Supporting information

Expanded Methods

The study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edition) and to European directives (86/609/CEE).

ECG recording in conscious mice. For telemetric ECG recordings, adult male mice were anesthetized with 2% isoflurane (Forene®, Abbott, UK). A midline incision was made on the back along the spine to insert a telemetric transmitter (TA10EA-F20, Data Sciences International; St. Paul, Minnesota, USA) into a subcutaneous pocket with paired wire electrodes placed over the thorax (chest bipolar ECG lead). Local anesthesia was obtained with lidocaine (1%) injected subcutaneously at the sites of electrodes and transmitter implantation. To manage possible post-surgery pain, Advil (paracetamol and ibuprofene, 7 mL/l) was added to the drinking water for 4 days after implantation. Experiments were initiated 10 days after recovery from surgical implantation, when mice had recovered the body weight recorded before surgery. Mice were housed in individual cages with free access to food and water and were exposed to 12-hour light/dark cycles (light, 8:30 to 20:30 h) in a thermostatically controlled room. ECG signals were recorded using a telemetry receiver and an analog-to-digital conversion data acquisition system for display and analysis by Dataquest™ A.R.T.™ software (Data Sciences International). Heart rates were determined from RR intervals. Mean heart rate values were obtained in each mouse for an overall 24-h period. Recordings started at 8:30 a.m. and were stopped at 8:30 a.m. of the following day. For evaluating drug effects, heart rate was recorded for

a total period of 8-h. Heart rate before drug injection was averaged over a 2-h period following a 2-h stabilization period. Following drug injection, mean heart rate values were calculated in each mouse by analyzing periods of 5-min at different time points corresponding to the peak effect of the drug. ECG parameters were measured with ECG Auto 1.5.7 software (EMKA Technologies, Paris, France).

ECG recording in sedated mice. Six-lead surface ECGs were recorded with 25-gauge subcutaneous electrodes through an analog-digital converter (IOX®, EMKA Technologies) for monitoring and later analysis (ECG Auto®, EMKA Technologies). Mice were anesthetized with 2-2.5% isoflurane. Body temperature was maintained at 37°C using a retro-controlled heating pad (Harvard Apparatus, USA). Standard criteria were used to measure RR, PR, QRS and QT intervals. The QT interval was corrected for heart rate with Bazett's formula adapted to mouse sinus rate (1), *i.e.* $QTc = QT/(RR/100)^{1/2}$, with QT and RR, expressed in ms.

Intracardiac recording and pacing. After ECG recording, a mouse electrophysiology octapolar catheter (2 F) was positioned in the right atrium and ventricle via the right internal jugular vein (Biosense Webster, Diamond Bar, CA, USA). ECGs were used as a guide for catheter positioning. Surface ECG (lead I) and intracardiac electrograms were recorded on a computer through an analog-digital converter (EMKA Technologies) for monitoring and offline analysis (EMKA Technologies). Intracardiac ECGs were filtered between 0.5 and 500 Hz. Pacing was performed with a modified Biotronik® UHS20 stimulator (Berlin, Germany) and a digital stimulator (DS8000, World Precision Instruments, Sarasota, FL, USA). Standard pacing protocols were used to determine electrophysiologic parameters. Stimulus amplitude and

duration were set at 1.5 times the excitation threshold and 2 ms, respectively. To assess anterograde AV nodal conduction properties, we determined the pacing cycle resulting in the Wenckebach phenomenon by atrial stimulation at progressively increasing rates. Atrial and atrio-ventricular effective refractory periods (AERP and AVERP) were first determined by delivering an 8-stimulus (S1) drive train at a cycle length of 100 ms, followed by a premature stimulus (S2) progressively decremented in 2-ms intervals from an initial coupling interval of 80 ms. The AVERP was defined as the longest S1-S2 coupling interval at which the atrial beat triggered by S2 failed to propagate to ventricles. The AERP was defined as the longest S1-S2 coupling interval that failed to generate a propagated atrial beat with S2. This protocol was performed twice and also allowed to evaluate the susceptibility to trigger atrial arrhythmias. Indeed, S2 premature stimuli at S1-S2 coupling intervals longer than AERP could trigger arrhythmias. Once the AERP was determined, a second pacing protocol was performed which consisted in delivering S1 trains followed by a S2 set at a S1-S2 coupling interval 10 ms longer than the AERP and by one S3 premature stimulus progressively decremented in 2-ms intervals (initial coupling interval of 80 ms) until S3 failed to trigger an atrial complex (AERP2). This protocol was also performed twice.A third pacing protocol consisted of S1 trains followed by one S2, one S3 with a coupling interval of 10 ms above AERP2 and a S4 progressively decremented in 2-ms intervals (initial coupling interval of 80 ms). This protocol was also performed twice. Finally, single, double and triple premature stimulation protocols were also performed under sinus rhythm, without 8-stimulus drive trains. Atrial tachycardia was defined as a salvo of at least 4 atrial ectopic beats leading to P waves and atrial electrograms with a shape different from those recorded under sinus rate. We considered atrial tachycardia as non-sustained when it lasted less than 10 complexes. Atrial fibrillation was defined as an atrial tachyarrhythmia with marked

disorganization both on the surface ECG (with no distinguishable P waves) and on the intracardiac recording, with random atrioventricular conduction.

SAN function was evaluated by measuring the resting SAN cycle length and the SAN recovery time. Atrial pacing was applied for a period of 30 s at cycle lengths of 100 ms and 80 ms. For each pacing cycle length, SAN recovery time (SNRT) was determined as the longest pause from the last paced atrial depolarization to the first sinus return cycle. Corrected SNRT (cSNRT) was calculated by subtracting the intrinsic SAN cycle length from the recovery interval. The SAN conduction time (SACT) was determined with the Narula method consisting of 8-stimulus trains at a cycle length slightly shorter than the spontaneous SAN cycle length to avoid overdrive suppression. SACT was determined by subtracting the intrinsic SAN cycle length from the interval between the last stimulus and the first spontaneous atrial complex.

Langendorff-perfused hearts. Excised hearts were quickly mounted on a Langendorff apparatus (Isolated heart system; EMKA Technologies) at a pressure of 70-80 mm Hg with normal Tyrode's solution. Perfused hearts were immersed in the water-jacked bath and maintained at 36°C. The ECG was continuously recorded by Ag-AgCl electrodes positioned on the epicardial side of the right atrium close to the SAN area and near the apex. The heart rate was allowed to stabilize for at least 30 min before perfusion of acetylcholine (ACh).

Isolation of SAN cells. SAN cells were isolated as described previously (2). Briefly, mice were killed by cervical dislocation under general anesthesia consisting of 0.01 mg/g xylazine (2% Rompun; Bayer AG) and 0.1 mg/g ketamine (Imalgène; Merial), and beating hearts were quickly removed. The SAN region was excised in warmed (35°C) Tyrode's solution containing (in mM):

140.0 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.0 HEPES-NaOH, and 5.5 d-glucose (adjusted to pH 7.4 with NaOH) and cut in tissue strips. Strips were then transferred into a low- Ca^{2+}, low-Mg^{2+} solution containing (in mM): 140.0 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 0.2 $CaCl_2$, 1.2 KH_2PO_4, 50.0 taurine, 5.5 d-glucose, 1.0 mg/ml BSA, and 5.0 HEPES-NaOH (adjusted to pH 6.9 with NaOH). The tissue was enzymatically digested by adding 229 U/ml collagenase type II (Worthington Biochemical Corporation), 1.9 U/ml elastase (Boehringer Mannheim), 0.9 U/ml protease (Sigma-Aldrich), 1 mg/ml BSA, and 200 μM $CaCl_2$. Tissue digestion was performed for a variable time of 9–13 min at 35°C with manual agitation using a flame-forged Pasteur pipette. Tissue strips were then washed and transferred into a medium containing (in mM): 70.0 l-glutamic acid, 20.0 KCl, 80.0 KOH, 10.0 (±) D-b-OH-butyric acid, 10.0 KH_2PO4, 10.0 taurine, 1 mg/ml BSA, and 10.0 HEPES-KOH, pH 7.4 with KOH. SAN cells were manually dissociated in KB solution at 35°C for 2 min. Cellular automaticity was recovered by readapting the cells to physiological extracellular Na^+ and Ca^{2+} concentrations by adding aliquots of solutions containing (in mM): 10.0 NaCl, 1.8 $CaCl_2$, and, subsequently, normal Tyrode's solution containing 1 mg/ml BSA. The final storage solution contained (in mM): 100.0 NaCl, 35.0 KCl, 1.3 $CaCl_2$, 0.7 $MgCl_2$, 14.0 l-glutamic acid, 2.0 (±)D-b-OH-butyric acid, 2.0 KH_2PO_4, 2.0 taurine, and 1.0 mg/ml BSA, pH 7.4. Cells were then stored at room temperature until use. For electrophysiological recording, SAN cells in the storage solution were harvested in special custom-made recording plexiglas chambers with glass bottoms for proper cell attachment. The storage solution was continuously rinsed with normal Tyrode's solution warmed at 36°C before recording.

Patch-clamp recordings of SAN myocytes. The extracellular Tyrode's solution used in all

recordings contained (in mM): 140.0 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.0 HEPES-NaOH, 5.5 and d-glucose (adjusted to pH 7.4 with NaOH). Pacemaker activity of SAN cells was recorded under perforated patch conditions by adding 50 μM b-escin to the pipette solution. Patch-clamp electrodes had a resistance of 4–5 MΩ when filled with an intracellular solution containing (in mM): 130.0 K^+-aspartate; 10.0 NaCl; 2.0 ATP-Na^+ salt, 6.6 creatine phosphate, 0.1 GTP-Mg^{2+}, 0.04 $CaCl_2$ (pCa = 7.0), and 10.0 HEPES-KOH (adjusted to pH 7.2 with KOH). All experiments were performed at 36°C.

Drugs. Atropine Sulfate (0.25 mg/ml) was obtained from Aguettant (Lyon, France). Propranolol (5 mg/5 ml) was obtained from Astra Zeneca (Reims, France). All other chemicals were obtained from Sigma-Aldrich (St Quentin Fallavier, France).

Supplemental references

1. Gehrmann J, et al. (2002) Impaired parasympathetic heart rate control in mice with a reduction of functional G protein betagamma-subunits. Am J Physiol Heart Circ Physiol 282(2):H445-456.
2. Mangoni ME, Nargeot J (2001) Properties of the hyperpolarization-activated current (I_f) in isolated mouse sino-atrial cells. Cardiovasc Res 52(1):51-64.
3. Thireau J, Zhang BL, Poisson D, Babuty D (2008) Heart rate variability in mice: a theoretical and practical guide. Exp Physiol 93(1):83-94.

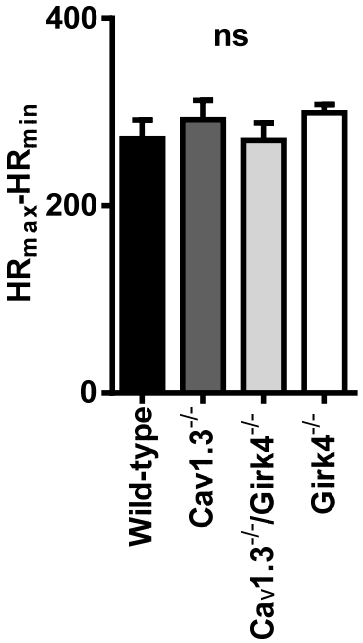

Fig. S1. Concurrent ablation of I_{KACh} does not diminish the relative degree of heart rate regulation in freely-moving mice. Range of heart rate regulation calculated from the differences between maximum and minimum recorded over 24-h ECG recordings in n=8 wild-type, n=8 *$Ca_v1.3^{-/-}$*, n=6 *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* and n=13 *Girk4$^{-/-}$* mice. Statistics: one-way ANOVA followed by Tukey's multiple comparisons test.

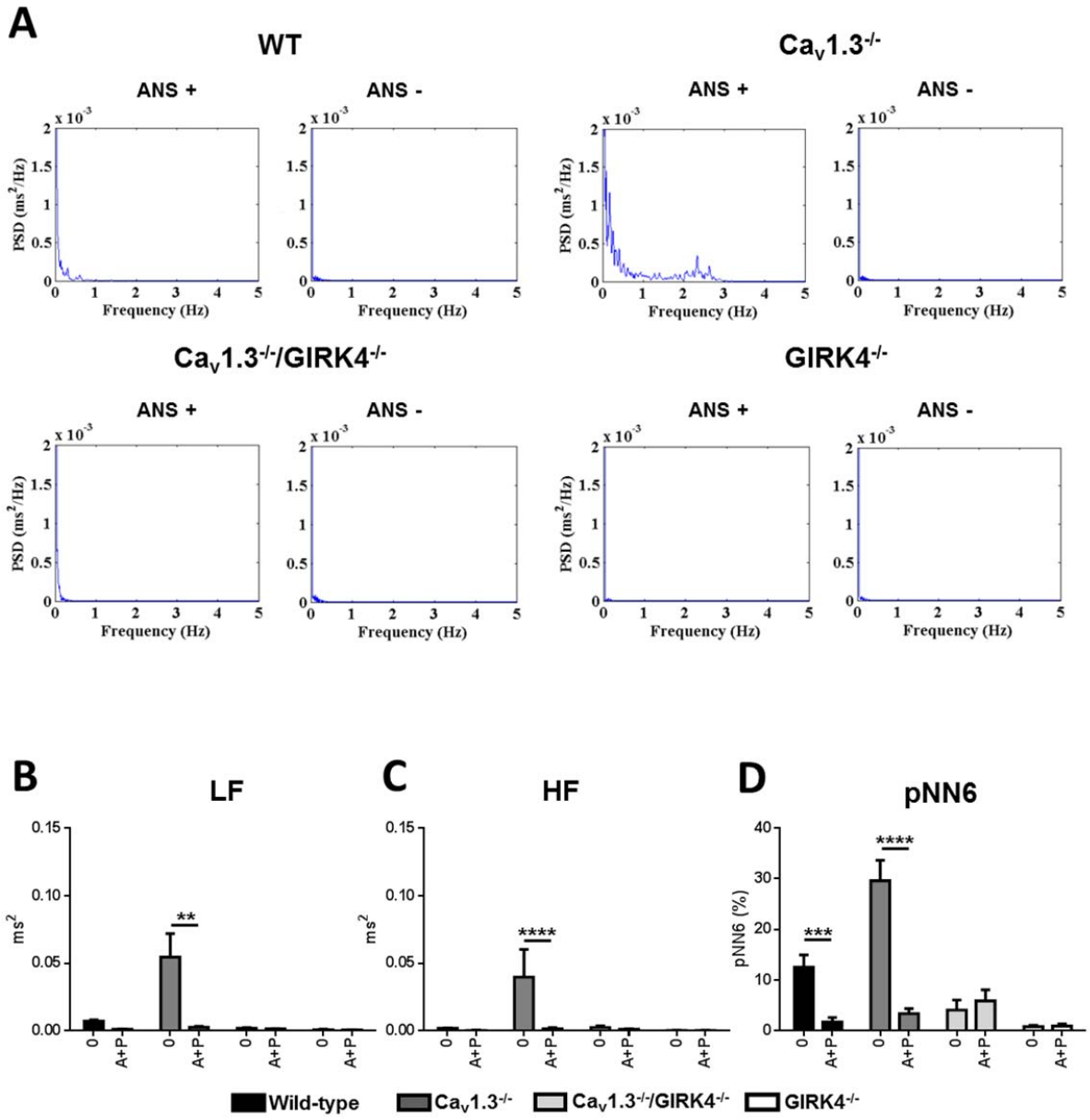

Fig. S2. Heart rate variability (HRV) of wild-type, *$Ca_v1.3^{-/-}$*, *$Girk4^{-/-}$* and *$Ca_v1.3^{-/-}/Girk4^{-/-}$* mice under control basal conditions and after combined injection of atropine and propranolol. (A) Samples of Fourier's spectra used for calculating HRV under basal conditions (ANS+) or after inhibition of the autonomic nervous system input by atropine and propranolol (ANS-). (**B**) Low-frequency (LF), (**C**) high-frequency (HF) spectra and pNN6 (**D**) of HRV

measured over 5-min telemetric ECG recording interval in n=12 wild-type, n=9 $Ca_v1.3^{-/-}$, n= 9 $Girk4^{-/-}$ and n=10 $Ca_v1.3^{-/-}/Girk4^{-/-}$ under control conditions (0) or after injection of atropine and propranolol (A+P). The high frequency of AV blocks and SAN pauses causes high HRV in $Ca_v1.3^{-/-}$ mice. However following genetic ablation of I_{KACh}, $Ca_v1.3^{-/-}/Girk4^{-/-}$ mice show HRV that is statistically similar to wild-type counterparts (see also Fig S2, inset). pNN6: percentage of consecutive R-R intervals differing by >6 ms (reflecting cardiac parasympathetic activity (3). Statistics: two-way ANOVA followed by Sidak's multiple comparisons test. For clarity, we showed in the figure only the statistical significance before and after injection of atropine and propranolol within each genotype.

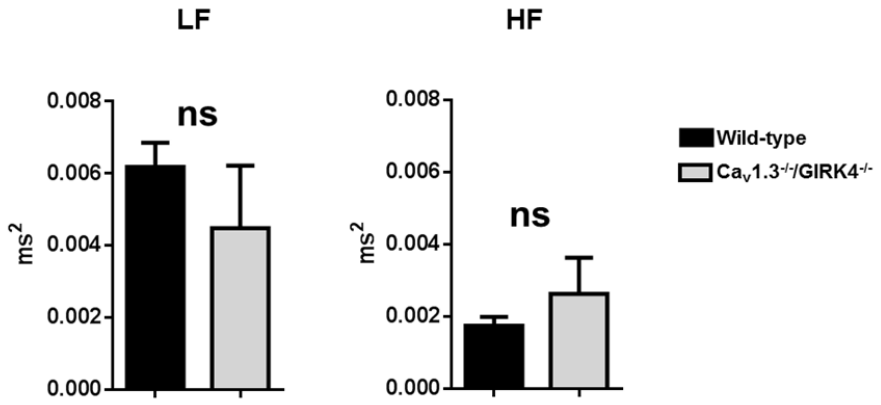

Fig. S2, *inset*. Comparison of HRV between wild-type (black bar) and $Ca_v1.3^{-/-}/Girk4^{-/-}$ mice. Same data as in Fig. S1 B and C. The LF and HF component are shown. Statistics: unpaired t-test; ns, non significant.

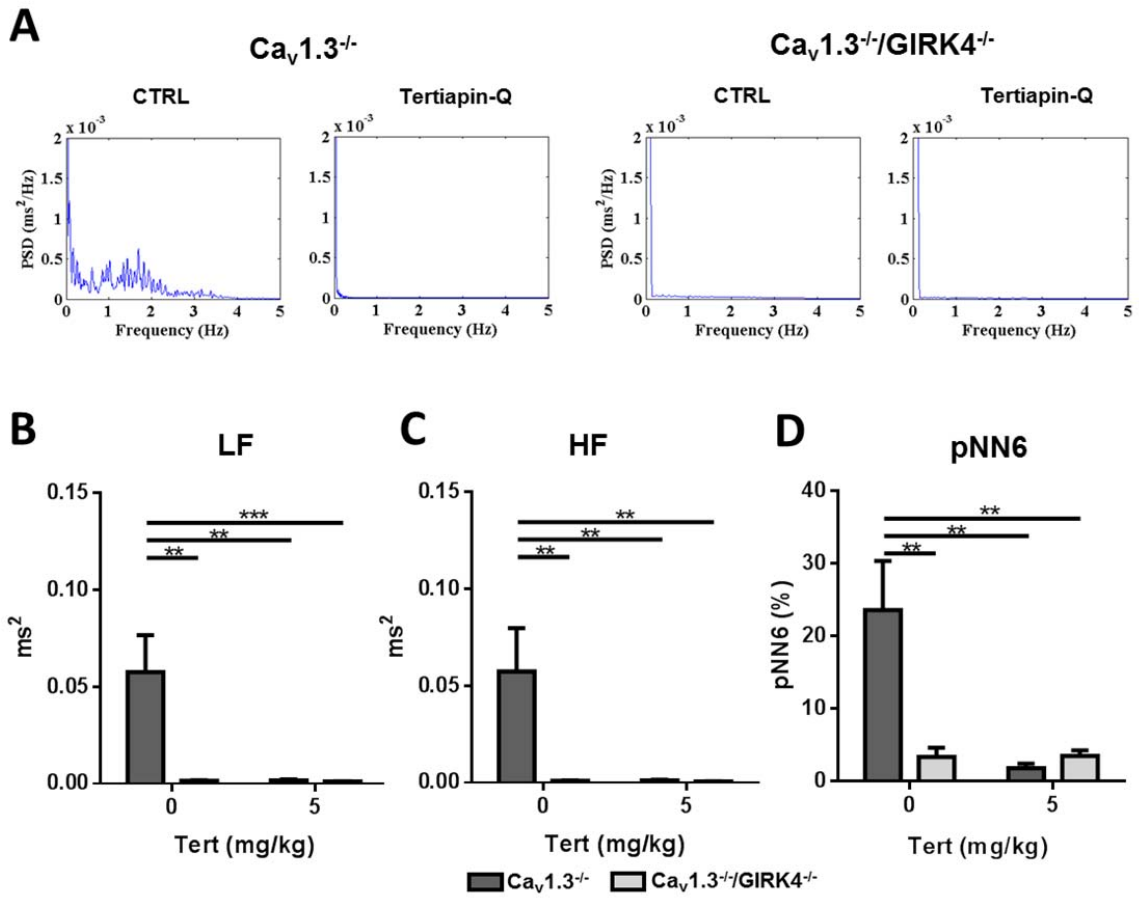

Fig. S3. Heart rate variability (HRV) of *$Ca_v1.3^{-/-}$* and *$Ca_v1.3^{-/-}/Girk4^{-/-}$* mice under control conditions and after injection of tertiapin-Q (Tert). (A) Samples of Fourier's spectra used for calculating HRV under basal conditions (CTRL) or after injection of tertiapin-Q. (**B**) Low-frequency (LF), (**C**) high-frequency (HF) spectra and pNN6 (**D**) of HRV measured over 5-min telemetric ECG recording interval in n=6 *$Ca_v1.3^{-/-}$* and in n=7 *$Ca_v1.3^{-/-}/Girk4^{-/-}$*. The high frequency of AV blocks and SAN pauses causes high HRV in *$Ca_v1.3^{-/-}$* mice. Note however, that following genetic ablation of $I_{KACh,}$ *$Ca_v1.3^{-/-}/Girk4^{-/-}$* mice show HRV spectra that are similar to *$Ca_v1.3^{-/-}$* mice injected with tertiapin-Q. pNN6: percentage of consecutive R-R intervals differing by >6 ms (reflecting cardiac parasympathetic activity (3). Statistics: two-way ANOVA followed by Tukey's multiple comparisons test.

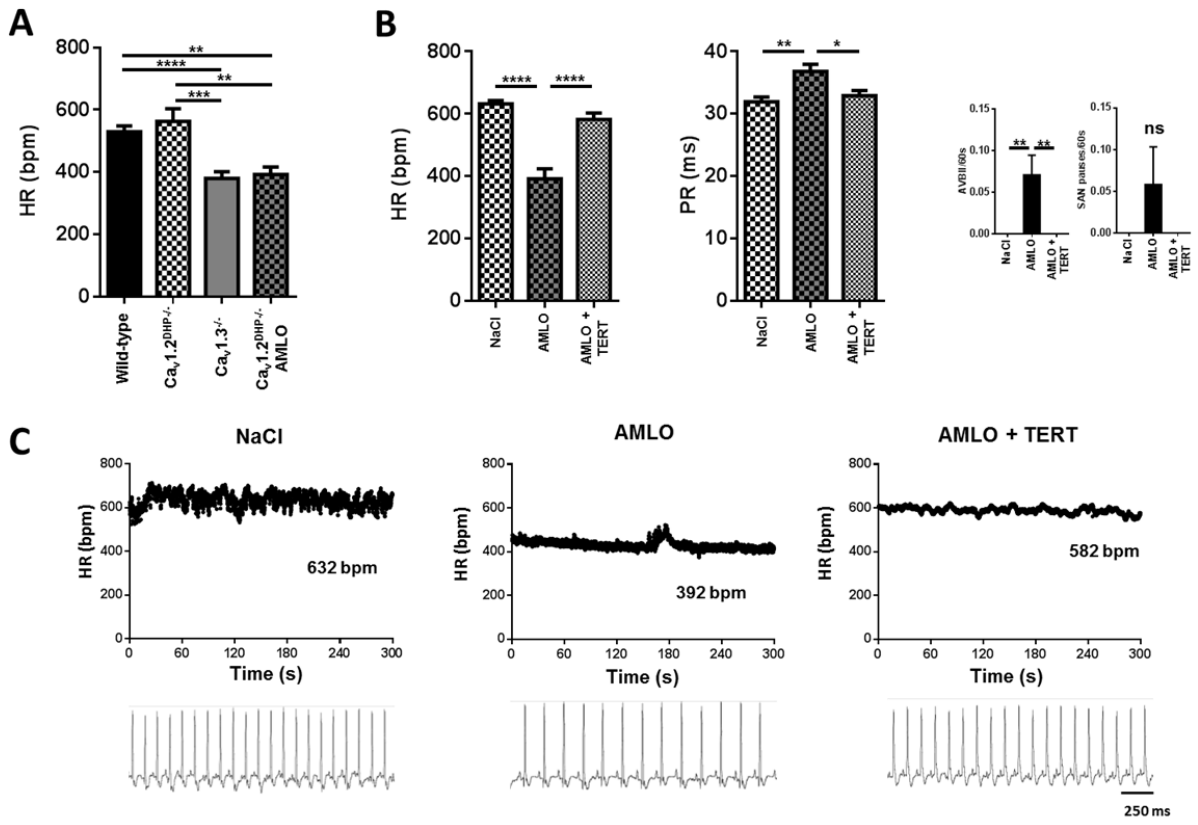

Fig. S4. Tertiapin-Q antagonizes slowing of heart rate and atrioventricular conduction induced by selective inhibition of $Ca_v1.3$ channels in $Ca_v1.2^{DHP-/-}$ mice. (A) Comparison between averaged heart rates of n=13 wild-type, n=6 $Ca_v1.2^{DHP-/-}$, n=16 $Ca_v1.3^{-/-}$ and n=6 $Ca_v1.2^{DHP-/-}$ mice injected with amlodipine (AMLO, 10 mg/Kg). While untreated $Ca_v1.2^{DHP-/-}$ show heart rates similar to those of wild-type mice, $Ca_v1.2^{DHP-/-}$ mice injected with amolodipine display heart rate reduction to levels similar to those of $Ca_v1.3^{-/-}$ counterparts. **(B)** Averaged heart rates (left panel) or atrioventricular conduction intervals (PR interval) of $Ca_v1.2^{DHP-/-}$ mice injected with control vehicle solution (NaCl), amlodipine or concomitant injection of amlodopine and tertiapin-Q (TERT, 5 mg/Kg). The insets show the frequency of 2nd –degree avtrioventricular blocks (AVBII) and the occurrence of SAN pauses. Statistics: one-way

ANOVA followed by Tukey's multiple comparisons test. (**C**) Dot plot of beat-to-beat variability (HR in b.p.m.) of heart rate (top) and representative samples of telemetric ECG recordings (bottom) from $Ca_v1.2^{DHP-/-}$ mice after injection of vehicle NaCl solution (left), after injection of amolodipine (center) or combined injection of amlodipine and tertiapin-Q (right).

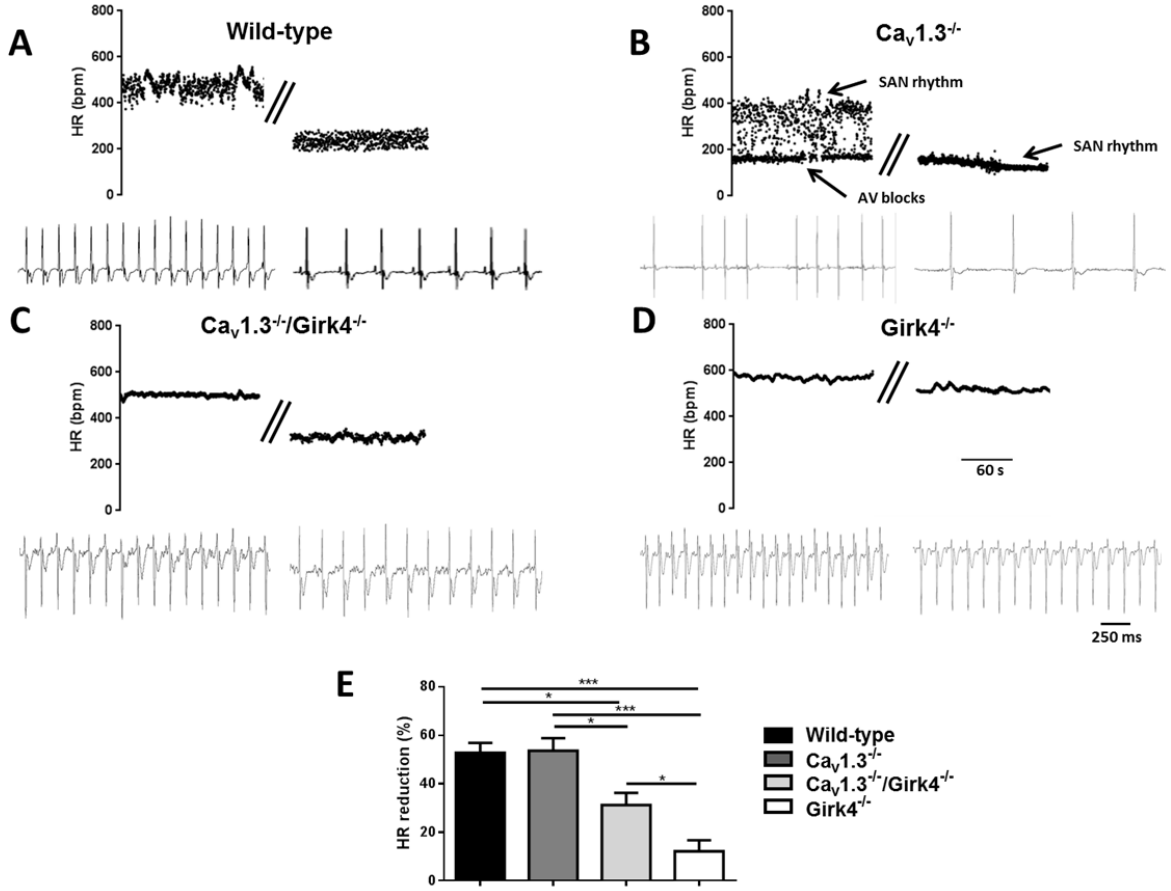

Fig. S5. I_{KACh} ablation attenuates bradycardia induced by activation of A_1 adenosine receptors. Heart rate slowing by intraperitoneal injection of the A_1R-selective agonist CCPA (0.1 mg/Kg) in wild-type (**A**), *$Ca_v1.3^{-/-}$* (**B**), *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* (**C**) and *Girk4$^{-/-}$* (**D**) mice. Panels show dot plots of beat-to-beat variability (HR in b.p.m.) of heart rate (top) and representative samples of ECG recordings (bottom). Arrows in (**B**) indicates ventricular beats driven by the SAN (SAN rhythm) or ventricular beats following AV block (AV blocks). We did not record AV blocks in the other genotypes and all wild-type, *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$*, and *Girk4$^{-/-}$* mice were under SAN rhythm. (**E**). Histogram showing the percentages of heart rate slowing in the different genotypes (n=7 for wild-type, n=5 for *$Ca_v1.3^{-/-}$*, n=15 *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* and n=8 for *Girk4$^{-/-}$*). Statistics: one-way ANOVA followed by Tukey's multiple comparisons test.

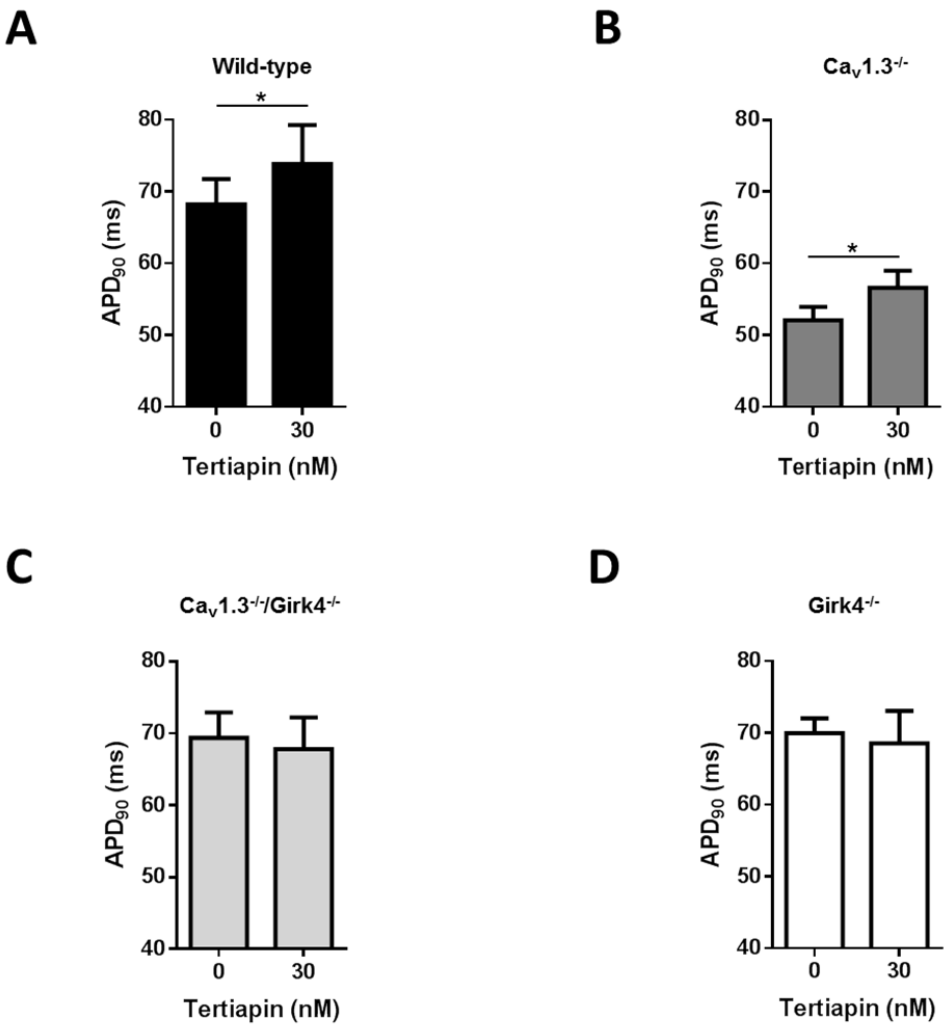

Fig. S6. Effect of tertiapin-Q on the action potential duration (APD_{90}) of atrial myocytes in the different genotypes. Tertiapin-Q significantly prolonged the action potential duration of wild-type (**A**, n=7) and *$Ca_v1.3^{-/-}$* (**B**, n=13) mice indicating that a fraction of I_{KACh} is constitutively active in atrial myocytes. In contrast, tertiapin-Q did not affect the action potential duration of *$Ca_v1.3^{-/-}/Girk4^{-/-}$* (**C**, n=12) and *$Girk4^{-/-}$* (**D**, n=13) atrial myocytes. Statistics: paired Student's *t* test.

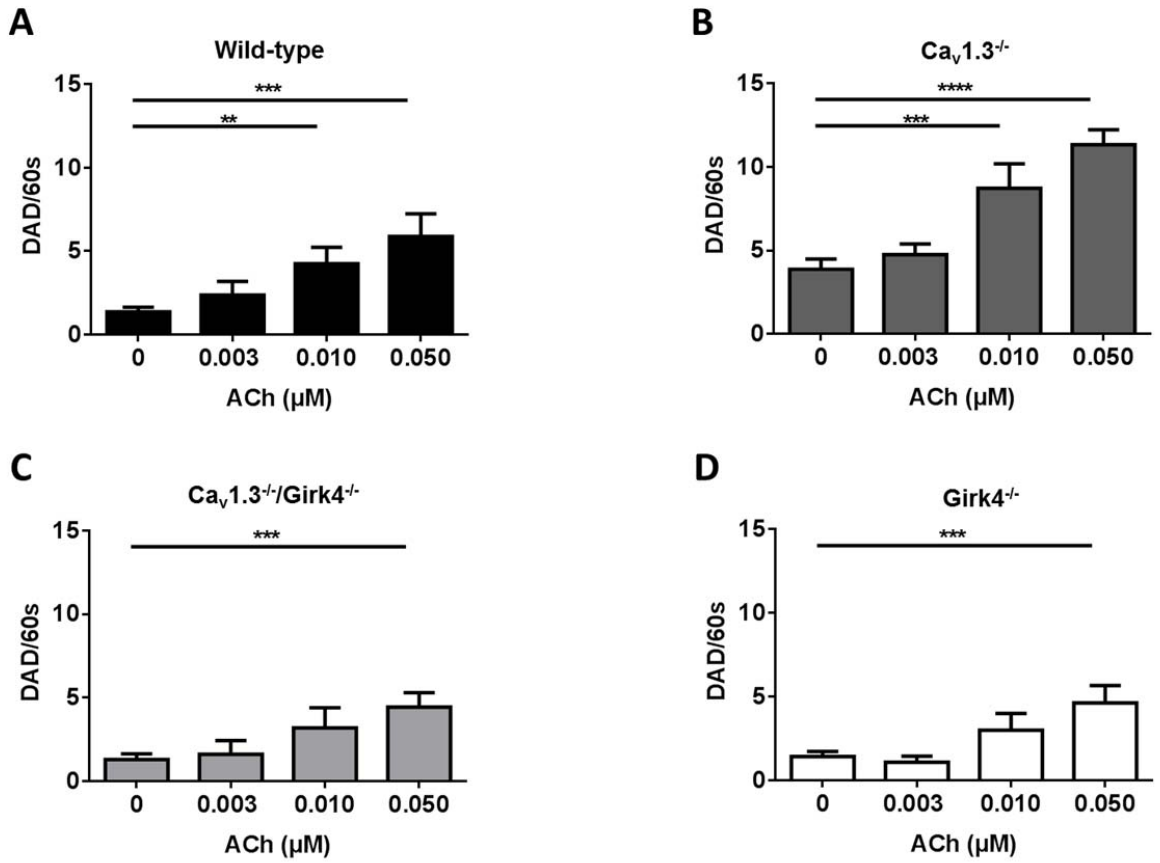

Fig. S7. Delayed after depolarizations (DADs) in SAN myocytes of control and mutant mice. Frequency of DADs after application of different ACh concentrations in isolated SAN myocytes from wild-type (n=8-32 **A**), *$Ca_v1.3^{-/-}$* (n=6-17; **B**), *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* (n=8-24; **C**) and *Girk4$^{-/-}$* (n=11-35; **D**) mice. Statistics: one-way ANOVA followed by Dunnett's multiple comparisons test.

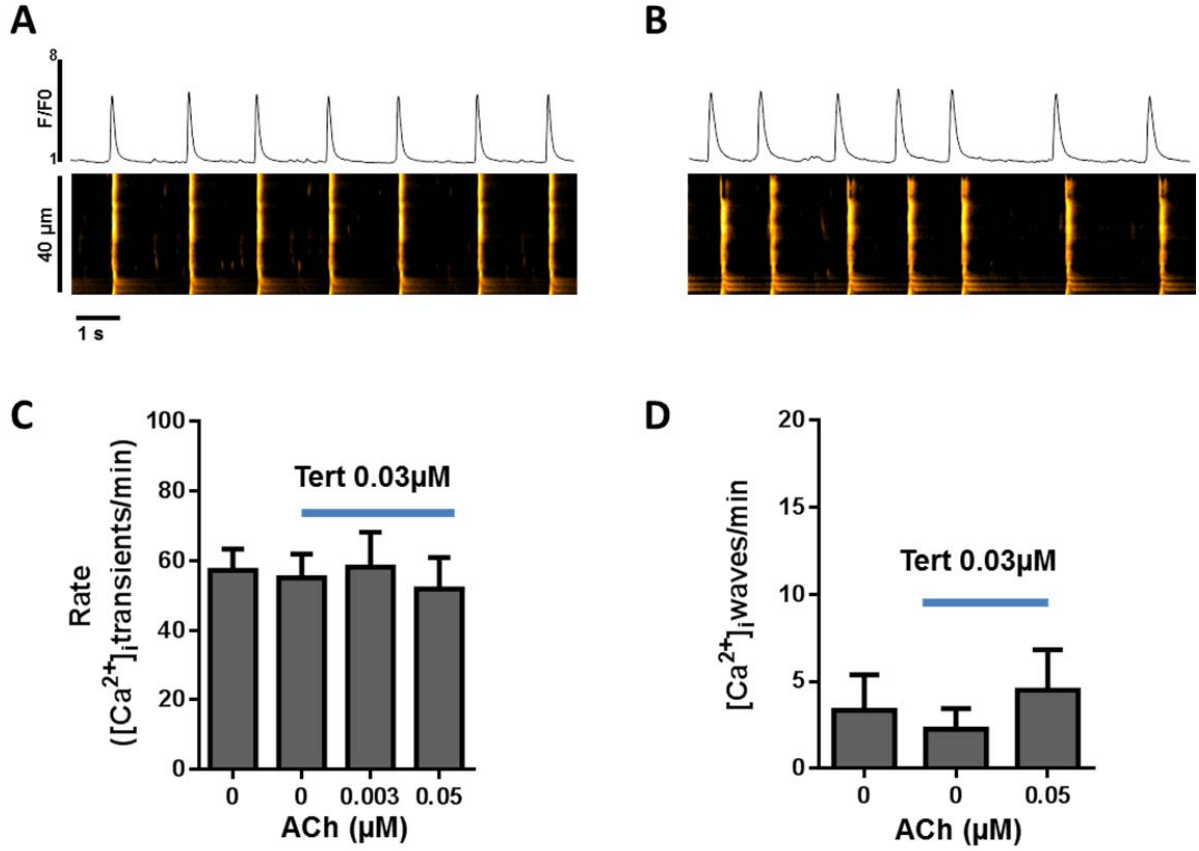

Fig. S8. Tertiapin-Q prevents ACh-induced slowing of pacemaker activity measured as the frequency of spontaneous $[Ca^{2+}]_i$ transients and formation of $[Ca^{2+}]_i$ waves in *$Ca_v1.3^{-/-}$* SAN myocytes. Spontaneous $[Ca^{2+}]_i$ transients in *$Ca_v1.3^{-/-}$* SAN myocytes before (**A**) and after (**B**) combined perfusion of ACh (0.05 µM) and tertiapin-Q (0.03 µM). (**C**) Averaged frequency of spontaneous $[Ca^{2+}]_i$ transients in n=7 SAN myocytes at the indicated concentrations of ACh, in the presence of tertiapin-Q. (**D**) Averaged frequency of $[Ca^{2+}]_i$ waves. Statistics: one-way ANOVA followed by Tukey's multiple comparisons test.

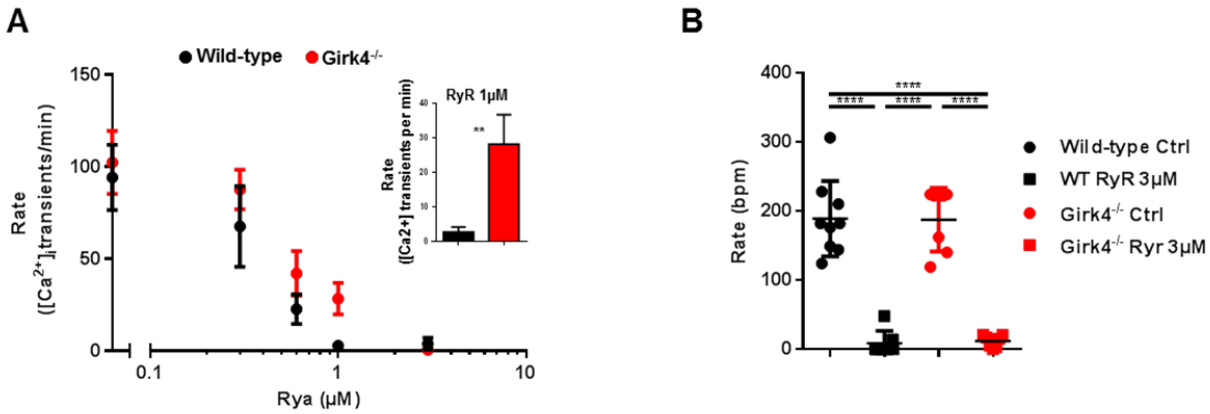

Fig. S9. Genetic ablation of I_{KACh} antagonizes slowing of pacemaker activity of SAN cells mediated by ryanodine. (A) Dose-response relationship of the frequency of spontaneous $[Ca^{2+}]_i$ transients to ryanodine (Rya) in n=8 wild-type and n=7 *Girk4*$^{-/-}$ SAN cells. The inset shows that spontaneous activity of *Girk4*$^{-/-}$ SAN cells is significantly faster than that of wild-type counterparts at 1 μM ryanodine. (**B**) The same concentration of ryanodine blocking spontaneous $[Ca^{2+}]_i$ transients also drastically reduced the frequency of spontaneous action potentials in n=9 wild-type and n=7 Girk4$^{-/-}$ SAN cells. Statistics (A, *inset*) unpaired t-test; (B) two-way ANOVA, followed by Sidak's multiple comparison test.

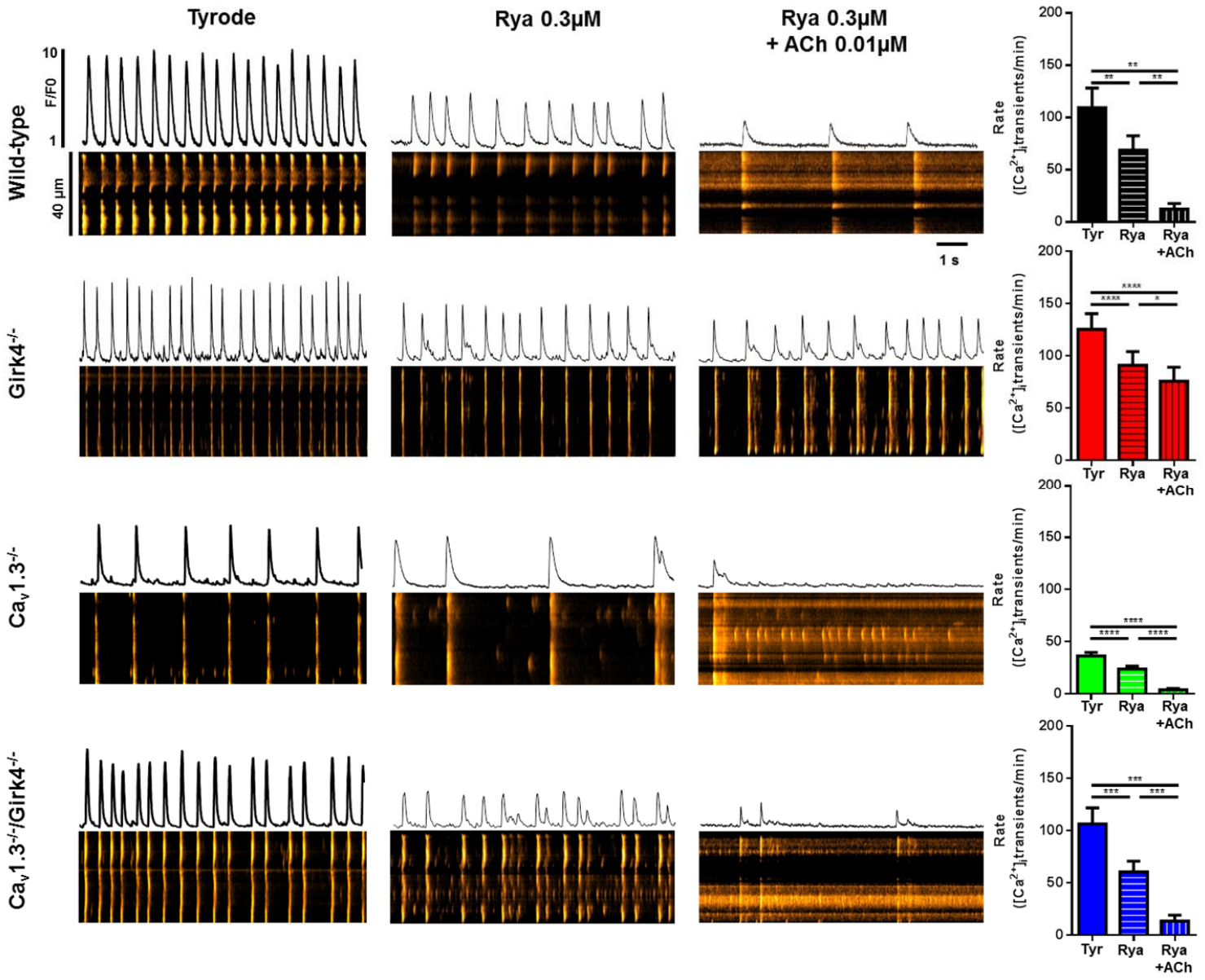
Tyrode
Rya 0.3μM
Rya 0.3μM
+ ACh 0.01μM
Wild-type
Girk4-/-
Cav1.3-/-
Cav1.3-/-/Girk4-/-
10
F/F0
1
40 μm
1 s
Rate
([Ca2+]i transients/min)
200
150
100
50
0
Tyr
Rya
Rya
+ACh
**
**
**
****
****
*
****
****
****
***
***
***

Fig. S10. Sample line scans of spontaneous $[Ca^{2+}]_i$ transients in wild-type, *$Ca_V1.3^{-/-}$*, *$Girk4^{-/-}$* and *$Ca_V1.3^{-/-}/Girk4^{-/-}$* SAN cells under control conditions (Tyr), after perfusion of ryanodine (Rya, 0.3 μM) alone, or combined perfusion of ACh (0.01 μM) and ryanodine. Bar plots in the right column shows statistical comparisons within each genotype considered. Statistics: one-way ANOVA followed by Holm-Sidak's multiple comparisons test.

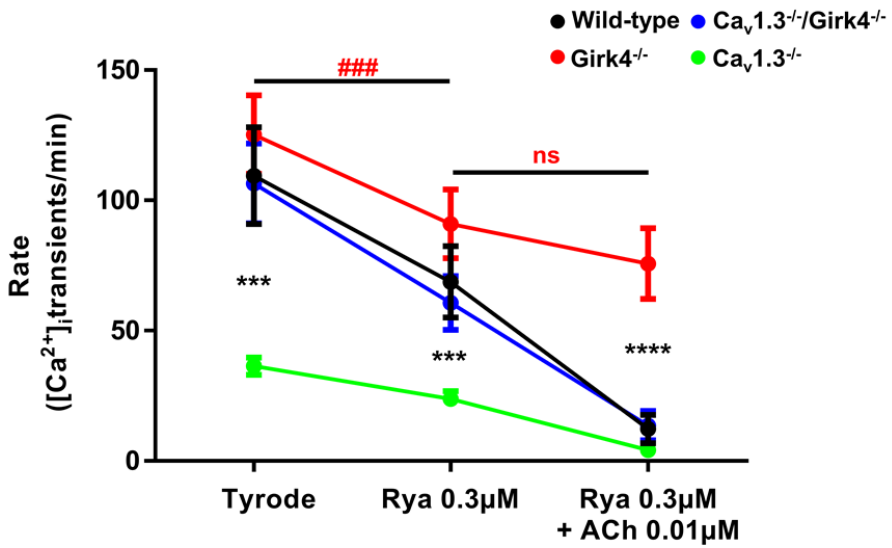

Fig. S11. Comparison between averaged frequencies of spontaneous $[Ca^{2+}]_i$ transients of n=8 wild-type, n=11 *$Ca_v1.3^{-/-}$*, n=12 *$Girk4^{-/-}$* and n=11 *$Ca_v1.3^{-/-}/Girk4^{-/-}$* SAN cells after application of 0.3 µM ryanodine or concomitant application of ryanodine and ACh (0.1 µM). Ablation of I_{KACh} in *$Girk4^{-/-}$* SAN cells strongly antagonizes slowing of spontaneous $[Ca^{2+}]_i$ transients induced by ryanodine and ACh and maintains spontaneous activity of *$Ca_v1.3^{-/-}/Girk4^{-/-}$* SAN cells similar to that recorded in wild-type rather than in *$Ca_v1.3^{-/-}$* counterparts. Statistics: one-way ANOVA followed by Holm-Sidak's multiple comparisons test; ns, non significant.

Table S1: ECG interval values

		WT		$Ca_v1.3^{-/-}$		$Ca_v1.3^{-/-}$ /$Girk4^{-/-}$		$Girk4^{-/-}$		P	P	P	P	P	P
		A	n	B	n	C	n	D	n	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
ANS +	HR (bpm)	490 ± 17	10	331 ± 21	7	467 ± 14	11	549± $10^{\#}$	9	****	ns	ns	****	****	**
	PP (ms)	122 ± 2	10	193 ± 19	7	129 ± 4	11	113 ± 5	9	****	ns	ns	***	****	ns
	PR (ms)	38 ± 1	10	52 ± 3	7	46 ± 2	11	37 ± 2	9	****	**	ns	*	****	**
	QT (ms)	62 ± 2	10	64 ± 4	7	64 ± 2	11	61 ± 1	9	ns	ns	ns	ns	ns	ns
	QTc (ms)	59 ± 2	10	47 ± 2	7	56 ± 2	11	57 ± 2	9	***	ns	ns	*	**	ns
	SP/60s	0 ± 0	10	3 ± $1^{\#\#\#\#}$	7	0.3 ± 0.2	11	0	9	****	ns	ns	****	****	ns
	AVB II/60s	0	10	4 ± $1^{\#\#\#\#}$	7	0.4 ± 0.3	11	0	9	****	ns	ns	****	****	ns
ANS -	HR (bpm)	443 ± 18	10	347 ± 17	7	421 ± 18	11	475 ± 15	9	**	ns	ns	*	****	ns
	PP (ms	132 ± 5	10	198 ± 22	7	141 ± 6	11	126 ± 3	9	****	ns	ns	****	****	ns
	PR (ms)	38 ± 1	10	48 ± 3	7	46 ± 2	11	37 ± 1	9	***	**	ns	ns	***	***
	QT (ms)	65 ± 3	10	66 ± 4	7	63 ± 2	11	61 ± 2	9	ns	ns	ns	ns	ns	ns
	QTc (ms)	57 ± 2	10	47 ± 3	7	53 ± 2	11	54 ± 1	9	**	ns	ns	ns	ns	ns
	SP/60s	0	10	0	7	0	11	0	9	ns	ns	ns	ns	ns	ns
	AVB II/60s	0	10	0	7	0	11	0	9	ns	ns	ns	ns	ns	ns

Summary table of the ECG interval values together with frequency of sinus pauses (SP) and second degree atrioventricular blocks (AVBII) recorded in the different mouse strains before and after injection of A+P Statistics: two-way ANOVA followed by Sidak multiple comparisons test. * P<0.05, ** P<0.01, ***P<0.001, P<0.0001 (genotype effect); # P<0.05, #### P<0.0001 (ANS inhibition effect). Abbreviations: SP=

sinus pauses; AVB II= II degree atrioventricular blocks of Mobitz 1 (MOBI) or Mobitz 2 (MOBII) with different numbers of isolated P waves for each QRS complex (MOBI 2:1; MOBI 3:1; MOBII 2:1; MOBII 3:1).

Table S2: ECG interval values before and after atropine injection

		WT		$Ca_v1.3^{-/-}$		$Ca_v1.3^{-/-}$ $Girk4^{-/-}$		$Girk4^{-/-}$		p	p	p	p	p	p
		A	n	B	n	C	n	D	n	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
PRE	HR (bpm)	507 ± 22$	10	352 ± 29$	9	450 ± 16$	13	534 ± 20$	9	****	ns	ns	****	****	ns
	PR (ms)	37 ± 1	10	54 ± 4##	9	44 ± 1	13	36 ± 1	9	****	*	ns	***	****	**
	QT (ms)	58 ± 2	10	62 ± 3	9	61 ± 1	13	56 ± 1	9	ns	ns	ns	ns	ns	ns
	QTc (ms)	55 ± 2	10	46 ± 2$	9	56 ± 1	13	56 ± 1	9	**	ns	ns	****	***	ns
	SP/60s	0	10	4 ± 1$	9	0.2 ± 0.2	13	0	9	****	ns	ns	****	****	ns
	AVB II/60s	0	13	4 ± 1$	7	0.2 ± 0.2	13	0	9	****	ns	ns	****	****	ns
POST	HR (bpm)	716 ± 8	10	603 ± 12	9	588 ± 13	13	747 ± 10	9	ns	ns	ns	ns	ns	ns
	PR (ms)	37 ± 1	10	46 ± 2	9	42 ± 1	13	33 ± 1	9	**	ns	ns	ns	****	**
	QT (ms)	56 ± 1	10	60 ± 2	9	60 ± 1	13	55 ± 1	9	ns	ns	ns	ns	ns	ns
	QTc (ms)	58 ± 1	10	56 ± 2	9	56 ± 1	13	57 ± 1	9	ns	ns	ns	ns	ns	ns
	SP/60s	0	13	0.1 ± 0.1	9	0.2 ± 0.2	13	0	9	ns	ns	ns	ns	ns	ns
	AVB II/60s	0	13	0	7	0.2 ± 0.2	13	0	9	ns	ns	ns	ns	ns	ns

Effects of injection of 0.5 mg/kg atropine (POST) on ECG parameters. Statistics: two-way ANOVA followed by Sidak multiple comparisons test. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 (genotype effect); ## P<0.01, $ P<0.0001 (Atropine effect); PRE= baseline values before atropine injection; SP= sinus pauses; AVB II= II degree atrioventricular block (MOBI 2:1; MOBI 3:1; MOBII 2:1; MOBII 3:1).

Table S3: ECG interval values before and after Tertiapin-Q injection

		WT		Girk4$^{-/-}$		$Ca_v1.3^{-/-}$		$Ca_v1.3^{-/-}$ Girk4$^{-/-}$		p	p	p	p	p	p
		A	n	B	n	C	n	D	n	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
PRE	RR (ms)	106 ± 3	5	95 ± 1	6	139 ± 8$^{####}$	6	116 ± 8	7	ns	***	ns	****	*	**
	PR (ms)	34 ± 1	5	31 ± 1	6	53 ± 2$^{####}$	6	40 ± 1	7	ns	****	ns	****	**	****
	QT (ms)	57 ± 2	5	53 ± 1	6	59 ± 2	6	57 ± 2	7	ns	ns	ns	ns	ns	ns
	QTc (ms)	56 ± 2	5	54 ± 1	6	51 ± 3	6	53 ± 2	7	ns	ns	ns	ns	ns	ns
	SP/60s	0	5	0	6	5 ± 2$^{####}$	6	0	7	ns	****	ns	****	ns	****
	AVB II/60s	0	5	0	6	7 ± 2$^{####}$	6	0	7	ns	****	ns	****	ns	****
POST	RR (ms)	97 ± 2	5	91 ± 2	6	115 ± 6	6	115 ± 6	7	ns	ns	ns	**	**	ns
	PR (ms)	35 ± 1	5	32 ± 1	6	45 ± 3	6	40 ± 2	7	ns	***	ns	****	**	ns
	QT (ms)	56 ± 1	5	56 ± 2	6	56 ± 3	6	60 ± 2	7	ns	ns	ns	ns	ns	ns
	QTc (ms)	57 ± 1	5	58 ± 3	6	52 ± 3	6	56 ± 2	7	ns	ns	ns	ns	ns	ns
	SP/60s	0	5	0	6	0	6	0	7	ns	ns	ns	ns	ns	ns
	AVB II/60s	0	5	0	6	0	6	0	7	ns	ns	ns	ns	ns	ns

* p<0.05; ** p<0.01; ***p<0.001; ****p<0.0001 (genotype effect); #### p<0.0001; (Tertiapine effect); SP= sinus pauses; AVB II= II degree Atrioventricular blocks (MOBI 2:1; MOBI 3:1; MOBII 2:1; MOBII 3:1).

Table S4: Cardiac electrophysiological parameters of wild-type (WT), *$Ca_v1.3^{-/-}$* and *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* mice under baseline conditions during intracardiac catheterization procedures

	WT		$Ca_v1.3^{-/-}$		$Ca_v1.3^{-/-}$ Girk4$^{-/-}$		p	p	p
	A	n	B	n	C	n	A vs B	A vs C	B vs C
RR (ms)	141 ± 4	25	217 ± 20	20	171 ± 6	20	***	ns	*
P wave (ms)	14 ± 1	25	17 ± 1	20	15 ± 1	20	****	*	ns
PR (ms)	40 ± 1	25	57 ± 2	20	48 ± 1	20	****	****	***
QRS (ms)	13 ± 1	25	13 ± 1	20	13 ± 1	20	ns	ns	ns
QT (ms)	61 ± 2	25	71 ± 3	20	64 ± 2	20	**	ns	ns
QTc	52 ± 1	25	50 ± 2	20	49 ± 1	20	ns	ns	ns
AERP (ms)	33 ± 1	25	33 ± 2	20	37 ± 2	20	ns	ns	ns
AVERP (ms)	50 ± 3	25	77 ± 3	20	63 ± 2	20	****	**	**
Wenckebach CL (ms)	78 ± 3	25	118 ± 8	20	89 ± 2	20	****	ns	***

AERP= atrial effective refractory period; AVERP= atrioventricular effective refractory period at a basal cycle length of 100 ms; CL: cycle length. Statistics: one-way ANOVA followed by Tukey's multiple comparisons test. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Table S5. Corrected SAN recovery time (cSNRT) and SAN-atrium conduction times (SACT) in $Ca_v1.3^{-/-}$ and *$Ca_v1.3^{-/-}$/Girk4$^{-/-}$* mice

	cSNRT (ms)	SACT (ms)
WT	**27.5 ± 8.1 (6)**	**15.4 ± 1.8 (8)**
$Ca_v1.3^{-/-}$	**62.9 ± 10.0 * (9)**	**29.7 ± 3.8 ** (10)**
$Ca_v1.3^{-/-}$/Girk4$^{-/-}$	**36.1 ± 5.8 § (7)**	**14.9 ± 1.8 §§ (8)**

The number of experiments is given within parentheses. *, **, p< 0.05 and p< 0.01, respectively, versus wild type mice; §, §§, p< 0.05 and p< 0.01, respectively, *versus* *$Ca_v1.3^{-/-}$* mice (ANOVA and Student-Newman-Keuls test for pairwise multiple comparison).

Table S6. Type, duration and reproducibility of atrial tachyarrhythmias

	Number of mice with arrhythmias	Type of arrhythmias (number of mice)	Maximum duration (sec)	Number of episodes
WT	**1/8**	**NS AT (1)**	**0.2**	**3**
$Ca_v1.3^{-/-}$	**8/12**	**NS AT (1)**	**0.25**	**22**
		AT (5)	**1.1-19**	**5-45**
		AF (2)	**0.7-20**	**13-15**
$Ca_v1.3^{-/-}$/Girk4$^{-/-}$	**2/10**	**NS AT (1)**	**0.4**	**2**
		AT (1)	**115 ***	**2**

Abbreviations: NS, non-sustained; AT, atrial tachycardia; AF, atrial fibrillation.

Table S7: Action potential parameters recorded in isolated SAN cells

a)

WT	0		3		10		50		p					
	A	n	B	n	C	n	D	n	AvsB	AvsC	AvsD	BvsC	BvsD	CvsD
Rate (bpm)	158 ± 10	28	116 ± 8	7	34 ± 8	14	2 ± 2	7	ns	****	****	***	****	ns
MDP (mV) T	-62 ± 1	28	-60 ± 2	7	-67 ± 2	14	-66 ± 1	7	ns	ns	ns	ns	ns	ns
Eth (mV)	-40 ± 1	28	-40 ± 1	7	-42 ± 1	11			ns	ns		ns		
SLDD (mV/ms)	0.04 ± 0.01	28	0.02 ± 0.01	7	0.02 ± 0.01	11			ns	ns		ns		
SEDD (mV/ms)	0.6 ± 0.1	28	0.4 ± 0.1	7	0.4 ± 0.1	11			ns	ns		ns		
APA (mV)	89 ± 2	28	86 ± 5	7	90 ± 3	11			ns	ns		ns		
dV/dt (mV/ms)	29 ± 3	28	27 ± 5	7	28 ± 3	11			ns	ns		ns		
APD30 (ms)	26 ± 2	28	27 ± 5	7	18 ± 4	11			ns	ns		ns		
APD50 (ms)	41 ± 3	28	38 ± 7	7	37 ± 6	11			ns	ns		ns		
APD70 (ms)	70 ± 4	28	74 ± 9	7	66 ± 6	11			ns	ns		ns		
APD90 (ms)	103 ± 5	28	98 ± 10	7	106 ± 6	11			ns	ns		ns		

b)

$Ca_v1.3^{-/-}$	0		3		10		50		p					
	A	n	B	n	C	n	D	n	AvsB	AvsC	AvsD	BvsC	BvsD	CvsD
Rate (bpm)	104 ± 7	23	65 ± 12	7	52 ± 13	9	2 ± 11	7	ns	**	****	ns	**	*
MDP (mV)	-61 ± 1	23	-62 ± 2	7	-61 ± 1	9	-68 ± 3	7	ns	ns	*	ns	ns	ns
Eth (mV)	-40 ± 1	23	-40 ± 2	7	-41 ± 1	9	-42 ± 6	2	ns	ns		ns		
SLDD (mV/ms)	0.05 ± 0.01	23	0.04 ± 0.01	7	0.03 ± 0.01	9	0.03 ± 0.01	2	ns	ns		ns		
SEDD (mV/ms)	0.45 ± 0.05	23	0.40 ± 0.05	7	0.35 ± 0.05	9	0.30 ± 0.02	2	ns	ns		ns		
APA (mV)	94 ± 2	23	95 ± 6	7	91 ± 2	9	100 ± 8	2	ns	ns		ns		
dV/dt (mV/ms)	35 ± 4	23	37 ± 7	7	28 ± 5	9	36 ± 6	2	ns	ns		ns		
APD30 (ms)	26 ± 3	23	26 ± 4	7	23 ± 4	9			ns	ns		ns		
APD50 (ms)	45 ± 3	23	43 ± 5	7	40 ± 5	9			ns	ns		ns		
APD70 (ms)	78 ± 5	23	73 ± 3	7	74 ± 4	9			ns	ns		ns		
APD90 (ms)	129 ± 10	23	118 ± 4	7	125 ± 8	9			ns	ns		ns		

c)

$Ca_v1.3^{-/-}/Girk4^{-/-}$	0		3		10		50		p					
	A	n	B	n	C	n	D	n	AvsB	AvsC	AvsD	BvsC	BvsD	CvsD
Rate (bpm)	149 ± 6	23	140 ± 14	6	133 ± 14	7	134 ± 9	15	ns	ns	ns	ns	ns	ns
MDP (mV)	-61 ± 2	23	-64 ± 3	6	-64 ± 4	7	-63 ± 2	15	ns	ns	ns	ns	ns	ns
Eth (mV)	-41 ± 2	23	-44 ± 3	6	-43 ± 4	7	-43 ± 2	15	ns	ns	ns	ns	ns	ns
SLDD (mV/ms)	0.03 ± 0.01	23	0.03 ± 0.01	6	0.02 ± 0.01	7	0.02 ± 0.01	15	ns	ns	ns	ns	ns	ns
SEDD (mV/ms)	0.5 ± 0.1	23	0.6 ± 0.1	6	0.40 ± 0.05	7	0.40 ± 0.05	15	ns	ns	ns	ns	ns	ns
APA (mV)	94 ± 3	23	97 ± 4	6	96 ± 5	7	96 ± 5	15	ns	ns	ns	ns	ns	ns
dV/dt (mV/ms)	31 ± 5	23	33 ± 6	6	33 ± 9	7	32 ± 5	15	ns	ns	ns	ns	ns	ns
APD30 (ms)	29 ± 2	23	25 ± 3	6	24 ± 1	7	27 ± 2	15	ns	ns	ns	ns	ns	ns
APD50 (ms)	48 ± 2	23	41 ± 4	6	44 ± 1	7	49 ± 2	15	ns	ns	ns	ns	ns	ns
APD70 (ms)	74 ± 3	23	66 ± 8	6	71 ± 7	7	79 ± 3	15	ns	ns	ns	ns	ns	ns
APD90 (ms)	108 ± 5	23	98 ± 14	6	109 ± 12	7	117 ± 7	15	ns	ns	ns	ns	ns	ns

d)

$Girk4^{-/-}$	0		3		10		50		p					
	A	n	B	n	C	n	D	n	AvsB	AvsC	AvsD	BvsC	BvsD	CvsD
Rate (bpm)	163 ± 7	25	153 ± 9	8	101 ± 16	10	92 ± 17	7	ns	***	***	*	**	ns
MDP (mV)	-62 ± 2	25	-58 ± 3	8	-66 ± 3	10	-67 ± 2	7	ns	ns	ns	ns	ns	ns
Eth (mV)	-40 ± 1	25	-39 ± 3	8	-43 ± 2	10	-43 ± 3	7	ns	ns	ns	ns	ns	ns
SLDD (mV/ms)	0.06 ± 0.01	25	0.05 ± 0.01	8	0.02 ± 0.01	10	0.02 ± 0.01	7	ns	ns	ns	ns	ns	ns
SEDD (mV/ms)	0.50 ± 0.05	25	0.50 ± 0.05	8	0.40 ± 0.05	9	0.4 ± 0.1	7	ns	ns	ns	ns	ns	ns
APA (mV)	89 ± 3	25	87 ± 4	8	91 ± 5	10	94 ± 4	7	ns	ns	ns	ns	ns	ns
dV/dt (mV/ms)	33 ± 4	25	29 ± 4	8	30 ± 6	10	34 ± 8	7	ns	ns	ns	ns	ns	ns
APD_{30} (ms)	26 ± 3	25	25 ± 6	8	26 ± 4	10	20 ± 6	7	ns	ns	ns	ns	ns	ns
APD_{50} (ms)	52 ± 5	25	51 ± 9	8	51 ± 5	10	46 ± 11	7	ns	ns	ns	ns	ns	ns
APD_{70} (ms)	80 ± 5	25	80 ± 9	8	85 ± 4	10	80 ± 10	7	ns	ns	ns	ns	ns	ns
APD_{90} (ms)	119 ± 6	25	117 ± 10	8	129 ± 6	10	123 ± 11	7	ns	ns	ns	ns	ns	ns

Action potential parameters in WT (a), $Ca_v1.3^{-/-}$(b), $Ca_v1.3^{-/-}/Girk4^{-/-}$ (c) and $Girk4^{-/-}$ (d) isolated SAN cells under basal conditions (A) and perfusion with 3 (B), 10 (C) and 50 (D) nM ACh. Statistics: one-way ANOVA followed by Tukey multiple comparisons test. * P<0.05, **

P<0.01, *** P<0.001, **** P<0.0001. Abbreviations: MDP = maximum diastolic potential; Eth = action potential threshold; SLDD = linear diastolic depolarization slope; SEDD = exponential diastolic depolarization slope; APA = action potential amplitude; dV/dt = action potential upstroke velocity; APD = action potential duration at 90%, 70% 50%, or 30% repolarization.