Novel *SCN5A* Mutation in Amiodarone-responsive Multifocal Ventricular Ectopy-associated Cardiomyopathy

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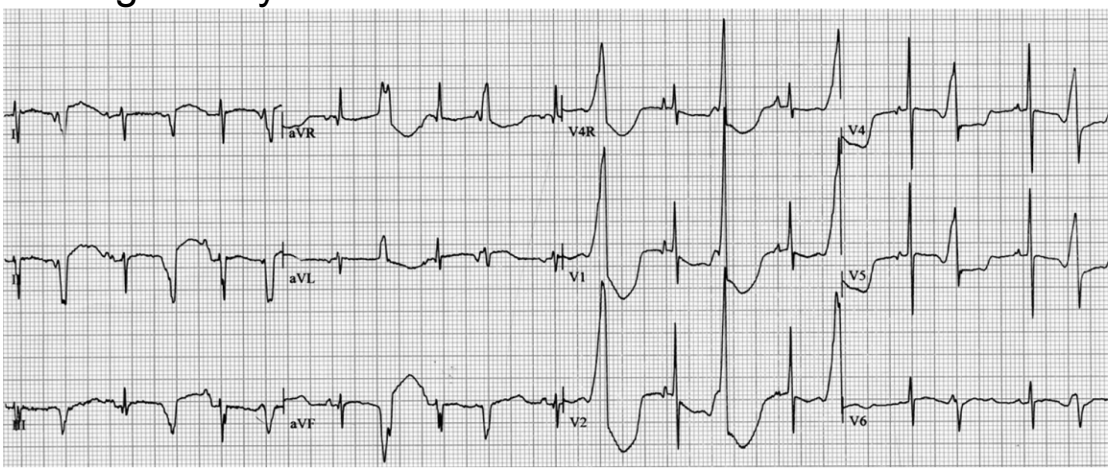
SUPPLEMENTAL INFORMATION

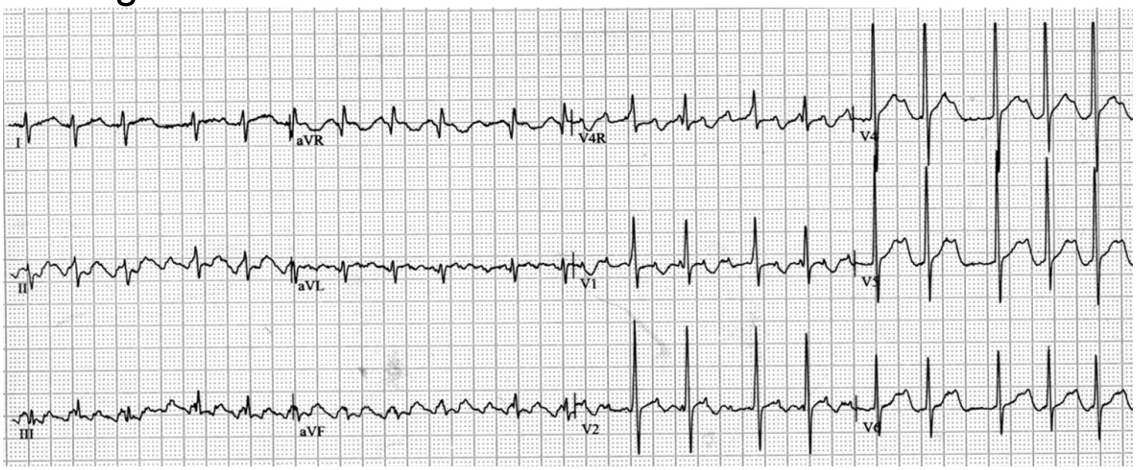
Supplemental Figures:

Supplemental Tables:

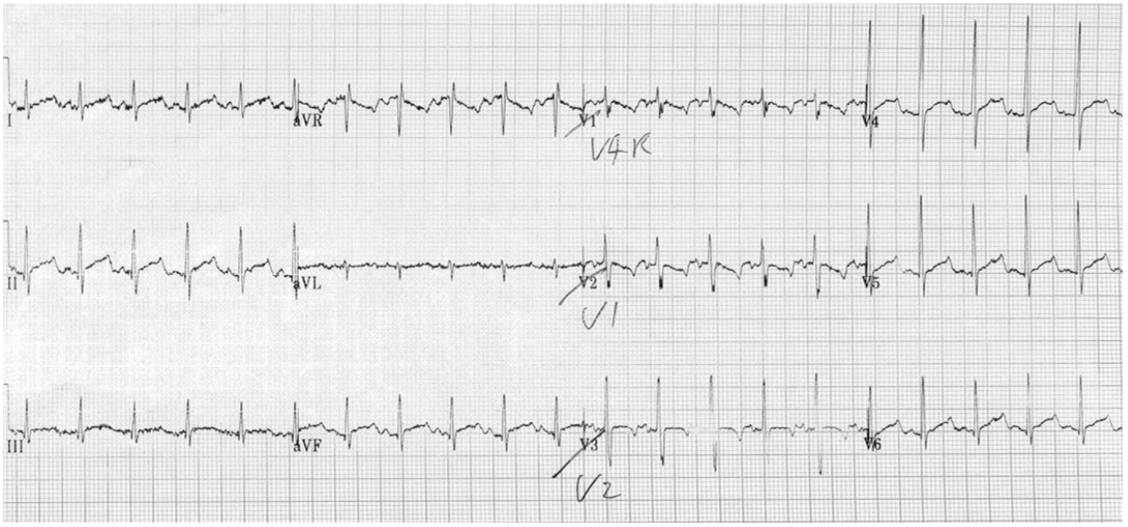
A. Age 3 days



B. Age 7 weeks

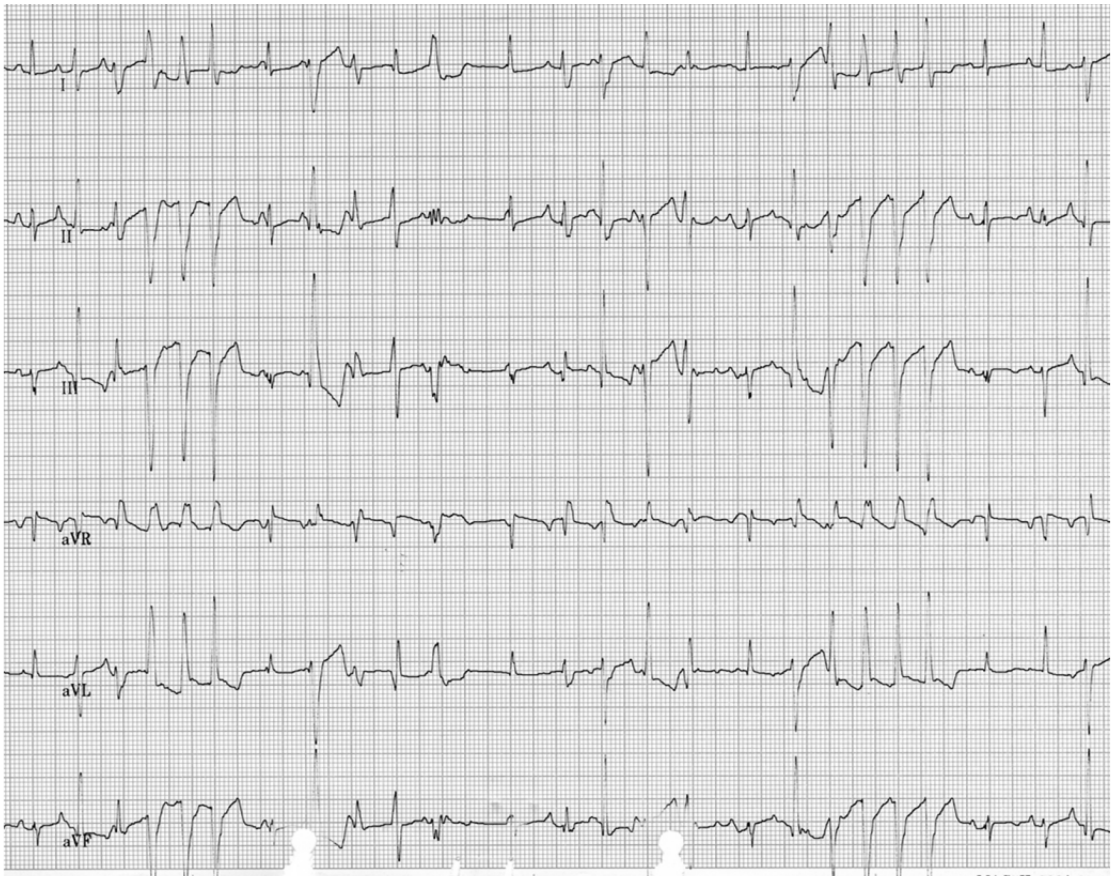


C. Age 6.5 months

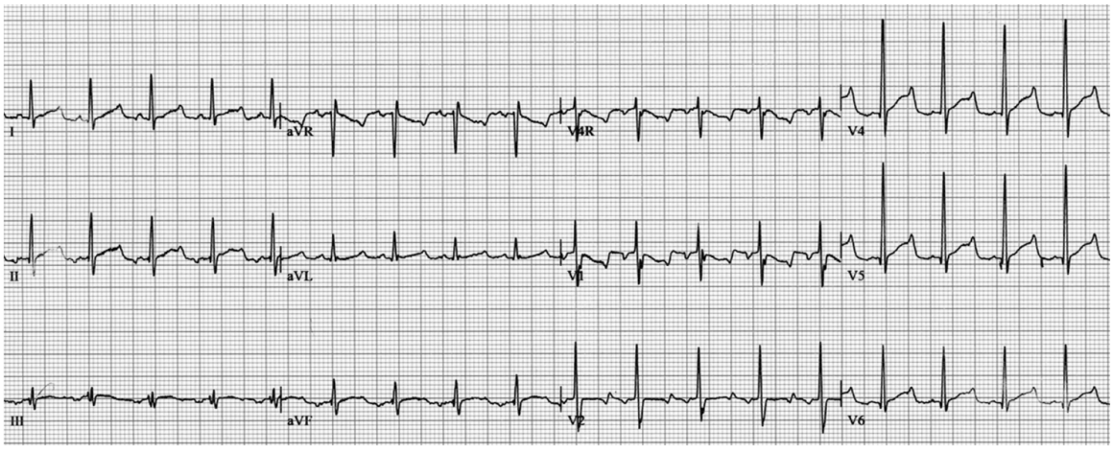


Supplemental Fig. S1. Additional electrocardiographic recordings of the proband.

D. Age 9 months



E. Age 20 months



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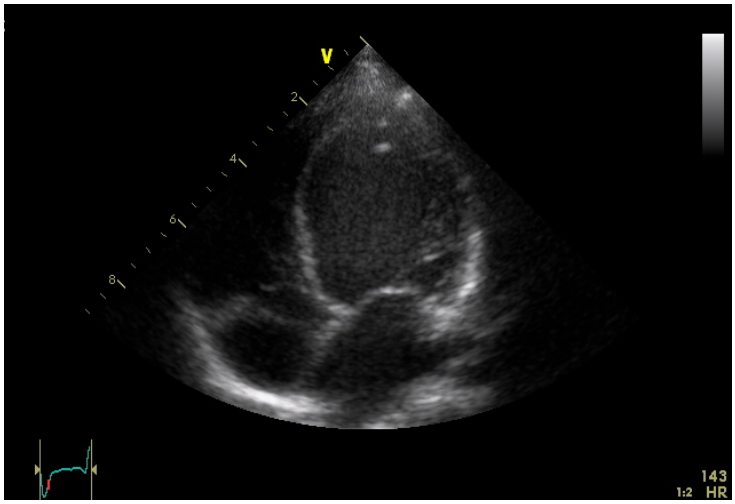
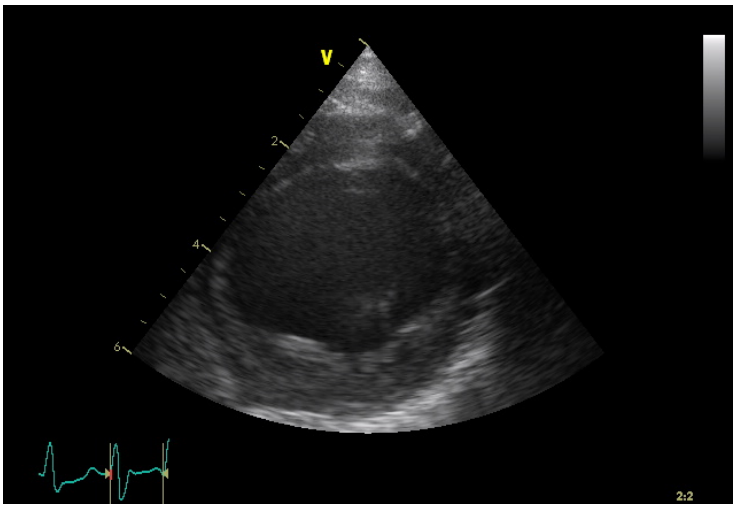
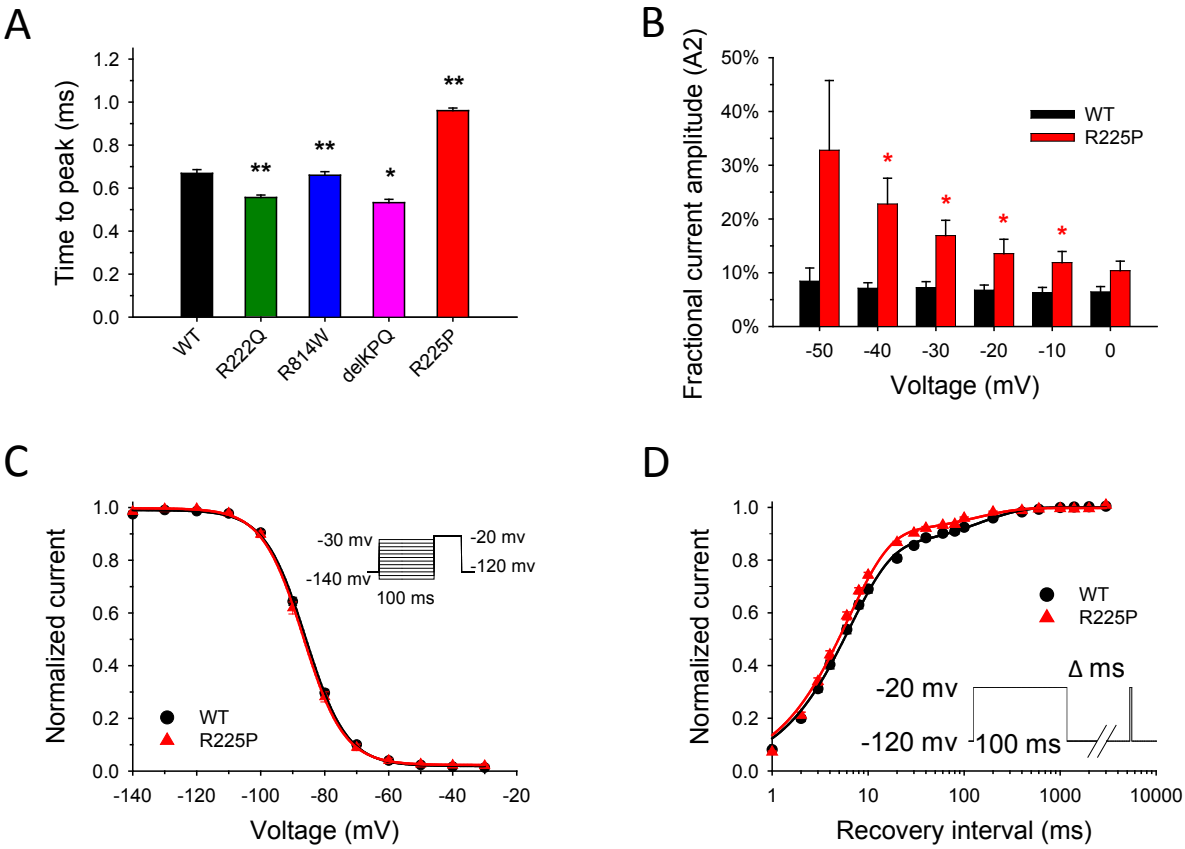
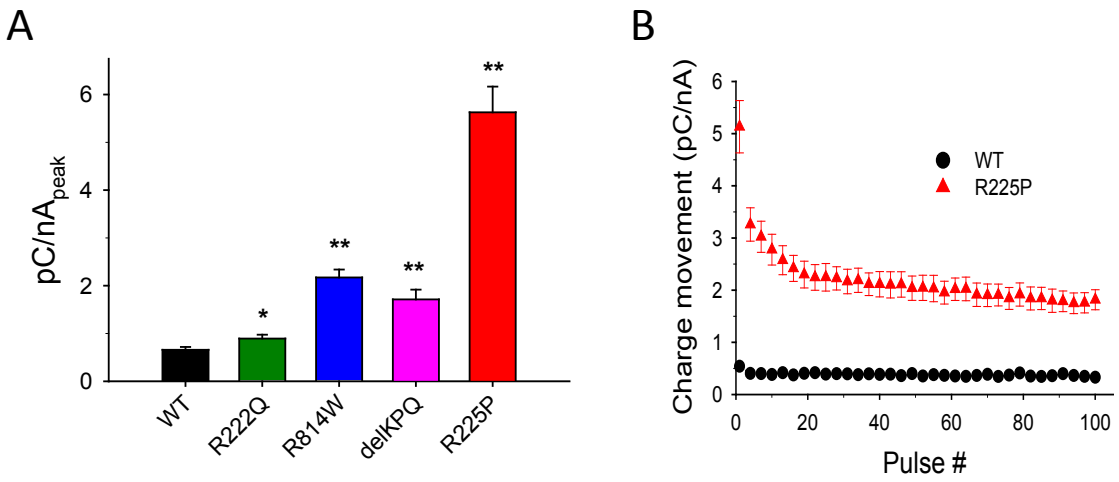




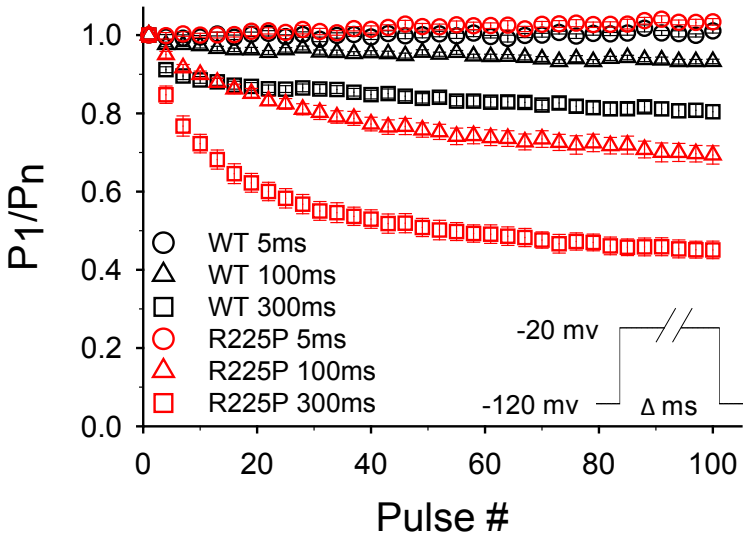
Supplemental Fig. S2. Echocardiographic images of the proband (weight 8.4 kg, height 69 cm) illustrating dilation of all cardiac chambers.



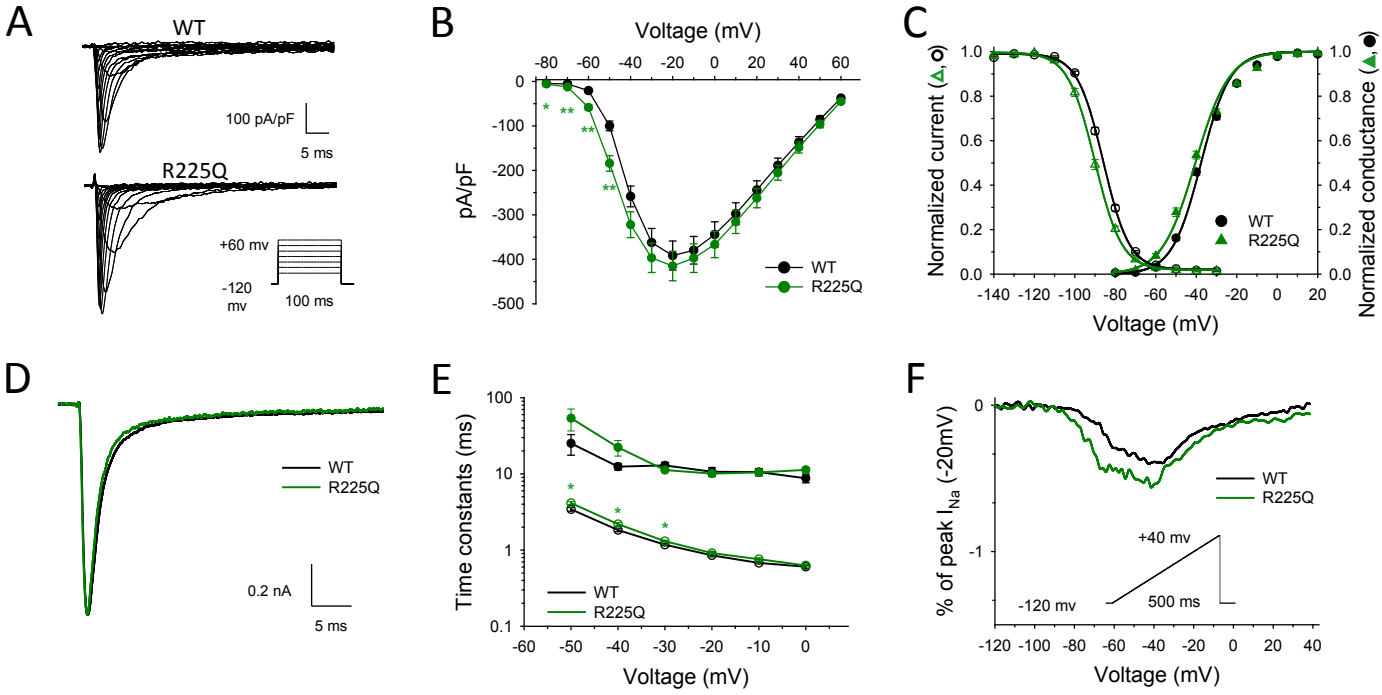
Supplemental Fig. S3. Activation and inactivation of R225P. (A) Activation kinetics measured as time to peak current derived from activating pulses to -20mV (holding potential -120mV) for WT and mutant channels. (B) Percentage of current inactivation associated with the slow component (time constant, τ_2) of current decay for WT and R225P. (C) Channel availability of WT and R225P. (D). Recovery from fast inactivation. All data are represented as mean ± S.E.M. for n= 8 - 9 cells.



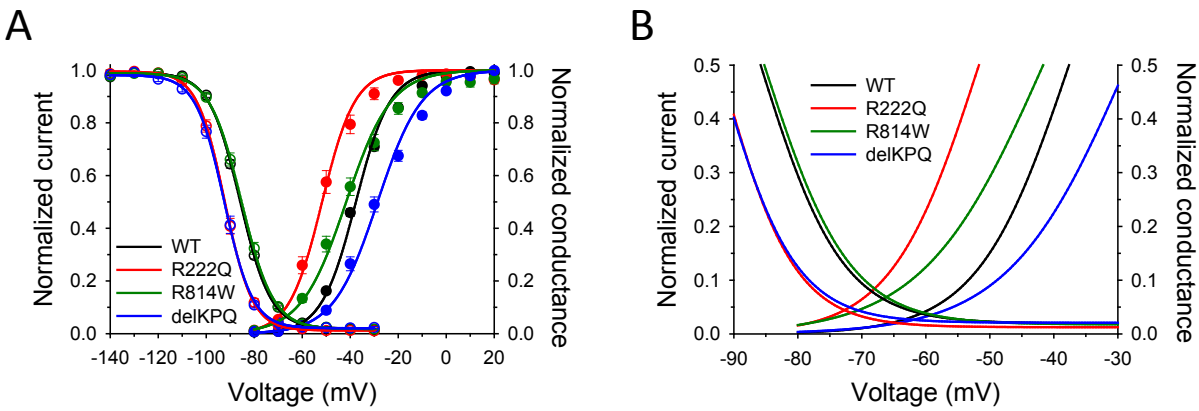
Supplemental figure S4. Ramp-current analysis. (A) Net charge movement during slow voltage-ramps of WT and mutant channels. (B) Charge movement in response to consecutive 500 ms ramp-current protocols (1.1 Hz; 100 pulses). All data are represented as mean ± S.E.M. for n = 7 - 16 cells.



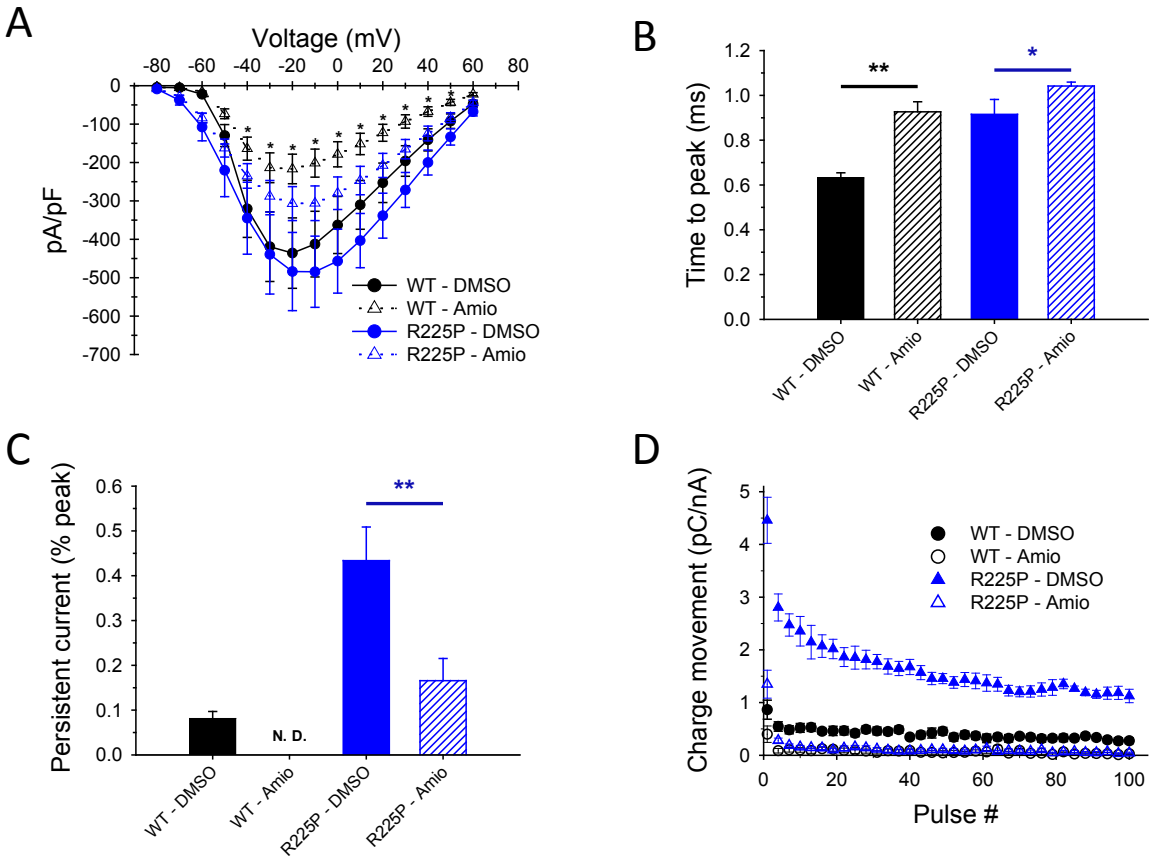
Supplemental Fig. S5. Frequency-dependent rundown. Pulse trains (2Hz; -20mV) illustrated for WT (black) and R225P (red) channels with activating pulses of 5 ms (open circles), 100 ms (open triangles), or 300 ms (open square). All data are represented as mean ± S.E.M. for n = 11 - 15 cells.



Supplemental Fig. S6. Biophysical properties of $Na_V1.5$-R225Q. (A) Representative traces of WT (top) and R225Q (bottom) sodium channels. (B) Current-density/voltage plots of WT and R225Q. (C) Voltage dependence of activation and channel availability for WT and R225Q. (D) Representative traces of WT and R225Q at -20 mV. (E) Voltage-dependence of inactivation time constants (open symbols represent fast component; closed symbols represent slow component) for WT and R225Q. (F) Normalized TTX-subtracted average ramp-currents (0.32 mV/ms) of WT and R225Q measured as a percentage of peak I_{Na} (-20 mV). All data are represented as mean ± S.E.M for n = 7 - 15 cells.



Supplemental Fig. S7. Window-currents. (A) Overlay of Boltzmann-fitted conductance – voltage (filled symbols) and channel availability (open symbols) curves of WT and mutations R222Q, R814W, and delKPQ. (B) Panel A enhanced to emphasize window-currents. All data are represented as mean ± S.E.M. for n = 7 - 12 cells.



Supplemental Fig S8. Effects of amiodarone on $Na_V1.5$-R225P. (A) Current-density – voltage plots of WT and R225P channels in the presence of 0.01% DMSO (control, solid symbols) or amiodarone (open symbols). (B) Activation kinetics measured as time to peak current following an activation pulse to -20 mV (holding potential -120 mV). (C) Persistent current as a percentage of peak I_{Na} from WT and R225P in the presence of DMSO or amiodarone. WT persistent current non-determinable (N. D.) in the presence of amiodarone. (D) Charge movement in response to consecutive 500 ms ramp-current protocols (1.1 Hz; 100 pulses) in DMSO (closed symbols) and amiodarone (open symbols) for WT (black circles) and R225P blue (triangles).

Table S1: Biophysical properties of WT-$Na_V1.5$ and mutant channels.

		WT	R225P	R222Q	R814W	R225Q	delKPQ
Activation	$V_{1/2}$	-37.3 ± 0.6	-37.1 ± 1.0	-51.2 ± 1.5**	-41.1 ± 1.6*	-40.2 ± 0.7*	-28.4 ± 1.2**
	k	8.4 ± 0.2	12.3 ± 0.3**	7.6 ± 0.4	10.7 ± 0.4**	9.8 ± 0.2**	10.4 ± 0.1**
	n	14	11	13	15	13	12
Inactivation	$V_{1/2}$	-86.0 ± 0.4	-86.8 ± 0.6	-92.7 ± 0.8**	-84.9 ± 0.8	-90.2 ± 0.6**	-92.5 ± 0.8**
	k	-6.4 ± 0.1	-6.3 ± 0.1	-5.6 ± 0.1**	-6.5 ± 0.2	-6.6 ± 0.2	-5.6 ± 0.1**
	n	11	10	14	14	10	14
Window-current peak	*Voltage*	-60.4 ± 0.4	-67.0 ± 1.2**	-74.7 ± 0.4**	-65.3 ± 0.9**	-65.6 ± 0.7**	-60.7 ± 1.5
	Activity	0.037 ± 0.001	0.063 ± 0.002**	0.050 ± 0.002**	0.064 ± 0.003**	0.038 ± 0.002	0.025 ± 0.002**
	n	10	7	8	12	11	10
Time to peak (-20 mV)	*Peak (ms)*	0.67 ± 0.02	0.96 ± 0.01**	0.56 ± 0.01**	0.66 ± 0.02	0.75 ± 0.03*	0.53 ± 0.02**
	n	18	10	7	7	14	8
Recovery from fast inactivation	*A1*	0.85 ± 0.01	0.90 ± 0.01*	0.87 ± 0.02	0.88 ± 0.01	0.85 ± 0.02	0.86 ± 0.02
	τ_1	6.5 ± 0.3	6.2 ± 0.3	7.0 ± 0.3	6.8 ± 0.5	7.8 ± 0.7	4.9 ± 0.2**
	A2	0.15 ± 0.01	0.10 ± 0.01**	0.14 ± 0.02	0.11 ± 0.01*	0.15 ± 0.02	0.14 ± 0.02
	τ_2	178 ± 18	147 ± 14	153 ± 17	190 ± 29	209 ± 289	265 ± 49
	n	18	9	10	8	8	10
500ms Ramps	*Peak (mV)*	45.7 ± 2.7	-57.1 ± 3.0*	-70.9 ± 0.9**	-64.9 ± 1.4**	-56.7 ± 3.4*	-35.0 ± 1.9*
	% peak	-0.60 ± 0.04	-4.9 ± 0.3**	-1.0 ± 0.1**	-2.4 ± 0.1**	-0.9 ± 0.1**	-1.0 ± 0.1**
	Area (pC/nA)	0.66 ± 0.1	5.86 ± 0.6**	0.89 ± 0.1	2.17 ± 0.2**	1.01 ± 0.2	1.71 ± 0.2**
	n	15	11	8	7	12	8
Ramp-current activation threshold	*Voltage*	-77.2 ± 1.2	-96.5 ± 1.2**	-97.3 ± 1.6**	-95.4 ± 2.3**	-86.42 ± 1.6**	-68.5 ± 1.2**
	n	13	16	8	7	11	7
Persistent I_{Na}	*% peak*	0.13 ± 0.02	0.87 ± 0.06**	0.09 ± 0.03	0.17 ± 0.03	0.24 ± 0.03*	0.65 ± 0.04**
	n	18	10	7	7	14	8

Versus WT

* = P < 0.05

** = P < 0.005

Table S2: Frequency-dependent rundown and slow inactivation properties of WT-$Na_V1.5$ and R225P.

			WT	R225P
Frequency dependent rundown 2 Hz	5 ms pulse length	P_1/P_{100}	1.01 ± 0.01	1.03 ± 0.01*
		n	11	12
	100 ms pulse length	P_1/P_{100}	0.93 ± 0.01	0.69 ± 0.02**
		n	15	15
	300 ms pulse length	P_1/P_{100}	0.80 ± 0.02	0.47 ± 0.02**
		n	14	12
Onset of slow inactivation		*A1*	0.21 ± 0.03	0.21 ± 0.04
		τ_1	314.1 ± 41.5	373.3 ± 81.5
		A2	0.45 ± 0.04	0.7 ± 0.04**
		τ_2	9973.5 ± 1601.2	10321.1 ± 224.5
		y0	0.32 ± 0.02	0.08 ± 0.003**
		n	8	7
Recovery from inactivation (500 ms pre-pulse)		*A1*	0.82 ± 0.01	0.83 ± 0.01
		τ_1	8.3 ± 0.6	7.3 ± 0.7
		A2	0.17 ± 0.01	0.13 ± 0.01*
		τ_2	219.6 ± 22.5	185.71 ± 24.29
		n	10	9
Recovery from slow inactivation (3s pre-pulse)		*A1*	0.69 ± 0.01	0.63 ± 0.02*
		τ_1	10.1 ± 0.9	14.0 ± 1.1*
		A2	0.31 ± 0.01	0.35 ± 0.02
		τ_2	352.3 ± 24.4	2261.9 ± 251.8**
		n	9	9
Voltage dependence of slow inactivation		$V_{1/2}$	-68.9 ± 5.2	-83.5 ± 0.8*
		k	-32.9 ± 2.2	-12.0 ± 0.4**
		n	8	8
1.1 Hz ramp train (P_{100})		*Area (pC/nA)*	0.33 ± 0.04	1.82 ± 0.19**
		n	8	13

Versus WT

* = P < 0.05

** = P < 0.005

Table S3: Effects of amiodarone on WT-$Na_V1.5$ and R225P channels.

Amiodarone (3μM)		WT - DMSO	WT - Amio	R225P - DMSO	R225P - Amio
Activation	***$V_{1/2}$***	-40.4 ± 1.1	-41.4 ± 0.7	-34.5 ± 1.5*	***-41.0 ± 1.2***
	k	6.7 ± 0.3	6.8 ± 0.2	11.1 ± 0.5**	10.7 ± 0.4**
	n	7	11	6	10
Inactivation	***$V_{1/2}$***	-85.0 ± 0.8	-92.2 ± 1.8**	-85.2 ± 1.1	**-91.6 ± 1.9***
	k	-6.2 ± 0.1	-10.1 ± 0.7**	-6.8 ± 0.2*	***-10.8 ± 0.7****
	n	8	10	8	12
Window-currents	***Voltage***	-61.00 ± 0.03	-61.6 ± 0.7	-63.2 ± 1.8	**-68.0 ± 1.3****
	Activity	0.034 ± 0.001	0.044 ± 0.002**	0.072 ± 0.004**	***0.093 ± 0.003****
	n	5	7	7	9
Recovery from inactivation	***A1***	0.87 ± 0.01	0.19 ± 0.07**	0.88 ± 0.02	***0.13 ± 0.03****
	τ_1	8.0± 1.1	6.8 ± 2.7	10.3 ± 1.0	5.6 ± 1.4
	A2	0.13 ± 0.01	1.03 ± 0.06**	0.12 ± 0.02	***1.04 ± 0.05****
	τ_2	217 ± 36	2130 ± 434**	245 ± 39	***1940 ± 293****
	n	7	8	8	8
Persistent I_{Na}	***% peak***	0.08 ± 0.02	-0.15 ± 1.16	0.43 ± 0.08**	**0.17± 0.05**
	n	5	8	8	7
Time to peak (-20 mV)	***Peak (ms)***	0.63 ± 0.02	0.93 ± 0.05**	0.92 ± 0.05*	**1.04 ± 0.02***
	n	5	8	9	14
500ms Ramps (Paired)	***mV***	-49.0 ± 3.6	-59.7 ± 1.7**	-57.2 ± 2.5	***-62.4 ± 1.0***
	% peak	-0.9 ± 0.1	-0.5 ± 0.1**	-4.6 ± 0.5**	-3.5 ± 0.5**
	Area (pC/nA)	1.2 ± 0.2	1.1 ± 0.3	5.8 ± 0.9**	**2.8 ± 0.3****
	n	10	10	11	11
Frequency dependent rundown 100ms	***1 Hz*** ***P_1/P_{100}***	0.96± 0.04	0.30 ± 0.01**	0.86 ± 0.03**	***0.18 ± 0.01****
	n	10	10	10	10
	2 Hz ***P_1/P_{100}***	0.89 ± 0.03	0.25 ± 0.01**	0.69 ± 0.02**	***0.10 ± 0.01****
	n	7	8	8	12
1.1 Hz ramp train (P_{100})	***Area (pC/nA)***	0.30 ± 0.5	0.02 ± 0.01*	1.12 ± 0.13**	***0.04 ± 0.02****
	n	6	6	6	7

Versus WT (DMSO)
* = P < 0.05
** = P < 0.005

Versus R225P (DMSO)
Bold P < 0.05
Bold, italices P < 0.005