therapies.

The Ca\textsuperscript{2+}-binding protein calmodulin (CaM) has emerged as a pivotal player in tuning cardiac Na\textsuperscript{+} channel function, although the impact of this regulation for cardiac function and disease remains to be fully resolved. CaM regulation of Na\textsubscript{v} channels has been the subject of intense scrutiny since the recognition of a CaM binding site within the IQ motif on the Na\textsubscript{v}1.2 C-terminal domain two decades ago (8). CaM has multiple points of contact on Na\textsubscript{v}1.5. The C-terminal domain of Na\textsubscript{v}1.5 contains a canonical apoCaM “IQ” binding motif (Ile1908 and Gln1909) that the CaM C-lobe buries (9-12). Indeed, mutations of the IQ motif that disrupt CaM interaction increased late Na\textsuperscript{+} current in cells heterologously expressing Na\textsubscript{v}1.5, suggesting a role for CaM in Na\textsubscript{v} inactivation (13). Human mutations within the IQ motif or in close vicinity within the CaM binding pocket including Q1909R, E1901Q and R1913H are associated with LQT3 and cause increased late Na\textsuperscript{+} current when expressed in heterologous cells (14). CaM over-expression readily diminished late Na\textsuperscript{+} currents for these mutants, further supporting a functional role of apoCaM in tuning late Na current (14). Beyond the IQ motif, some reports also proposed a Ca\textsuperscript{2+}-CaM interaction with the III-IV linker (15-17), that the III-IV linker and C-terminal domain interaction is enhanced by CaM (13), and a role for a Ca\textsuperscript{2+}-dependent interaction in Na\textsubscript{v}1.5 inactivation (15-17). We found, however, no effect of Ca\textsuperscript{2+}-CaM on Na\textsubscript{v}1.5 channel inactivation (13, 18). These findings all hint at CaM as a major regulator of Na\textsubscript{v}1.5 channel function, with disruption of CaM
interaction with the C-terminal domain potentially unveiling pathogenic late Na current predicted to trigger cardiac instability. Even so, the functional manifestation of this regulation and its potential pathophysiological role in the context of cardiomyocytes has never been established. This gap in understanding is critical, as ion channel function and regulation are exquisitely tuned by a rich repertoire of modulatory proteins that may be present in cardiomyocytes but not in heterologous expression systems.

Knock-in mice are valuable in the assessment of physiology and pathophysiology in cardiomyocytes, although compared to humans, there are acknowledged differences in the ion channel profile and electrophysiologic properties, especially of repolarizing currents. Here, we developed an alternative approach of using transgenic mice expressing doxycycline-inducible, tissue-specific, FLAG-epitope-tagged tetrodotoxin (TTX)-sensitive NaV1.5 channels. Since the expression of these channels is inducible and cardiac-specific, there is a low likelihood of developmental or other compensatory mechanisms affecting the phenotype. Moreover, from the same cardiomyocyte or animal, we can assess the electrophysiological characteristics of the endogenous and transgenically-expressed channels. These channels when expressed in cardiomyocytes demonstrated electrophysiologic properties similar to endogenous channels. In this background, we created additional transgenic mice expressing Ala substitutions for IQ motif (IQ/AA), which prevents CaM binding to the C-terminus of NaV1.5. We found that the NaV1.5 IQ/AA mutant channels in cardiomyocytes did not appreciably increase late Na\(^+\) current hinting at the presence of endogenous protective mechanisms. By comparison to cardiomyocytes, the NaV1.5 IQ/AA channels exhibit strong late Na\(^+\) current when expressed heterologously. To determine regulatory factors that prevent late Na\(^+\) current for IQ/AA mutant, we considered fibroblast growth factor homologous factors (FHFs), which are within the NaV1.5 proteomic subdomain in heart, can bind to the C-terminal domain of NaV1.5, and have been implicated in the regulation of late Na\(^+\) current (11, 12, 19-21). We find that FGF13, the predominant cardiac isoform in rodents (22), diminishes late current of the IQ/AA mutant, suggesting that endogenous FHFs may serve to prevent late Na\(^+\) current in cardiomyocytes. These findings highlight a protective role for endogenous regulatory proteins in preventing pathogenic late Na\(^+\) current in the heart, a potential pathway that may be leveraged to devise next-generation anti-arrhythmics.
Results

Generation of inducible, cardiac specific Naᵥ1.5 transgenic mice

We created transgenic mice with tetO-3XFLAG-epitope-tagged, TTX-sensitive (C374Y) transgenic mice (designated pseudo-wild-type, pWT) and tetO-3XFLAG-epitope-tagged, TTX-sensitive IQ/AA transgenic mice (designated IQ/AA) (Figure 1B). The channel was engineered to be TTX-sensitive with the substitution of C374Y (23) as Naᵥ1.5 channels are relatively resistant to TTX compared to neuronal and skeletal muscle isoforms. The tetO-Naᵥ1.5 mice were crossed to transgenic mice expressing a reverse transactivator (rTA) controlled by the cardiac-specific αMHC promoter (Figure 1B). The bitransgenic tetracycline-regulated system enables robust expression of the FLAG-tagged transgenic Naᵥ1.5 channels only when both transgenes and doxycycline are present.

Expression of the FLAG-tagged transgenic Naᵥ1.5 channels in ventricles was assessed using an anti-FLAG antibody. In cardiomyocytes isolated from non-transgenic mice, no signal was detected. Both the FLAG-tagged pWT and FLAG-tagged IQ/AA Naᵥ1.5 channels were detected in ventricular myocytes isolated from bi-transgenic mice (Figure 1C). For most experiments, mice were fed doxycycline for one to five days. In one of the two IQ/AA lines, doxycycline was not required for expression although expression of rTA was required, likely due to a low basal binding of rTA protein to the Tet operator sequences (so called “leak”) (24). Confirming the expression of the transgenic Naᵥ1.5, immunofluorescence staining of fixed cardiomyocytes from pWT and IQ/AA mutant transgenic mice with an anti-FLAG antibody showed a membrane distribution consistent with that of endogenously expressed Naᵥ1.5 channels (Figure 1D).

The total peak current in the transgenic cardiomyocytes is the sum of the endogenous Na⁺ currents and the transgenic Naᵥ1.5 currents. Endogenous cardiac Naᵥ1.5 are largely resistant to TTX. Consistent with this observation, 20 nM TTX had no effect on peak endogenous Na⁺ currents in cardiomyocytes isolated from non-transgenic animals (Figure 1E, H). In cardiomyocytes isolated from pWT or IQ/AA transgenic mice, in contrast, 20 nM TTX reduced the peak Na⁺ current implying that the transgenic channels were expressed, inserted in the membrane and were functional (Fig 1F-L, Supplemental Data Figure 1A-B, D-E). The difference between the total current and the remaining current after TTX infusion represents the current carried by the transgenic pWT or IQ/AA channels (Supplemental Data Figure 1C-F). The mean fraction of TTX-sensitive current, which can be attributed to the transgenic Na⁺ channels, was 69% and 71% for pWT and IQ/AA respectively (Figure 1I).

Late Na⁺ current is not increased in IQ/AA cardiomyocytes
As late Na$^+$ current is an inherent channel property that reflects alterations in channel inactivation, its magnitude is typically normalized to the peak current. This maneuver is essential as it accounts for changes in peak current that may occur due to variability in channel expression in a transgenic model. The low magnitude of late current in relation to the peak (<1%), however, poses two experimental challenges for robust quantification. First, non-selective membrane leak or digitization artifacts could corrupt accurate resolution of small amplitude late Na$^+$ current. Second, when late current is amplified by large ionic gradients, the amplitude of the peak current becomes large and vulnerable to voltage-clamp artifacts. To overcome these limitations, we devised a protocol whereby the late current is measured using 100 mM Na$^+$ and peak current using 3 or 5 mM Na$^+$ in the extracellular solution. For the measurement of late current, we quantify the difference in currents during the final 10 ms of a 190-ms depolarization with 100 mM Na$^+$ in the extracellular solution, before and after application of either TTX or Ranolazine, a selective blocker of late Na$^+$ current (Figure 2A-D). After wash-out of Ranolazine or TTX, peak current is measured with 3 mM Na$^+$ in the extracellular solution in the absence and presence of 20 nM TTX for pWT or IQ/AA transgenic mice (Figure 1E-G) or 3 mM lidocaine for F1759A transgenic mice (Supplemental Data Figure 2). Normalization of late Na$^+$ current to the peak current yields a late current ratio enabling comparison between transgenic models. All recordings were performed with CsF for patch stability, and with BAPTA in the patch pipette to chelate intracellular Ca$^{2+}$, thus querying the role of apoCaM.

The late current ratio was not different between non-transgenic and pWT cardiomyocytes, implying that over-expression of TTX-sensitive Na$_{v}1.5$ in mice does not alter the ratio of late to peak current (Figure 2E). Similarly, the late current ratio was not increased in the IQ/AA mice compared to either the late current ratio in NTG or pWT mice (Figure 2E). This finding was unexpected since LQT3 mutations that disrupt CaM interaction cause increase late current when measured in a heterologous expression system (14) and contrasts with our findings from F1759A lidocaine-resistant transgenic channels in which the late current ratio was markedly increased compared to non-transgenic, pWT and IQ/AA mice (Figure 2D-E).

To further scrutinize late Na$^+$ current in the pWT, IQ/AA, and F1759A transgenic models, we undertook cell-attached multichannel recordings to directly measure late channel openings (25, 26). A 300 ms depolarizing pulse is used to elicit channel openings as evident in a patch from pWT transgenic mice (Figure 2F). Rapid activation followed by inactivation of multiple channels within the patch result in stacked channel openings reflecting near-macroscopic peak current. As late openings still occur to the unitary current level, they can be easily distinguished from instrument noise and baseline level. For channels from pWT myocytes, we observed sparse late
channel openings consistent with near-complete inactivation (Figure 2F, shaded area and inset). For each patch we obtain 50-100 stochastic traces to compute an ensemble average current, which is subsequently divided by the unitary current level (i) to obtain NPo. To determine late current, we normalize NPo trace by its peak value to obtain a normalized Po waveform for each patch (Figure 2G) and compute the mean value following 50 ms of depolarization (Figure 2L). Similar to pWT, multichannel recordings from IQ/AA transgenic cardiomyocytes also showed minimal late channel openings as evident from exemplar trace (Figure 2H) and ensemble average (Figure 2I). By comparison, Na+ channels from F1759A cardiomyocytes show incomplete inactivation with frequent channel openings even 50 ms following depolarization (Figure 2J-K). The ratio of late to peak open probability was significantly increased for F1759A channels but not for IQ/AA channels compared to the control channels (Figure 2L). These findings contrast with reports from studies of IQ/AA mutant channels expressed in HEK cells where the IQ/AA mutation results in a nearly 7-fold increase in late current (13).

**Repolarization not prolonged in IQ/AA transgenic mice**

To assess whether disruption of apoCaM binding to NaV1.5 affected electrophysiological properties of the heart in vivo, we performed electrocardiographic (ECG) analyses on the mice. Increased late Na+ current from NaV1.5 manifests as a prolonged QT interval on the ECG and is the basis for LQT3 in humans. Compared to NTG mice, the heart rate (R-R interval) in pWT and IQ/AA mice were unchanged (Figure 3A-B). The PR interval, which represents the conduction time from the sinus node through initial activation of the ventricles via the atrioventricular node was shorter in both pWT and IQ/AA transgenic mice (Figure 3A,C), consistent with previously created transgenic mouse models with wild-type NaV1.5 over-expression (27). The PR was not shortened in the F1759A-NaV1.5 mice (Figure 3C) compared to NTG controls, perhaps reflecting the balance between Na+ channel overexpression and prolonged repolarization. In some patients with LQT3, prolonged AV conduction time and AV block has been reported (28). The QT interval in IQ/AA was not increased compared to NTG or pWT (Figure 3A,D), unexpected in light of reported LQT3 mutations (and corresponding increased QT interval) that disrupt CaM interaction. In comparison, expression of mutant F1759A-NaV1.5 channels that enhances late Na+ current in a heterologous system causes prolonged QT interval (Figure 3A,D) (5).

Epicardial surface optical voltage mapping of the anterior surface of Langendorff-perfused IQ/AA and F1759A-NaV1.5 hearts was used to assess ventricular repolarization (Figure 3E-G). As we have done previously (29), the Langendorff apparatus mounted hearts were perfused with a hyperkalemic solution to terminate the spontaneous arrhythmias in order to elucidate the
underlying electrophysiologic substrate. Thereafter, a normokalemic solution was infused and the ventricular APD was assessed. In the F1759A mutant mice, we observed APD prolongation (Figure 3E-F). As we have previously described for the atria (5, 30), there was considerable heterogeneity in the APD, likely caused by the variable expression of the F1759A-Na\(^+\) channels. In contrast, the IQ/AA transgenic mice had uniformly normal ventricular repolarization (Figure 3F-G). We hypothesized that inhomogeneity of the prolonged ventricular APD can form the substrate for initiating and sustaining ventricular arrhythmias in F1759A mice. We optically acquired voltage maps of Langendorff-perfused hearts before and after burst pacing to induce ventricular arrhythmias, and used phase mapping to quantify rotational reentry dynamics including phase singularity points. The F1759A transgenic hearts were readily inducible into rotational reentry-dependent ventricular tachycardia/fibrillation (Figure 3H), which was not observed in the IQ/AA hearts. Consistent with this finding, spontaneous premature ventricular contractions were frequently observed on 12-lead ECG of F1759A mice but not in IQ/AA mice. Taken together, the absence of QT interval and cardiac action potential prolongation is consistent with the lack of increased late current in the IQ/AA mice, suggesting that in vivo loss of CaM binding can be fully compensated in mice.

**FHF and CaM in complex with Na\(_V\)1.5 in cardiomyocytes**

We adapted for application to cardiomyocytes the ascorbate peroxidase (APEX2) proximity labeling method, originally developed for the identification of the mitochondrial proteome (31, 32). We recently used this approach to obtain a comprehensive proteome of Ca\(_V\)1.2 in cardiomyocytes (33). We generated transgenic mice with doxycycline-inducible, cardiomyocyte-specific expression of TTX-sensitive Na\(_V\)1.5 proteins with APEX2 and a V5 epitope conjugated to the N-terminus, enabling biotin labelling of proteins within approximately 20-nm radius. Fusing APEX2 to Na\(_V\)1.5 did not affect Na\(_V\)1.5 subcellular localization and function, as assessed by cellular electrophysiology and anti-V5 antibody immunofluorescence (Figure 4A-B). Incubating isolated ventricular cardiomyocytes with a solution containing biotin-phenol induced robust biotinylation of proteins at the sarcolemma, intercalated disk, and in a striated z-disk pattern, coinciding with the pattern of transgenic Na\(_V\)1.5 expression (Figure 4B). Western blots showed the biotinylation and streptavidin purification of both CaM and FGF13 (Figure 4C), confirming prior studies showing FGF13 and Na\(_V\)1.5 interact (22, 34), and consistent with known cellular distribution of FHF13 in transverse-tubules and the sarcolemma (35). As FHF proteins have been shown to modify multiple aspects of Na\(_V\) channel inactivation and reduce late current in heterologously expressed C-terminal-deleted Na\(_V\)1.5 at residue 1885, which deletes the C-terminus including the IQ-motif
(21), we reasoned that FGF13 may be in part responsible for attenuating late current of IQ/AA mutant in vivo. FGF13 binds to the C-terminus of Na\textsubscript{v}1.5 with an isothermal calorimetry (ITC)-determined affinity of 16.6 nM (Supplemental Data Figure 3), which is approximately 10-fold higher than the affinity of FGF12B (11), which is the FHF isoform expressed in human cardiomyocytes.

**Expression of FGF13 reduces late current caused by IQ/AA but not F1759A mutations**

As HEK cells lack endogenous FHFs, we tested whether FGF13 overexpression in these cells might recapitulate the differential penetrance of late Na\textsuperscript{+} current that we observed for the IQ/AA versus F1759A mutant in cardiomyocytes. As control, we quantified late channel openings from wild-type Na\textsubscript{v}1.5 channels in the presence and absence of FGF13 using multichannel cell-attached. At baseline, wild-type channels exhibited minimal late channel openings (Figure 4D-E), and FGF13 co-expression had no appreciable effect (Figure 4F-G, L-M). Expression of Na\textsubscript{v}1.5 IQ/AA mutant alone revealed late channel openings (Figure 4H-I) with an 11-fold increase in late current (Figure 4L-M), consistent with previous studies (13). To mimic the co-localization of FHF with Na\textsubscript{v}1.5 in cardiomyocytes, we co-expressed FGF13 with Na\textsubscript{v}1.5 IQ/AA mutant. This maneuver resulted in a complete reversal of late current to wild-type levels (Figure 4J-M), suggesting that the addition of FGF13 eliminated late current when the IQ/AA motif was completely ablated, and reminiscent of the absence of late Na\textsuperscript{+} current in IQ/AA transgenic cardiomyocytes in which FGF13 is present (Figure 2). These findings suggest that endogenous FGF13 in cardiomyocytes may serve a protective function by preventing pathogenic late Na\textsuperscript{+} channel openings.

In cardiomyocytes, F1759A mutants exhibit substantial late Na\textsuperscript{+} current that causes cardiac arrhythmias in mice, raising the possibility that these channels are less sensitive to endogenous protective mechanisms. To test this possibility, we expressed the Na\textsubscript{v}1.5 F1759A channels in HEK293 cells and undertook multichannel recordings. Similar to cardiomyocytes, recombinantly expressed F1759A mutant display markedly increased late openings (Figure 5A-B) as previously reported (36). Interestingly, the amount of late current for F1759A is similar to the IQ/AA mutant in the absence of FHFs. In contrast to IQ/AA mutant, however, FGF13 over-expression exerted negligible effect on late current of F1759A mutation (Figure 5C-F), thus confirming the reduced sensitivity of these channels for FGF13. In all, the differential modulation of IQ/AA versus F1759A mutant by FGF13 further corroborates the emerging role of FGF13 in tuning pathogenic late current of cardiac Na\textsubscript{v} channels.
Discussion

In cardiomyocytes, late Na\(^+\) current caused by delayed or incomplete inactivation of Na\(_V\)1.5 channels underlies diverse cardiac disorders. Our previous studies using a transgenic Na\(_V\)1.5 F1759A mutant channel illustrated the contribution of late current for cardiac disease pathogenesis (5, 30). Although diminutive compared to the peak Na\(^+\) current that initiates the cardiac action potential, the depolarizing late current counteracts repolarization resulting in heterogeneous action potential prolongation that may trigger atrial (5) or ventricular arrhythmias (Figure 3). Sustained Na\(^+\) influx also causes structural abnormalities such as those linked to dilated cardiomyopathy (3). In heterologous expression systems, CaM has emerged as an important regulator of late Na\(_V\)1.5 current (13, 14). LQT3-linked and structure-guided mutations in the Na\(_V\)1.5 carboxy-terminus that disrupt CaM interaction cause a marked increase in late Na\(^+\) current (13, 14) to levels comparable to those seen in the F1759A mutant. To probe for the functional relevance of CaM for late Na\(_V\)1.5 current in cardiomyocytes, we generated a transgenic mouse model expressing IQ/AA mutant Na\(_V\)1.5 channels with disrupted CaM binding. Surprisingly, we found that IQ/AA mutant did not increase late Na\(^+\) current in cardiomyocytes. These findings reveal the existence of endogenous protective mechanisms that counteract the increase in late current that occurs with loss of CaM binding (Figure 5G). Further biochemical and mechanistic analysis demonstrated that FGF13, a component of the Na\(_V\)1.5 neighborhood in cardiomyocytes, fully reverses the late current of IQ/AA, but minimally perturbs F1759A mutant in HEK293 cells.

CaM regulation of Na\(_V\) channels is multifaceted and isoform specific, although a vast majority of functional effects thus far have been deduced through heterologous expression of recombinant Na\(_V\) channels (13-15, 21, 37, 38) Both Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound CaM interact with Na\(_V\)1.5 channels with a high affinity (9-12, 15). The Ca\(^{2+}\)/CaM mediated effects of Na\(_V\)1.5 have been variable, with some studies finding a depolarizing shift in steady-state inactivation properties depending on CaM interaction with the III-IV linker (15, 38). Prior analysis of endogenous Na\(_V\)1.5 in guinea pig ventricular myocytes, however, revealed no Ca\(^{2+}\)-dependent effects leading us to focus here on effects of apoCaM (18). Indeed, loss of apoCaM pre-association to the Na\(_V\)1.5 IQ motif results in a marked elevation of late current in heterologous systems caused by a structural uncoupling of the III-IV linker from the C-terminal domain (13, 21). Our present study, however, demonstrates that this is not the case for CaM-deficient IQ/AA mutant channels in mouse cardiomyocytes. Thus, the manifestation of CaM regulation of Na\(_V\) channels diverges considerably in the native setting compared to heterologous systems.
The absence of both apo and Ca\textsuperscript{2+}-CaM dependent modulation of Na\textsubscript{v}1.5 in cardiomyocytes raises new questions regarding the functional role of CaM for these channels. This is critical as human mutations in the CaM binding interface of Na\textsubscript{v}1.5 are linked to LQT3 (14), although augmentation of late Na\textsuperscript{+} current is likely not the main pathophysiological mechanism responsible for the LQTS phenotype observed in patients with CaM mutations (39). One possibility is that CaM plays a redundant role also served by FHF to ensure fail-safe inactivation (21), a critical factor for proper cardiac function (Figure 5G). Yet, if FHF and CaM cooperatively ensure fail-safe inactivation, why should the human mutation Q1909R (40), which decreases CaM affinity, lead to LQT3 and arrhythmias in humans? No apparent phenotype would be expected based upon the findings for the IQ/AA mutant in mouse cardiomyocytes. There are several possible explanations: (1) Mice and humans express different FGF isoforms: mice express FGF13 and humans express predominantly FGF12 (35). The affinity of FGF13, \sim 16 nM, for the Na\textsubscript{v}1.5 C-terminal domain (Supplemental Data Figure 3) is approximately 10-fold higher relative to FGF12 (11). Thus, mice may be more “protected” than humans to CaM-depletion induced Na\textsubscript{v}1.5-mediated arrhythmias, because of expression of FGF13 rather than FGF12. Studies of inducible pluripotent stem cell-derived differentiated cardiomyocytes may further delineate the role of FGF12 in tuning CaM-deficient Na\textsubscript{v}1.5 channels in humans. (2) FHF expression and interactions with Na\textsubscript{v}1.5 may be tuned by transcriptional or post-translation modifications (34, 41), that unveils latent CaM-dependent modulation. Perhaps the phenotype of Q1909R becomes manifest only in specific circumstances, which may not be apparent in healthy sedentary laboratory animals. For instance, a bevy of other cellular factors and conditions have been shown to upregulate late Na\textsuperscript{+} current (42, 43) including, heart failure (44), hypoxia (45, 46), reactive oxygen species (45), CaMKII-dependent phosphorylation (47, 48), and altered interaction with other regulatory proteins (49, 50). It is possible that some of these processes may be linked to FHF modulation of Na\textsubscript{v}1.5. For example, CaMKII-dependent phosphorylation of the Na\textsubscript{v} carboxy-tail has been shown to disrupt FHF interaction (34). Finally, FHF-CaM regulation of Na\textsubscript{v} channels can be synergistic or antagonistic depending on the physiological setting. For skeletal muscle Na\textsubscript{v}1.4 channels, CaM regulation manifests as rapid Ca\textsuperscript{2+}-dependent inhibition of the peak current (18). FHF co-expression with Na\textsubscript{v}1.4 antagonizes Ca\textsuperscript{2+}/CaM-dependent feedback of these channels (51). This functional plasticity may help ensure that Na\textsuperscript{+} influx is precisely tuned to match varied physiological demands.

The relative insensitivity of F1759A mutant for FGF13 could be predicted by the presence of late current in cardiomyocytes. Structurally, F1759 residue is located within the transmembrane region in a central cavity on the intracellular side of the selectivity filter (7). This location is within
the membrane, rather than on the C-terminal domain, which is the primary FHF binding site (11). As such, we expect intact FHF binding to the F1759A mutant. \( \text{Na}_V \) channels in Purkinje neurons exhibit resurgent current that results from open-state block by intracellular endogenous proteins including FGF14 and the cytosolic domain of the \( \text{Na}_V \beta 4 \) subunit (52). Interestingly, such endogenous open-channel blocking proteins antagonize lidocaine action suggesting that the two sites may be coupled (53).

Future studies that knock out FGF13 in the IQ/AA transgenic model will help ascertain the precise contribution of FGF13 in attenuating late current of FGF13. In our multichannel recordings, the peak-normalized late open probability of wild-type \( \text{Na}_V1.5 \) channels bound to FGF13 in HEK cells are nearly an order of magnitude higher than that of the native cardiac setting. Careful scrutiny of late channel openings of F1759A mutant in cardiomyocytes reveal a “late-scattered” phenotype, while FHF co-expression with F1759A mutant in HEK cells show “burst mode” openings, two distinct mechanisms for late current (42). These differences in channel gating behavior suggests that there may be as-yet-unidentified regulatory proteins in addition to FHF that may also be involved in vivo. Proteomic studies utilizing the \( \text{Na}_V1.5\)-APEX mice could help identify other regulatory proteins that diminish late current.

In all, our results reveal a surprisingly complete protection from late \( \text{Na}^+ \) current in murine cardiomyocytes expressing \( \text{Na}_V1.5 \) devoid of apo-CaM binding. Heterologous expression studies suggest that the protection is due to FGF13, which is co-localized with \( \text{Na}_V1.5 \) in murine cardiomyocytes. Leveraging endogenous mechanisms may furnish an alternative avenue for developing novel pharmacology that selectively blunts late \( \text{Na}^+ \) current, a highly sought-after drug target (42, 43).
Methods

General experimental approaches:

All experimental procedures and analysis were performed in a blinded fashion. No data points, samples or mice were excluded from the study.

Mouse models:

The pWT, IQ/AA, F1759A, and V5-APEX2 lines were generated by fusing human heart Na\(^+\) channel \(\alpha\)-subunit cDNA (hH1) (54) to a vector containing the modified murine \(\alpha\)-MHC, tetracycline-inducible promoter gift of Jeffrey Robbins and Jeffrey Molkentin (University of Cincinnati, Cincinnati, OH) (55, 56). SCN5A was engineered to be either TTX-sensitive by inserting a C374Y mutation or lidocaine-resistant by insertion a F1759A mutation. A 3X FLAG epitope was ligated in-frame to the N-terminus. These mice, in a B6CBA/F2 hybrid background, were bred with cardiac-specific rtTA mice in a FVB/N background, obtained via MMRRC (24), to generate doxycycline-inducible transgenic mice. The V5 epitope and APEX2 cDNA (57, 58), created by gene-synthesis, were conjugated to the N-terminus of human heart Na\(^+\) channel \(\alpha\)-subunit. Both male and female mice were used in all experiments. Gender had no effect on the outcomes of any experiment.

ECG Analysis:

Subcutaneous four-lead electrocardiograms of isoflurane-anesthetized mice were performed using EMKA ECG and recorded using IOX software (Emka Technologies). PR, RR and QT intervals were measured manually using Ponemah 3 software (Data Sciences International).

Isolation of cardiac myocytes from adult mice:

Mice ventricular myocytes were isolated by enzymatic digestion using a Langendorff perfusion apparatus as previously described (5, 33, 59-62). Cardiomyocytes were isolated from 8-12 week-old non-transgenic and transgenic mice.

Cardiomyocyte patch clamp recordings:

Experiments were performed at room temperature. Membrane currents from rod-shaped cells with clear striations that were not spontaneously contracting were measured by the whole-cell patch-clamp method using a MultiClamp 700B amplifier and pCLAMP software (Axon Instruments, Molecular Devices). The pipette resistance was 0.4-1.0 M\(\Omega\) in order to minimize voltage clamp error. The cell capacitance currents were compensated. Series resistance was
compensated at 60%. Liquid junction potential (-10 mV) was corrected. The leak current was subtracted using a P/4 protocol. The intracellular pipette solution contained (in mM): 3 or 5 NaCl, 20 CsCl, 115 CsF, 10 HEPES, and 10 BAPTA (pH 7.4) titrated with CsOH. For late Na⁺ current determinations, the bath solution contained (in mM): 100 NaCl, 45 TEA-Cl, 10 HEPES, 1 MgCl₂, 0.25 CaCl₂, and 5 glucose (pH 7.4) titrated with CsOH. To determine late current, the cell membrane potential was held at -110 mM and stepped to -30 mV for 190 ms in the absence and in the presence of TTX, 40 µM (non-transgenic and F1759A mice), or ranolazine, 50 µM (pWT and IQ/AA mice). The mean value of the current during the last 10 ms of the 190-ms pulse was measured. The difference of these values was used as a measure of late Na⁺ current and later normalized to cell capacitance. The mean value of the late current was also normalized to the peak Na⁺ current for each cell. For measurement of peak Na⁺ current, the extracellular solution contained (in mM): 3 or 5 NaCl, 142 TEA-Cl, 10 HEPES, 1 MgCl₂, 0.25 CaCl₂, and 5 glucose (pH 7.4) titrated with CsOH. Peak transient currents in the F1759A mice were measured with 5 mM Na⁺ in both intracellular and extracellular solutions. For the cardiomyocytes isolated from F1759A mice, the fraction of transgenic current was assessed by applying 3 mM lidocaine as we have previously described (5). Peak transient currents in the cardiomyocytes isolated from the pWT and IQ/AA mice were measured with 3 mM Na⁺ in both intracellular and extracellular solutions by stepping the voltage from a holding potential of -110 mV to -30 mV. For these cardiomyocytes, the fraction of transgenic current was assessed by applying 20 nM TTX, which inhibits all mutant channels (IC₅₀ < 1 nM), but has no effect on endogenous, non-transgenic channels. The current voltage-relationship of transgenic Na⁺ currents for the pWT and IQ/AA cardiomyocytes were quantified as the difference between no TTX and 20 nM TTX across a range of voltages, from a holding potential of -110 mV to 0 mV (Supplemental Data Figure 1).

**Multi-channel analysis of late Na⁺ current:**

Multichannel records were obtained in the on-cell configuration with either HEK293 cells or in cardiomyocytes. The pipette contained (in mM): 140 NaCl; 10 HEPES; 0.5 CaCl₂; at 300 mOsm, adjusted with tetraethylammonium methanesulfonate; and pH 7.4 adjusted with tetraethylammonium hydroxide. To zero membrane potential, the bath contained (in mM): 132 K⁺-glutamate; 5 KCl; 5 NaCl; 3 MgCl₂; 2 EGTA; 10 glucose; 20 HEPES; at 300 mOsm adjusted with glucose; and pH 7.4 adjusted with NaOH. Data were acquired at room temperature using the integrating mode of an Axopatch 200A amplifier (Axon Instruments, Molecular Devices). Patch pipettes (3 – 10 MΩ) were pulled from ultra-thick-walled borosilicate glass (BF200-116-10; Sutter Instruments) using horizontal puller (P-97, Sutter Instruments), fire polished with a microforge
(Narishige), and coated with Sylgard (Dow Corning). Elementary currents were low-pass filtered at 2 kHz with a four-pole Bessel filter and digitized at 200 kHz with an ITC-18 unit (Instrutech), controlled by custom MATLAB software (Mathworks). For each pulse, we obtained P/8 leak pulses. Leak subtraction was performed using an automated algorithm which fit the kinetics of the leak current or the capacitive transient with convex optimization with L1 regularization of the following the objective function:

$$
\min_{e,s} \left( \frac{1}{2} |I_L - D e - s|^2 + \lambda |s|_1 \right)
$$

s.t. $e \geq 0, s \leq 0$, and $s(t < t_0) = 0$

Here, $I_L$ is the leak waveform that is to be fit as a sum of exponentials; $D$ is a matrix composed of a library of exponential functions with various time constants; $e$ is a sparse vector composed of the amplitude of each exponentials; $s$ is a vector representing an estimate of negative outliers from the baseline that correspond to actual channel openings; and $\lambda$ is a penalty term that controls the importance of regularization. For most fits, $\lambda$ was set to be 0.25. Following leak subtraction, the unitary current for each patch was estimated using an amplitude histogram. Each stochastic trace was subsequently idealized. The ensemble average from 50-100 stochastic traces was computed for each patch and normalized to the peak current. The average late current for each patch ($R_{\text{persist}}$) was computed as the average normalized $P_o$ following 50ms of depolarization.

**Cell Culture and Transfection:**

Human Na\textsubscript{v}1.5 pGW corresponds to clone M77235.1 (GenBank). To construct IQ/AA mutation, we perform overlap-extension PCR and ligated the mutated PCR product into the Na\textsubscript{v}1.5 wildtype pGW vector using Kpn I and Xba I restriction sites. The F1759 plasmid was constructed as previously described. Human FGF13 / FGF13 plasmid corresponding to NM_004114.5 (Genbank) was cloned pcDNA3 vector following PCR amplification using EcoRI and XbaI. For whole-cell patch clamp experiments, HEK293 cells (ATCC) were cultured on 6-cm plates, and channels transiently transfected by Ca\textsuperscript{2+} phosphate method as previously described. For Na\textsubscript{v}1.5 WT and IQ/AA channels, we transfected 4-8 µg of the $\alpha$-subunit, with 4 µg of YFP, and 1 µg of t-antigen to boost expression. For experiments evaluating effect of FHF, we transfected FGF13-pcDNA3 at 1:1 ratio as the $\alpha$-subunit.

**Proximity labeling biotinylation:**

Proximity labeling was performed as described (33, 58). Isolated ventricular cardiomyocytes were incubated in labeling solution with 0.5 µM biotin-phenol (Iris-biotech) for 30 minutes. During
the final 10 minutes of labeling, 1 µM isoproterenol (Sigma I5627) was added. To initiate labeling, 
H₂O₂ (Sigma H1009) was added to a final concentration of 1 mM for 1 min. Exactly 1 minute after 
H₂O₂ treatment, the labeling solution was decanted and cells were washed three times with cold 
quenching solution containing (in mM) 10 Sodium ascorbate (VWR 95035-692), 5 Trolox (Sigma 
238813), and 10 Sodium azide (Sigma S2002). After cells were harvested by centrifugation, the 
quenching solution was aspirated and the pellet was flash-frozen and stored at -80 °C until 
streptavidin pull-down.

The cells were lysed with a hand-held tip homogenizer in a solution containing (in mM), 50 
Tris (tris(hydroxymethyl)aminomethane), 150 NaCl, 10 EGTA, 10 EDTA, 1% Triton X-100 (v/v), 
0.1% SDS (w/v), 10 Sodium ascorbate, 5 Trolox, and 10 Sodium azide, phosphatase inhibitors 
(Sigma 4906845001), protease inhibitors (Sigma 4693159001), Calpain inhibitor I (Sigma A6185) 
and Calpain inhibitor II (Sigma A6060). Biotin labeling of the samples was confirmed by Western 
blot with Streptavidin-HRP (Sigma RABHRP3). Proteins were prepared as previously described 
(63). Proteins were precipitated with trichloroacetic acid (TCA; Sigma T9159) and then centrifuged 
at 21,130 × g at 4 °C for 10 minutes. The pellet was washed with -20 °C cold acetone (Sigma 
650501), vortexed, and centrifuged at 21,130 × g at 4 °C for 10 minutes. Following centrifugation, 
acetone was aspirated and the pellet was acetone-washed again three more times. After the last 
washing step, the pellet was resuspended in: 8M urea, 100 mM sodium phosphate pH 8, 100 mM 
NH₄HCO₃, and 1% SDS (w/v) and rotated at room temperature until fully dissolved. Re-
suspended proteins were centrifuged at 21,130 × g at room temperature for 10 minutes and the 
cleared supernatant was transferred to a new microcentrifuge tube. To reduce disulfides, 10 mM 
TCEP-HCl (Thermo Fisher Scientific PG82089) in Milli-Q water titrated to pH 7.5 with NaOH was 
added. To alkylate free Cys, freshly prepared 400 mM iodoacetamide (Thermo Fisher Scientific 
90034) stock solution in 50 mM ammonium bicarbonate was added to the supernatant to a final 
concentration of 20 mM, immediately vortexed, and incubated in the dark for 25 minutes at room 
temperature. After alkylation, freshly prepared DTT (dithiothreitol) stock solution was added to 50 
mM final concentration to quench alkylation. Water was added to each sample to reach a final 
concentration of 4 M urea and 0.5% (w/v) of SDS.

For each sample, a 100 µL suspension of streptavidin magnetic beads (Thermo Fisher 
Scientific #88817) was washed twice with 4 M urea, 0.5% SDS (w/v), 100 mM sodium phosphate 
pH 8. The liquid was aspirated and the beads were added to each ~ 1 mg sample, thereafter 
diluting each sample with an equal amount of water to reach a final concentration of 2 M urea, 
0.25% SDS (w/v), 50 mM sodium phosphate pH 8 during pulldown. The tubes were rotated 
overnight at 4 °C. Following streptavidin pull-down, the magnetic beads were washed three times
with 4 M urea, 0.5% SDS (w/v), 100 mM sodium phosphate pH 8, and three times with the same buffer without SDS. The beads were transferred to new tubes for the last wash step.

**Immunoblots:**

Cardiomyocytes were homogenized in a 1% Triton X-100 buffer containing (in mM): 50 Tris-HCl (pH 7.4) 150 NaCl, 10 EDTA, 10 EGTA and protease inhibitors. The lysates were incubated on ice for 30 min, centrifuged at 14K rpm at 4 °C for 10 min and supernatants collected. Proteins were size-fractionated on SDS-PAGE, transferred to nitrocellulose membranes and probed with anti-FLAG antibody (Sigma, catalog A8592), a custom-made anti-FGF13 (FGF13) antibody (22), and an anti-calmodulin antibody (Millipore Sigma 05-173). Image collection was performed with a CCD camera (Carestream Imaging) and quantification was performed using ImageQuant software.

**Immunofluorescence:**

For proximity labeling, isolated cardiomyocytes were first exposed to biotin-phenol and H₂O₂ as described above. After quenching, the cells were fixed for 15 minutes in 4% paraformaldehyde, washed with glycine/PBS (phosphate-buffered saline) twice, treated with PBST (0.1% Triton X-100 (v/v) in PBS) for 5 minutes, and blocked with 3% BSA (w/v) in PBS for 1 hour. Indirect immunofluorescence was performed using a 1:500 anti-V5 antibody (Sigma) and 1:200 Alexa594-labeled goat-anti-mouse antibody (Sigma), and 1:800 streptavidin-Alexa Fluor 488 conjugate. For immunofluorescence without proximity labeling, isolated cardiomyocytes were fixed for 15 min in 4% paraformaldehyde. Indirect immunofluorescence was performed using either a 1:200 rabbit anti-FLAG antibody (Sigma) or anti-NaV1.5 antibody (Alomone; ASC-005), and 1:400 FITC-labeled goat-anti-rabbit antibody (Thermo-Fisher A-11034). Images were acquired using a confocal microscope.

**Optical mapping protocol and data processing:**

Mice were heparinized and isolated hearts were perfused via a Langendorff apparatus with warm oxygenated Krebs-Henseleit buffer (pH 7.4; 95% O₂, 5% CO₂, 36–38°C). The hearts were also superfused in a glass chamber filled with Tyrode. One AgCl wire was attached to the metal aortic cannula, and another AgCl wire was positioned near the surface of the heart to record an ECG. Blebbistatin (5-10 µM) was perfused to reduce motion, and Di-4-ANEPPS (100 µM) was perfused to optically record membrane potentials. (30). Hearts were uniformly illuminated with green excitation lasers (532 nm) to excite Di-4-ANEPPS. Emitted fluorescence was captured
through a 580-nm pass filter using a complementary metal-oxide-semiconductor (CMOS) camera (MICAM Ultima, SciMedia). Movies were acquired at 1000 frames per second for a duration of 4-5 sec, with 100 x 100-pixel resolution (0.095 mm per pixel). Susceptibility to pacing-induced ventricular arrhythmia was assessed by 3 attempts of burst pacing at the apex at twice the excitation threshold (Pulsar 6i, FHM, Brunswick, ME) of the left ventricle (20 Hz, amplitude 0.5–2.0 mA, 5 ms). To convert the F1759A mice to sinus rhythm, the arrhythmias were terminated by infusion of a hyperkalemia solution. After conversion to sinus rhythm, a normokalemic solution was perfused and the optical maps were obtained.

Recorded optical movies were processed using custom software based on PV-WAVE (Precision Visuals - Workstation Analysis and Visualization Environment, Visual Numerics, Inc) (64, 65). The background fluorescence was subtracted from each frame, and spatial (5 X 5 pixels) and temporal (9 frames) conical convolution filters were used to increase signal-to-noise ratio. Movies recorded during pacing were averaged to improve signal-to-noise ratio. The optical APD were measured in each pixel at 50% repolarization level at 10Hz pacing. Phase movies and phase singularities locations were obtained after Hilbert transformation of the fluorescent signal. (66). Rotational activity of at least 1 cycle was classified as a rotor (64).

**Isothermal Titration Calorimetry:**

Experiments were performed with an ITC-200 (MicroCal) at 20°C as described (11). Solution containing Na\(_{\text{v1.5}}\) C-terminal domain (20-51 µM) were titrated with 20-30 10-µl injections of solution containing FGF13 (240-510 µM). ITC experiments were repeated with different preparations and different concentrations at least three times to confirm thermodynamic parameters and stoichiometry values. The binding isotherms were analyzed with a single site binding model using the Microcal Origin version 7.0 software package (Originlab Corporation). Results are presented as mean ± standard error.

**Statistics:**

Results are presented as mean ± SEM. Statistical analyses were performed using Prism 8 (Graphpad Software). Data were tested using D’Agostino-Pearson normality test. For non-normally distributed data requiring multiple comparisons, a Kruskal-Wallis test followed by a Dunn’s post-hoc test were performed. For normally distributed data that required multiple comparisons testing, a one-way ANOVA followed by a Dunnett’ test were performed. For comparisons between two groups, Student’s t-test was used for normally distributed data and a Mann-Whitney test was used for non-normally distributed data. Differences were considered
statistically significant at values of $P < 0.05$.

**Study Approvals:**

The Institutional Animal Care and Use Committee at Columbia University approved all animal experiments.
Acknowledgements: We thank Ben Haeffele and Po Wei Kang for automated leak subtraction algorithm.

Sources of funding: This publication was supported by R01 HL140934 to SOM, 1R01NS110672 to MBJ and 1R01HL152236 to EYW. Images were collected and analyzed in the Confocal and Specialized Microscopy Shared Resource of the Herbert Irving Comprehensive Cancer Center at Columbia University, supported by NIH grant P30 CA013696 (National Cancer Institute). Daniel Roybal was supported by T32 HL120826 and F31 HL142178. Jessica Hennessey was supported by T32 HL007854. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: The authors declare no competing interests.
References:
1. Yan Z, Zhou Q, Wang L, Wu J, Zhao Y, Huang G, et al. Structure of the Nav1.4-beta1 Complex from Electric Eel. *Cell.* 2017;170(3):470-82 e11.
2. Abriel H, Rougier JS, and Jalife J. Ion channel macromolecular complexes in cardiomyocytes: roles in sudden cardiac death. *Circ Res.* 2015;116(12):1971-88.
3. Wilde AAM, and Amin AS. Clinical Spectrum of SCN5A Mutations: Long QT Syndrome, Brugada Syndrome, and Cardiomyopathy. *JACC Clin Electrophysiol.* 2018;4(5):569-79.
4. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell.* 1995;80(5):805-11.
5. Wan E, Abrams J, Weinberg RL, Katchman AN, Bayne J, Zakharov SI, et al. Aberrant sodium influx causes cardiomyopathy and atrial fibrillation in mice. *J Clin Invest.* 2016;126(1):112-22.
6. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature.* 1998;392(6673):293-6.
7. Jiang D, Shi H, Tonggu L, Gamal El-Din TM, Lenaeus MJ, Zhao Y, et al. Structure of the Cardiac Sodium Channel. *Cell.* 2020;180(1):122-34 e10.
8. Mori M, Konno T, Ozawa T, Murata M, Imoto K, and Nagayama K. Novel interaction of the voltage-dependent sodium channel (VDSC) with calmodulin: does VDSC acquire calmodulin-mediated Ca2+-sensitivity? *Biochemistry.* 2000;39(6):1316-23.
9. Theoharis NT, Sorensen BR, Theisen-Toupal J, and Shea MA. The neuronal voltage-dependent sodium channel type II IQ motif lowers the calcium affinity of the C-domain of calmodulin. *Biochemistry.* 2008;47(1):112-23.
10. Gabelli SB, Boto A, Kuhns VH, Bianchet MA, Farinelli F, Aripirala S, et al. Regulation of the NaV1.5 cytoplasmic domain by calmodulin. *Nat Commun.* 2014;5:5126.
11. Wang C, Chung BC, Yan H, Lee SY, and Pitt GS. Crystal structure of the ternary complex of a NaV C-terminal domain, a fibroblast growth factor homologous factor, and calmodulin. *Structure.* 2012;20(7):1167-76.
12. Wang C, Chung BC, Yan H, Wang HG, Lee SY, and Pitt GS. Structural analyses of Ca(2)(+)/CaM interaction with NaV channel C-termini reveal mechanisms of calcium-dependent regulation. *Nat Commun.* 2014;5:4896.
13. Kim J, Ghosh S, Liu H, Tateyama M, Kass RS, and Pitt GS. Calmodulin mediates Ca2+ sensitivity of sodium channels. *J Biol Chem.* 2004;279(43):45004-12.
14. Yan H, Wang C, Marx SO, and Pitt GS. Calmodulin limits pathogenic Na+ channel persistent current. *J Gen Physiol.* 2017;149(2):277-93.
15. Sarhan MF, Tung CC, Van Petegem F, and Ahern CA. Crystallographic basis for calcium regulation of sodium channels. *Proc Natl Acad Sci U S A.* 2012;109(9):3558-63.
16. Sarhan MF, Van Petegem F, and Ahern CA. A double tyrosine motif in the cardiac sodium channel domain III-IV linker couples calcium-dependent calmodulin binding to inactivation gating. *J Biol Chem.* 2009;284(48):33265-74.
17. Johnson CN, Potet F, Thompson MK, Kroncke BM, Glazer AM, Voehler MW, et al. A Mechanism of Calmodulin Modulation of the Human Cardiac Sodium Channel. *Structure.* 2018;26(5):683-94 e3.
18. Ben-Johny M, Yang PS, Niu J, Yang W, Joshi-Mukherjee R, and Yue DT. Conservation of Ca2+/calmodulin regulation across Na and Ca2+ channels. *Cell.* 2014;157(7):1657-70.
19. Wang C, Wang C, Hoch EG, and Pitt GS. Identification of novel interaction sites that determine specificity between fibroblast growth factor homologous factors and voltage-gated sodium channels. *J Biol Chem.* 2011;286(27):24253-63.
20. Musa H, Kline CF, Sturm AC, Murphy N, Adelman S, Wang C, et al. SCN5A variant that blocks fibroblast growth factor homologous factor regulation causes human arrhythmia. *Proc Natl Acad Sci U S A.* 2015;112(40):12528-33.

21. Gade AR, Marx SO, and Pitt GS. An interaction between the III-IV linker and CTD in NaV1.5 confers regulation of inactivation by CaM and FHF. *J Gen Physiol.* 2020;152(2).

22. Wang C, Hennessey JA, Kirkton RD, Wang C, Graham V, Puranam RS, et al. Fibroblast growth factor homologous factor 13 regulates Na+ channels and conduction velocity in murine hearts. *Circ Res.* 2011;109(7):775-82.

23. Satin J, Kyle JW, Chen M, Bell P, Cribbs LL, Fozzard HA, et al. A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. *Science.* 1992;256(5060):1202-5.

24. Valencik ML, and McDonald JA. Codon optimization markedly improves doxycycline regulated gene expression in the mouse heart. *Transgenic Res.* 2001;10(3):269-75.

25. Kiyosue T, and Arita M. Late sodium current and its contribution to action potential configuration in guinea pig ventricular myocytes. *Circ Res.* 1989;64(2):389-97.

26. Maltsev VA, and Undrovinas AI. A multi-modal composition of the late Na+ current in human ventricular cardiomyocytes. *Cardiovasc Res.* 2006;69(1):116-27.

27. Zhang T, Yong SL, Tian XL, and Wang QK. Cardiac-specific overexpression of SCN5A gene leads to shorter P wave duration and PR interval in transgenic mice. *Biochem Biophys Res Commun.* 2007;355(2):444-50.

28. Lupoglazoff JM, Cheav T, Baroudi G, Berthet M, Denjoy I, Cauchemez B, et al. Homozygous SCN5A mutation in long-QT syndrome with functional two-to-one atrioventricular block. *Circ Res.* 2001;89(2):E16-21.

29. UM RA, Abrams J, Katchman A, Zakharov S, Mironov S, Bayne J, et al. Heterogeneity of the action potential duration is required for sustained atrial fibrillation. *JCI Insight.* 2019;5.

30. Avula UMR, Abrams J, Katchman A, Zakharov S, Mironov S, Bayne J, et al. Heterogeneity of the action potential duration is required for sustained atrial fibrillation. *JCI Insight.* 2019;5.

31. Rhee HW, Zou P, Udeshi ND, Martell JD, Moota VK, Carr SA, et al. Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science.* 2013;339(6125):1328-31.

32. Hung V, Zou P, Rhee HW, Udeshi ND, Cracan V, Svinkina T, et al. Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric APEX tagging. *Mol Cell.* 2014;55(2):332-41.

33. Liu G, Papa A, Katchman AN, Zakharov SI, Roybal D, Hennessey JA, et al. Mechanism of adrenergic CaV1.2 stimulation revealed by proximity proteomics. *Nature.* 2020;577(7792):695-700.

34. Burel S, Coyan FC, Lorenzini M, Meyer MR, Lichti CF, Brown JH, et al. C-terminal phosphorylation of NaV1.5 impairs FGF13-dependent regulation of channel inactivation. *J Biol Chem.* 2017;292(42):17431-48.

35. Hennessey JA, Wei EQ, and Pitt GS. Fibroblast growth factor homologous factors modulate cardiac calcium channels. *Circ Res.* 2013;113(4):381-8.

36. Carboni M, Zhang ZS, Nepliouvea V, Starmer CF, and Grant AO. Slow sodium channel inactivation and use-dependent block modulated by the same domain IV S6 residue. *J Membr Biol.* 2005;207(2):107-17.

37. Tan HL, Kupershemdit S, Zhang R, Stepanovic S, Roden DM, Wilde AA, et al. A calcium sensor in the sodium channel modulates cardiac excitability. *Nature.* 2002;415(6870):442-7.

38. Shah VN, Wingo TL, Weiss KL, Williams CK, Balser JR, and Chazin WJ. Calcium-dependent regulation of the voltage-gated sodium channel hH1: intrinsic and extrinsic
sensors use a common molecular switch. *Proc Natl Acad Sci U S A.* 2006;103(10):3592-7.

39. Yin G, Hassan F, Haroun AR, Murphy LL, Crotti L, Schwartz PJ, et al. Arrhythmogenic calmodulin mutations disrupt intracellular cardiomyocyte Ca2+ regulation by distinct mechanisms. *J Am Heart Assoc.* 2014;3(3):e000996.

40. Kapplinger JD, Giudicessi JR, Ye D, Tester DJ, Callis TE, Valdivia CR, et al. Enhanced Classification of Brugada Syndrome-Associated and Long-QT Syndrome-Associated Genetic Variants in the SCN5A-Encoded Na(v)1.5 Cardiac Sodium Channel. *Circ Cardiovasc Genet.* 2015;8(4):582-95.

41. Yang J, Wang Z, Sinden DS, Wang X, Shan B, Yu X, et al. FGF13 modulates the gating properties of the cardiac sodium channel Nav1.5 in an isofrom-specific manner. *Channels (Austin).* 2016;10(5):410-20.

42. Moreno JD, and Clancy CE. Pathophysiology of the cardiac late Na current and its potential as a drug target. *J Mol Cell Cardiol.* 2012;52(3):608-19.

43. Banyasz T, Szentandrassy N, Magyar J, Szabo Z, Nanasi PP, Chen-Izu Y, et al. An emerging antiarrhythmic target: late sodium current. *Curr Pharm Des.* 2015;21(8):1073-90.

44. Maltsev VA, and Undrovinas A. Late sodium current in failing heart: friend or foe? *Prog Biophys Mol Biol.* 2008;96(1-3):421-51.

45. Plant LD, Xiong D, Romero J, Dai H, and Goldstein SAN. Hypoxia Produces Pro-arrhythmic Late Sodium Current in Cardiac Myocytes by SUMOylation of NaV1.5 Channels. *Cell Rep.* 2020;30(7):2225-36 e4.

46. Ma J, Song Y, Shryock JC, Hu L, Wang W, Yan X, et al. Ranolazine attenuates hypoxia- and hydrogen peroxide-induced increases in sodium channel late openings in ventricular myocytes. *J Cardiovasc Pharmacol.* 2014;64(1):60-6.

47. Horvath B, Banyasz T, Jian Z, Hegyi B, Kistamas K, Nanasi PP, et al. Dynamics of the late Na(+) current during cardiac action potential and its contribution to afterdepolarizations. *J Mol Cell Cardiol.* 2013;64:59-68.

48. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, et al. Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels. *J Mol Cell Cardiol.* 2013;64:59-68.

49. Cheng J, Valdivia CR, Vaidyanathan R, Balijepalli RC, Ackerman MJ, and Makielski JC. Caveolin-3 suppresses late sodium current by inhibiting nNOS-dependent S-nitrosylation of SCN5A. *J Mol Cell Cardiol.* 2013;61:102-10.

50. Cheng J, Van Norstrand DW, Medeiros-Domingo A, Valdivia C, Tan BH, Ye B, et al. Alpha1-syntrophin mutations identified in sudden infant death syndrome cause an increase in late cardiac sodium current. *Circ Arrhythm Electrophysiol.* 2009;2(6):667-76.

51. Niu J, Dick IE, Yang W, Barmbye MA, Yue DT, Tomaselli G, et al. Allosteric regulators selectively prevent Ca(2+)-feedback of CaV and NaV channels. *Elife.* 2018;7.

52. White HV, Brown ST, Bozza TC, and Raman IM. Effects of FGF14 and NaVbeta4 deletion on transient and resurgent Na current in cerebellar Purkinje neurons. *J Gen Physiol.* 2019;151(11):1300-18.

53. Bant JS, Aman TK, and Raman IM. Antagonism of lidocaine inhibition by open-channel blockers that generate resurgent Na current. *J Neurosci.* 2013;33(11):4976-87.

54. Gellens ME, George AL, Jr., Chen LQ, Chahine M, Horn R, Barchi RL, et al. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc Natl Acad Sci U S A.* 1992;89(2):554-8.

55. Sanbe A, Gulick J, Hanks MC, Liang Q, Osinska H, and Robbins J. Reengineering inducible cardiac-specific transgenesis with an attenuated myosin heavy chain promoter. *Circ Res.* 2003;92(6):609-16.
Hambleton M, York A, Sargent MA, Kaiser RA, Lorenz JN, Robbins J, et al. Inducible and myocyte-specific inhibition of PKCalpha enhances cardiac contractility and protects against infarction-induced heart failure. *Am J Physiol Heart Circ Physiol.* 2007;293(6):H3768-71.

Hung V, Udeshi ND, Lam SS, Loh KH, Cox KJ, Pedram K, et al. Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2. *Nat Protoc.* 2016;11(3):456-75.

Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, Mootha VK, et al. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat Methods.* 2015;12(1):51-4.

Katchman A, Yang L, Zakharov SI, Kushner J, Abrams J, Chen BX, et al. Proteolytic cleavage and PKA phosphorylation of alpha1C subunit are not required for adrenergic regulation of CaV1.2 in the heart. *Proc Natl Acad Sci U S A.* 2017;114(34):9194-9.

Yang L, Katchman A, Kushner J, Kushnir A, Zakharov SI, Chen BX, et al. Cardiac CaV1.2 channels require beta subunits for beta-adrenergic-mediated modulation but not trafficking. *J Clin Invest.* 2019;129(2):647-58.

Yang L, Katchman A, Weinberg RL, Abrams J, Samad T, Wan E, et al. The PDZ motif of the alpha1C subunit is not required for surface trafficking and adrenergic modulation of CaV1.2 channel in the heart. *J Biol Chem.* 2015;290(4):2166-74.

Yang L, Katchman A, Samad T, Morrow JP, Weinberg RL, and Marx SO. beta-adrenergic regulation of the L-type Ca2+ channel does not require phosphorylation of alpha1C Ser1700. *Circ Res.* 2013;113(7):871-80.

Paek J, Kalocsay M, Staus DP, Wingler L, Pascolutti R, Paulo JA, et al. Multidimensional Tracking of GPCR Signaling via Peroxidase-Catalyzed Proximity Labeling. *Cell.* 2017;169(2):338-49 e11.

Yamazaki M, Avula UM, Bandaru K, Atreya A, Boppana VS, Honjo H, et al. Acute regional left atrial ischemia causes acceleration of atrial drivers during atrial fibrillation. *Heart Rhythm.* 2013;10(6):901-9.

Avula UMR, Hernandez JJ, Yamazaki M, Valdivia CR, Chu A, Rojas-Pena A, et al. Atrial Infarction-Induced Spontaneous Focal Discharges and Atrial Fibrillation in Sheep: Role of Dantrolene-Sensitive Aberrant Ryanodine Receptor Calcium Release. *Circ Arrhythm Electrophysiol.* 2018;11(3):e005659.

Tomii N, Yamazaki M, Arafune T, Honjo H, Shibata N, and Sakuma I. Detection Algorithm of Phase Singularity Using Phase Variance Analysis for Epicardial Optical Mapping Data. *IEEE Trans Biomed Eng.* 2016;63(9):1795-803.
Figure 1. Cardiac-specific, FLAG-tagged TTX-sensitive Nav1.5-expressing transgenic mice. (A) Diagram showing Nav1.5. The pore-forming α subunit is a pseudotetramer of transmembrane domains (I-IV) linked by intracellular loops. The channel’s inactivation gate is in the III-IV linker. The best-established CaM binding site is on the C-terminal domain, where the FHF binding site also resides. (B) Schematic of binary transgene system. The expression of reverse tetracycline-controlled transactivator (rtTA) is driven by the cardiac-specific α-myosin heavy chain promoter. The cDNAs for FLAG-F1759A-Na\textsubscript{v}1.5 or FLAG-TTX-sensitive-Na\textsubscript{v}1.5 were ligated behind 7 tandem tetO sequences. (C) Anti-FLAG antibody immunoblots of cleared lysates of hearts from pWT, IQ/AA and non-transgenic mice. Representative images of three independent experiments. (D) Immunostaining of non-transgenic, pWT and IQ/AA mice cardiomyocytes. Non-transgenic cardiomyocytes: primary antibody (Ab) - anti-Na\textsubscript{v}1.5 antibody; pWT and IQ/AA cardiomyocytes: primary Ab - anti-FLAG antibody. FITC-conjugated secondary antibody was used for all experiments. Scale bar = 5 µm. Representative of 20 cardiomyocytes from at least 3 independent cardiomyocyte isolations for all groups. (E-G) Exemplar whole cell Na\textsuperscript{+} current trace of ventricular cardiomyocyte from non-transgenic, pWT and IQ/AA transgenic mice in the absence (black) and presence (red) of 20 nM TTX. Representative of n=13, 21, 44 cells from left to right. Vertical scale bars = 10 pA/pF; horizontal scale bars = 5 ms. (H) Graph showing effect of 20 nM TTX on peak Na\textsuperscript{+} current. **** P< 0.0001 by paired t-test. For non-transgenic, P= 0.61. n=13, 21, 44 cells from left to right. (I) Graph of fraction transgenic Na\textsuperscript{+} current for pWT and IQ/AA. Mean + SEM. n=21, 44 cells from left to right. P =0.73 by t-test.
Figure 2. Late Na\(^+\) current is not increased in cardiomyocytes expressing IQ/AA-Na\(_{1.5}\). (A-D) Exemplar whole cell Na\(^+\) current traces of ventricular cardiomyocytes isolated from non-transgenic, pWT, IQ/AA and F1759A mice. Experiments designed to assess late Na\(^+\) current using a 190-ms depolarization from a holding potential of –110 to –30 mV in the absence and presence of 500 µM ranolazine or 40 µM TTX; intracellular solution contained 5 mM Na\(^+\) and extracellular solution contained 100 mM Na\(^+\). Horizontal scale bars = 50 ms; vertical scale bars = 10 pA/pF. (E) Graph of fraction of late Na\(^+\) current normalized to peak Na\(^+\) current. Mean ± SEM, *P* < 0.0001 by Kruskal-Wallis test, **** *P* < 0.0001 by Dunn’s multiple comparison test. n= 23, 25, 29, 31 cardiomyocytes from left to right. (F) Multi-channel record from pseudo-WT myocyte shows rapid Na\(^+\) channel activation and inactivation followed by a rare opening in the late phase, following 50 ms of depolarization (gray shaded region). Inset shows lone Na\(_{V1.5}\) opening to unitary current level (dashed line) in the late phase. Vertical scale bar = 10 pA, Horizontal scale bar = 100 ms. (G) Normalized ensemble-average open probability relation computed from 50-80 stochastic records. Inset shows low levels of late P\(_O\) following 50 ms of depolarization. Vertical scale bar = 25% for normalized P\(_O\) (P\(_O\)(t)/P\(_O\)peak). (H-I) Multi-channel recordings of Na\(^+\) channels from IQ/AA mice show minimal late current similar to pWT myocytes. Format as in F-G. (J-K) Appreiciable late Na\(^+\) channel openings were detected for F1759A mutant. Format as in F-G. (L) Graph of P\(_O\) normalized to peak P\(_O\). Mean ± SEM, *P* < 0.001 by Kruskal-Wallis test, ** *P* < 0.01 by Dunn’s multiple comparison test.
Figure 3. QT interval and ventricular repolarization is not prolonged in IQ/AA transgenic mice. (A) Representative limb-lead surface electrocardiograms of isoflurane-anesthetized littermate non-transgenic, pWT, IQ/AA and F1759A transgenic mice. (B-D) Bar graphs of RR, PR and QT intervals from isoflurane-anesthetized mice. Mean ± SEM. For RR interval, \( P = 0.12 \); for PR interval, \( P = 0.0004 \); for QT interval, \( P < 0.001 \) by one-way ANOVA. ** \( P < 0.01 \), *** \( P < 0.001 \) by Dunnett’s multiple comparison test. NTG, \( n = 5 \); pWT, \( n = 17 \); IQ/AA, \( n = 13 \); F1759A, \( n = 5 \). (E-G) Representative optical APD maps (E,G) and optical action potential tracings (F) from F1759A and IQ/AA mice. APD maps for F1759A-dTG were obtained after hyperkalemia-induced conversion to sinus rhythm. The circles in panels E and G mark the regions for which optical action potential tracings are displayed in F. Scale bar = 1 mm. Representative of 3 similar recordings. (H) Snapshot from phase movie of Langendorff-perfused F1759A-dTG hearts demonstrating rotor in the ventricle after burst-pacing induced ventricular arrhythmia. Representative of 3 similar experiments.
Figure 4. Expression of FGF13 reduces late Na\(^+\) current in IQ/AA Na\(_{\text{V} 1.5}\). (A) Exemplar whole-cell Na\(^+\) currents recorded from cardiomyocytes of TTX-sensitive Na\(_{\text{V} 1.5}\)-V5-APEx2 transgenic mice, before (black trace) and after (red trace) 20 nM TTX. Representative of 3 similar recordings. Vertical scale bar = 5 pA/pF; horizontal scale bar = 5 ms. (B) Immunofluorescence of cardiomyocytes isolated from mice expressing Na\(_{\text{V} 1.5}\)–V5–APEX2 exposed to biotin-phenol and H\(_2\)O\(_2\). Staining is with anti-V5 and Alexa594-conjugated secondary antibodies (upper) and streptavidin-conjugated Alexa488. Scale bar = 5 \(\mu\)m. (C) Immunoblots of biotin-labelled proteins from cardiomyocytes of Na\(_{\text{V} 1.5}\)-APEX2 mice. Na\(_{\text{V} 1.5}\), FGF13 (FGF13) and CaM are detected in streptavidin pulldown. Blots representative of 2 independent experiments. (D-E) Multichannel recordings show minimal late Na\(^+\) channel openings for WT-Na\(_{\text{V} 1.5}\) expressed in HEK293 cells. Format as in Figure 2F-G. Horizontal scale bar = 100 ms. Vertical scale bar in panel D = 10 pA; Vertical scale bar in panel E = 25% for normalized P\(_o\) (P\(_o\)(t)/P\(_o\)(peak)). (F-G) Late current of WT-Na\(_{\text{V} 1.5}\) is unaffected by FGF13 overexpression. Same format as in D-E. (H-I) IQ/AA-Na\(_{\text{V} 1.5}\) mutant channel show enhanced late channel openings compared to WT-Na\(_{\text{V} 1.5}\) in HEK293 cells. Same format as in D-E. (J-K) FGF13 co-expression with IQ/AA-Na\(_{\text{V} 1.5}\) reverses the increase in late current to wild-type levels. Same format as in D-E. (L) Dot plot summary of P\(_{o,\text{late}}\) for WT-Na\(_{\text{V} 1.5}\) and IQ/AA-Na\(_{\text{V} 1.5}\) in the presence and absence of FGF13. Mean ± SEM, \(P < 0.0001\) by Kruskal-Wallis test, ** \(P < 0.001\) by Dunn’s multiple comparison test. (M) Graph shows fold-change in P\(_{o,\text{late}}\) for WT-Na\(_{\text{V} 1.5}\) and IQ/AA-Na\(_{\text{V} 1.5}\) by FGF13. Mean ± SEM, computed from aggregate data in panel L.
Figure 5. Late Na\(^+\) current for F1759A-Na\(_v\)1.5 is minimally perturbed by FGF13. (A-B) Multichannel recordings show high late Na\(^+\) current for F1759A-Na\(_v\)1.5 mutant when expressed in HEK293 cells. Format as in Figure 2F-G. Horizontal scale bar = 100 ms. Vertical scale bar in panel A = 10 pA; Vertical scale bar in panel B = 25% for normalized P\(_O\) (P\(_O\)(t)/P\(_O\)peak). (C-D) FGF13 coexpression with F1759A-Na\(_v\)1.5 mutant. Horizontal scale bar = 100 ms. Vertical scale bar in panel C = 10 pA; Vertical scale bar in panel D = 25% for normalized P\(_O\) (P\(_O\)(t)/P\(_O\)peak). (E-F) Population data confirms minimal change in late P\(_O\) for F1759A-Na\(_v\)1.5 with FGF13. Format as in Figure 4L-M. Open probability of late Na\(^+\) current from heterologously expressed IQ/AA-Na\(_v\)1.5 (without FHF13) is shown by the dashed blue line. (G) Schematic depicting late Na\(^+\) current in the absence of both FGF13 and CaM binding to the C-terminal domain of Na\(_v\)1.5. Late Na\(^+\) current is not present when either FHF or CaM binds to the C-terminal domain of Na\(_v\)1.5.
Supplemental Data Figure 1. Methodology used for analysis of transgenic Na$^+$ current. A,D, Graphs of current-voltage relationship for pWT Na$\nu$1.5 and IQ/AA Na$\nu$1.5 transgenic mice cardiomyocytes. Whole cell current traces were recorded with 3 mM Na$^+$ in both extracellular and intracellular solutions, in the absence of TTX. Inset, current traces from holding potential of –110 to 0 mV. Horizontal scale bar: 5 ms; vertical scale bar: 10 pA/pF. Total Na$^+$ current = non-transgenic (NT) current + current from pWT transgenic channels. B,E, Graphs of current-voltage relationship for pWT Na$\nu$1.5 and IQ/AA Na$\nu$1.5 transgenic mice cardiomyocytes after 20 nM TTX. The Na$^+$ current is from the non-transgenic (NT), endogenous channels. C,F, Current-voltage relationship of the pWT or IQ/AA channels, derived from total current (I$o$) by subtraction of remaining current in the presence of 20 nM TTX. Representative of 21 pWT cells and 44 IQ/AA cells.
Supplemental Data Figure 2. Peak Na\(^+\) current in F1759A transgenic mice. A-B, Exemplar whole cell Na\(^+\) current traces of ventricular cardiomyocytes isolated from non-transgenic (A) and F1759A transgenic (B) before (black) and after 3 mM lidocaine (blue). Whole cell current traces were recorded with 5 mM Na\(^+\) in both extracellular and intracellular solutions. Horizontal scale bars = 5 ms; vertical scale bars = 10 pA/pF. Representative of 5 similar experiments.
Supplemental Data Figure 3. Isothermal titration calorimetry experiment of FGF13 binding to Na\textsubscript{v}1.5 C-terminal domain. The total heat exchanged during each injection is fit to a binding isotherm with n, K\textsubscript{D}, and ΔH° as independent parameters. K\textsubscript{D} =16.6 ± 0.7 nM.