Supplementary Appendix

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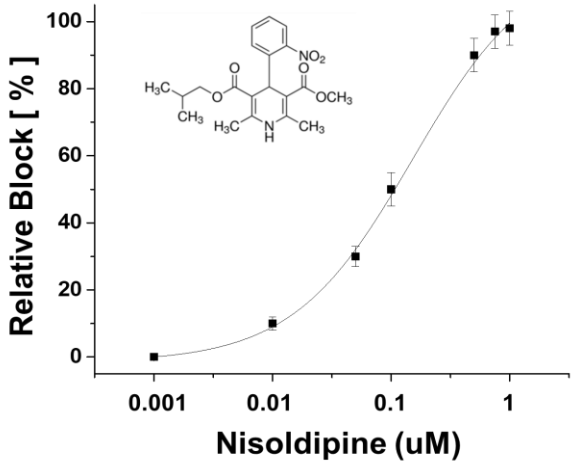
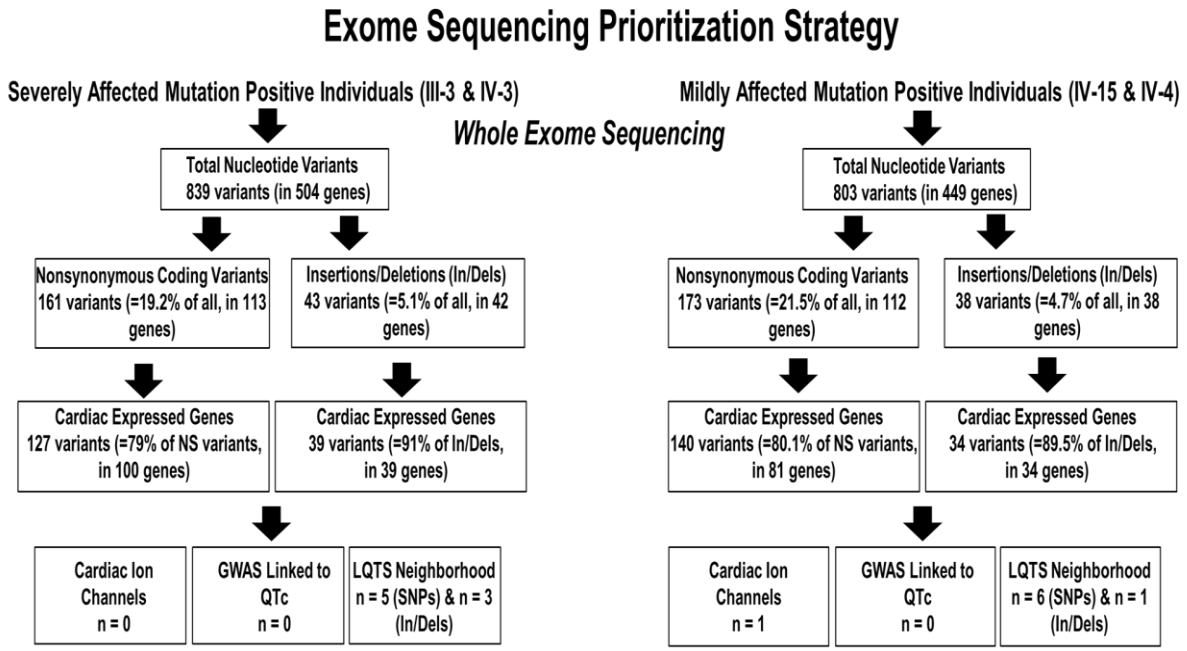
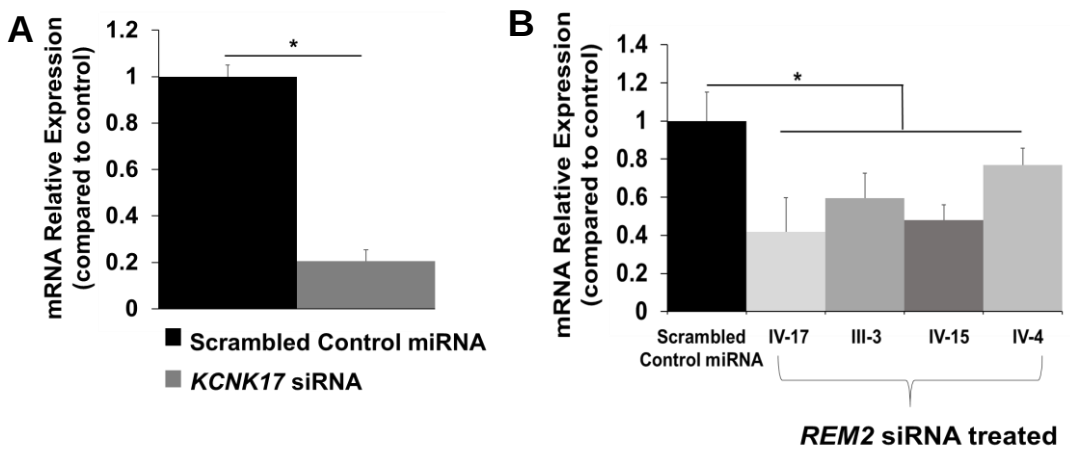


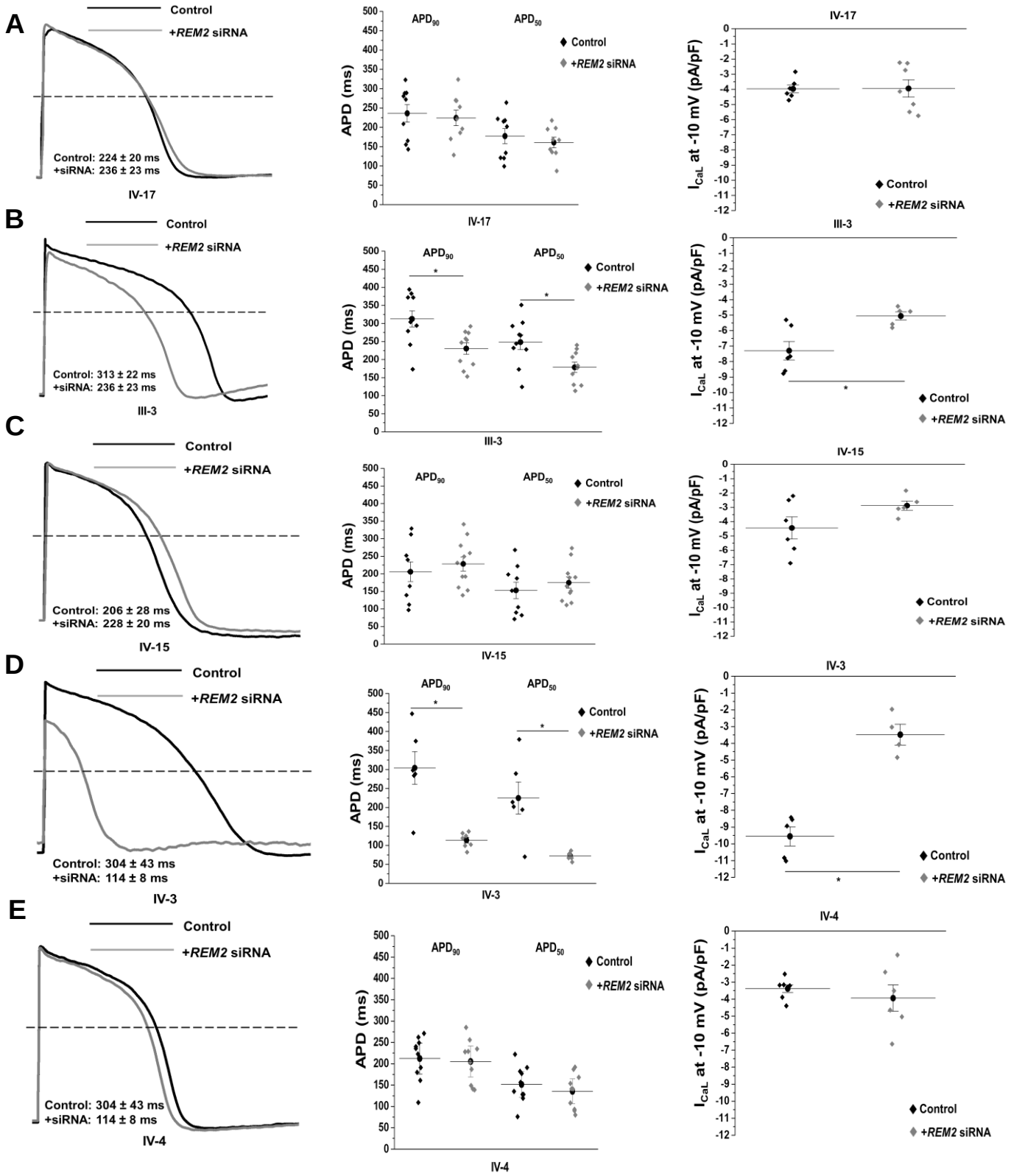
Supplemental Figure 1. Concentration-Response Relationship for Nisoldipine on iPSC-CMs. Concentration-response relationship for the effect of nisoldipine ($Ca_v1.2$ blocker) on I_{CaL} (L-type Ca^{2+} channel) was measured at -10 mV (between 3-7 cells, mean±SEM). EC_{50} value yielded 150 nM. Inset: structural schematic of nisoldipine.



Supplemental Figure 2. Exome Sequencing Prioritization Strategy. Four patients were exome sequenced (two in each group: IV-15 and IV-4 in the mildly affected and III-3 and IV-3 in the severely affected). The severely affected hERG R752W mutation-positive and mildly affected mutation-positive individuals were filtered looking for variants in genes exclusive to each cohort. Total coding variants describes all synonymous, nonsynonymous, and frameshift-inducing insertions or deletions as well as canonical splice site variants within captured exons (but does not include 5' or 3' untranslated regions and introns). From this group we filtered first for only nonsynonymous coding variants (i.e. variants resulting in an amino acid substitution) or for Insertion/Deletion. Next, we filtered for genes specific to cardiac tissue. Then, we filtered for cardiac ion channels or genes that were cross listed in the LQTS Neighborhood(22) or linked by GWAS to QT interval modulation(18, 19, 21).



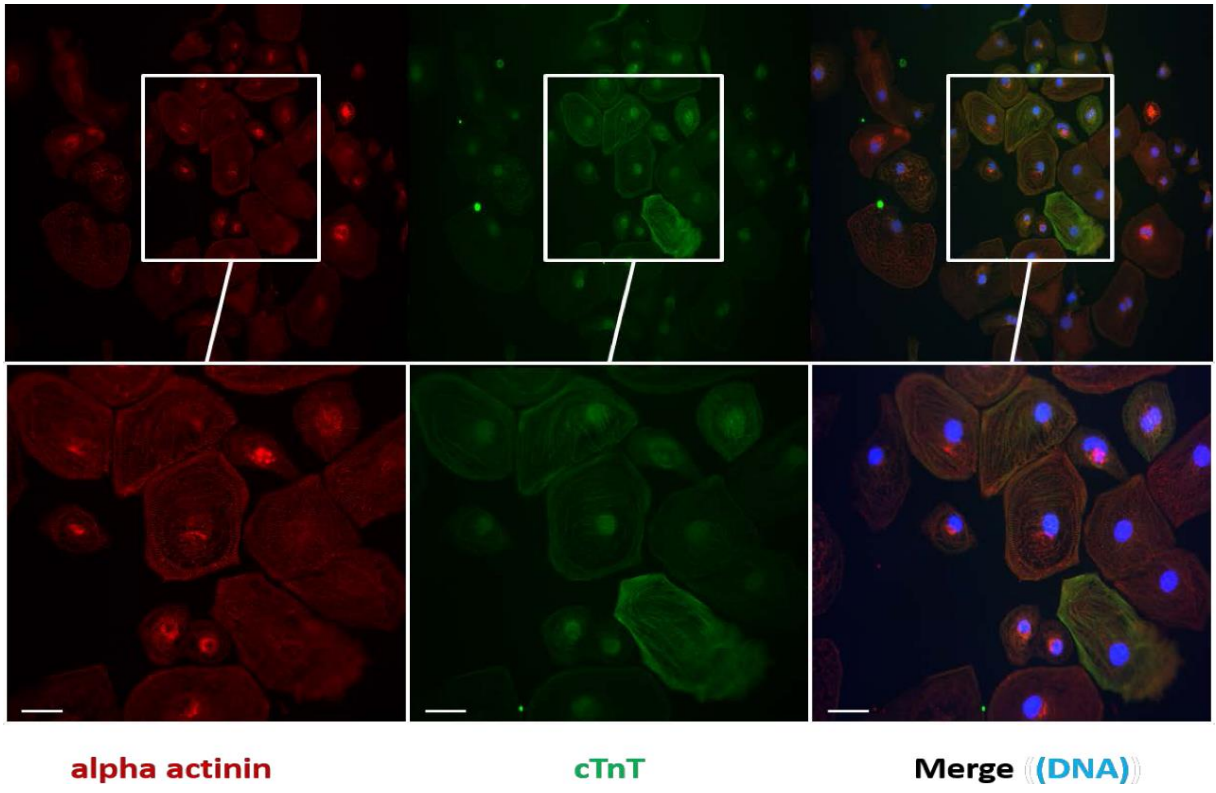
Supplemental Figure 3. siRNA knockdown of *KCNK17* and *REM2.* Panel A shows qPCR data of iCell® cardiomyocytes transfected with either scrambled control siRNA or *KCNK17* siRNA revealed ~5 fold knockdown of *KCNK17* transcript in the siRNA group. Panel B shows between ~1.3-2.4 fold knockdown of *REM2* transcript in 4 patient derived iPSC-CM lines in the siRNA treated group compared to scrambled control. * denotes $p < 0.05$ with unpaired Student's t-test used to assess significance in Panel A and ANOVA in Panel B.


A
Control
+REM2 siRNA
Control: 224 ± 20 ms
+siRNA: 236 ± 23 ms
IV-17
APD (ms)
APD90
APD50
Control
+REM2 siRNA
IV-17
ICaL at -10 mV (pA/pF)
IV-17
Control
+REM2 siRNA
B
Control
+REM2 siRNA
Control: 313 ± 22 ms
+siRNA: 236 ± 23 ms
III-3
APD (ms)
APD90
APD50
*
*
Control
+REM2 siRNA
III-3
ICaL at -10 mV (pA/pF)
III-3
*
Control
+REM2 siRNA
C
Control
+REM2 siRNA
Control: 206 ± 28 ms
+siRNA: 228 ± 20 ms
IV-15
APD (ms)
APD90
APD50
Control
+REM2 siRNA
IV-15
ICaL at -10 mV (pA/pF)
IV-15
Control
+REM2 siRNA
D
Control
+REM2 siRNA
Control: 304 ± 43 ms
+siRNA: 114 ± 8 ms
IV-3
APD (ms)
APD90
APD50
*
*
Control
+REM2 siRNA
IV-3
ICaL at -10 mV (pA/pF)
IV-3
*
Control
+REM2 siRNA
E
Control
+REM2 siRNA
Control: 304 ± 43 ms
+siRNA: 114 ± 8 ms
IV-4
APD (ms)
APD90
APD50
Control
+REM2 siRNA
IV-4
ICaL at -10 mV (pA/pF)
IV-4
Control
+REM2 siRNA

1

2

Supplemental Figure 4. Effect of *REM2* siRNA on APD and I_{CaL} in patient derived iPSC-CM. Panels A-E illustrate the effects of *REM2* siRNA on both patient iPSC-CM trios (IV-17, III-3, IV-15 and IV-17, IV-3 and IV-4). Data is depicted as macroscopic action potential recording, APD summary data, and I_{CaL} comparison between scrambled control and *REM2* siRNA. Between 6-11 cells were analyzed in the effects of *REM2* siRNA on APD and 5-7 cells for the effects of *REM2* siRNA on I_{CaL} in Panels A-E. Please refer to Supplemental Table 7 for numbers of replicate measures (n) and statistical analysis.



Supplemental Figure 5. Immunohistochemistry of patient derived iPSC-CMs. Immunostaining of iPSC-CM derived from III-3 reveal canonical markers of cardiomyocyte lineage (alpha actinin and the cardiac isoform of Troponin T) as well as normal sarcomeric orientation. Immunohistochemical assessment reproduces identical results from the mildly affected mutation-positive son and severely affected mutation-positive sister (data not shown). Scale bar is 40 μm.

Supplemental Tables

Cleveland LQT2 Family	
Number of Family Members Enrolled in LQT2 Study	n = 101
Number of hERG R752W Mutation Positive Individuals	n = 26 (26%, No. of Mutation Positive/No. of Enrolled Family Members)
Sex Distribution Amongst 26 hERG R752W Mutation Positive Individuals	Females (n = 12, 46%) Males (n = 14, 54%)
Average Age of ECG & Genotyping / QTc ± stdev (males) from hERG R752W Mutation Positive Individuals	29 / 438 ± 23
Average Age of ECG & Genotyping / QTc ± stdev (females) from hERG R752W Mutation Positive Individuals	34 / 481 ± 32

Supplemental Table 1. Cleveland LQT2 Family Summary Statistics.

Generation	Family Member Number	Sex	Phenotype
III	1	F	Severely affected
	2	F	Mildly affected
	3	M	Mildly affected
	4	**M**	**Severely affected**
	5 (deceased)	M	Mildly affected
IV	1	F	Mildly affected
	2	M	Mildly affected
	3	M	Mildly affected
	4	F	Mildly affected
	5	M	Mildly affected
	6	**F**	**Severely affected**
	7	**F**	**Mildly affected**
	8	F	Mildly affected
	9	**M**	**Mildly affected**
	10	F	Mildly affected
	12	F	Mildly affected
	13	M	Mildly affected
	13	M	Mildly affected
	14	M	Mildly affected
	15	F	Mildly affected
V	1	F	Mildly affected
	2	M	Mildly affected
	3	F	Mildly affected
	4	M	Mildly affected
	5	M	Mildly affected
	6	M	Mildly affected
	7	M	Mildly affected

Supplemental Table 2. Phenotype Details from All 26 living hERG R752W Mutation-Positive Individuals from Cleveland LQT2 Family. This table shows all 26 mutation-positive family members distributed across three generations involved in the study (and 1 deceased, Gen III, #5). Rows shaded in dark grey with bold font are the original 4 family members characterized by iPSC-CM and exome sequencing in the main text.

The light shaded grey rows indicate additional mutation carriers in the family that were additionally genotyped for *REM2* and *KCNK17.* Non-shaded rows are mutation carriers that remain uncharacterized. *Note:* these Generation + Family Member Number identifiers do not correspond to those in Figure 1 because for conciseness Figure 1 is a zoomed-in snapshot of the family pedigree and thus uses its own numbering system. Here in this table we are showing all the hERG R752W mutation carriers in the family starting from the first individual in each generation.

iPSC-CM Background	n	APD_{90} (msec)	APD_{50} (msec)	APA (mV)	MDP (mV)
IV-17 (Control)	71	226 ± 9	115 ± 12	109 ± 2	-60 ± 1
III-3 (Severely affected)	203	271 ± 11	158 ± 14	107 ± 3	-56 ± 2
IV-15 (Mildly affected)	143	215 ± 9	132 ± 9	106 ± 2	-57 ± 1
IV-17 (Control)	77	184 ± 13	72 ± 6	98 ± 2	-55 ± 1
IV-3 (Severely affected)	134	287 ± 17	166 ± 13	106 ± 2	-56 ± 1
IV-4 (Mildly affected)	94	169 ± 8	104 ± 8	104 ± 2	-56 ± 1

Supplemental Table 3. Comprehensive Action Potential Characteristics from Patient Specific iPSC-CMs from Figure 2. MDP: mean diastolic potential, APD_{90}: action potential duration at 90% of repolarization in milliseconds, APD_{50}: action potential duration at 50% of depolarization in milliseconds, APA: action potential amplitude.

iPSC-CM Background	n	APD_{90} (ms) -nisoldipine	APD_{90} (ms) +nisoldipine	p value	APD_{50} (ms) -nisoldipine	APD_{50} (ms) +nisoldipine	p value	n	I_{CaL} (pA/pF) - nisoldipine	I_{CaL} (pA/pF) +nisoldipine	p value
IV-17 (Control)	8	229 ± 25	181 ± 20	0.002	175 ± 18	132 ± 15	0.008	7	-4.6 ± 0.72	-3.4 ± 0.75	0.04
III-3 (Severely affected)	10	305 ± 25	221 ± 20	0.01	227 ± 16	158 ± 13	0.002	7	-7.1 ± 0.75	-5.2 ± 0.68	0.001
IV-15 (Mildly affected)	8	211 ± 19	151 ± 16	0.004	144 ± 19	96 ± 17	0.003	8	-4.0 ± 0.49	-2.9 ± 0.56	0.03
IV-3 (Severely affected)	8	305 ± 18	178 ± 14	0.006	247 ± 13	130 ± 18	0.002	6	-9.0 ± 0.77	-5.1 ± 0.66	0.001
IV-4 (Mildly affected)	11	210 ± 25	157 ± 18	0.0009	158 ± 23	106 ± 16	0.01	5	-4.6 ± 0.32	-3.0 ± 0.37	0.01

Supplemental Table 4. Summary data of action potential duration and I_{CaL} characteristics from patient specific iPSC-CMs with and without nisoldipine treatment from Figure 5**.** I_{CaL}: L-type Calcium Current. * denotes significance with p-value assessed by paired Student's t-test ($p < 0.05$ considered significant).

SNPs & Insertion/Deletions in Severely Affected vs. Mildly Affected hERG R752W Mutation Positive Individuals

Locus	SNP	Chr	Nucleotide Change	Amino Acid Change	MAF
SNPs in Severely Affected hERG R752W Mutation Positive Individuals (III-3 and IV-3)					
REM2	rs8014119	14	G287C	G96A	C = 0.10
ARHGAP10	rs115753644	4	G1183A	G395R	A = 0.0006
CAMKK2	rs3817190	12	A253T	T85S	A = 0.41
GRIN3A	rs62000403	9	A3218T	N1073I	A = 0.05
P2RX7	rs2230912	12	A1379G	Q460R	G = 0.07
In/Del in Severely Affected hERG R752W Mutation Positive Individuals (III-3 and IV-3)	**In/Del**	**Chr**	**Type & Nucleotide**	**Intronic or UTR**	**MAF**
DMD	rs3833412	X	Insertion of T	Intronic	T: 0.43
NRGN	rs11399333	11	Insertion of G	Introinc	G: 0.41
PALM2, PALM2-AKAP2	rs201053095	9	Insertion of T	UTR	T: 0.02
SNPs in Mildly Affected hERG R752W Mutation Positive Individuals (IV-15 and IV-4)	**SNP**	**Chr**	**Allele Change**	**Amino Acid Change**	**MAF**
KCNK17	rs10947804	6	A61G	S21G	A = 0.42
PPP1R18	rs9262143	6	C1015A	G339R	A = 0.02
PPP2R3A	rs9814557	3	A200G	D67G	G =0.14
PLCG1	rs753381	20	T2438C	I813T	T = 0.27
ADCY9	rs2230739	16	A2316G	I772M	G = 0.26
MYO5B	rs2298624	18	G2753A	R918H	T = 0.14
SPTA1	rs857725	1	A5077C	K1693Q	G = 0.25
In/Del in Mildly Affected hERG R752W Mutation Positive Individuals (IV-15 and IV-4)	**In/Del**	**Chr**	**Type & Nucleotide**	**Intronic or UTR**	**MAF**
KIF1B	rs146553372	1	Deletion of T	Intronic	T: 0.48

Supplemental Table 5. Exome Sequencing Gene Candidates. This table shows the complete list of candidate genes identified by exome sequencing in either the severely affected hERG R752W mutation-positive or mildly affected hERG R752W mutation-positive individuals. Applying the prioritization strategy shown in Supplemental Figure 2 we identified in the severely affected group SNPs in five genes and three genes with insertion/deletions. In the mildly affected group, SNPs were identified in seven genes along with one insertion/deletion. SNP: single nucleotide polymorphism, In/Del: insertion/deletion, Chr: chromosome, MAF: minor allele frequency (as reported by 1000 Genomes or ExAC Database), UTR: untranslated region.

III-3 (Severely affected Homozygous WT:S21)	MDP (mV)	APA (mV)
Scrambled Control (n = 15)	-62.52 ± 4.07	108.33 ± 2.40
KCNK17 siRNA treated (n =15)	-66.33 ± 4.31	105.00 ± 4.49
IV-4 (Mildly affected Heterozygote S21/G21)	**MDP (mV)**	**APA (mV)**
Scrambled Control (n = 21)	-65.27 ± 3.11	87.24 ± 2.18
KCNK17 siRNA treated (n = 27)	-61.33 ± 1.59	100.26 ± 2.73*

Supplemental Table 6. Additional Action Potential Characteristics from Effects of *KCNK17* siRNA on patient iPSC-CM (III-3 and IV-4) from Figure 6. Data shown as mean ± SEM, MDP: mean diastolic potential, APA: action potential amplitude, *p-value=0.0008 as determined by unpaired Student's t-test.

iPSC-CM Background	n	APD_{90} (ms) –REM2 siRNA	APD_{90} (ms) +REM2 siRNA	p value	APD_{50} (ms) -REM2 siRNA	APD_{50} (ms) +REM2 siRNA	p value	n	I_{CaL} (pA/pF) - REM2 siRNA	I_{CaL} (pA/pF) +REM2 siRNA	p value
IV-17 (Control)	9-10	236 ± 23	224 ± 20	0.70	181 ± 13	177 ± 20	0.50	6-7	-4.0 ± 0.31	-3.9 ± 0.28	0.61
III-3 (Severely affected)	10	313 ± 22	231 ± 16	0.007	248 ± 21	179 ± 14	0.01	5-6	-7.8 ± 0.68	-5.1 ± 0.22	0.02
IV-15 (Mildly affected)	9-11	228 ± 20	206 ± 28	0.51	175 ± 16	153 ± 23	0.44	5-6	-4.4 ± 0.65	-2.9 ± 0.31	0.11
IV-3 (Severely affected)	6-7	304 ± 43	114 ± 8	0.0006	225 ± 42	72 ± 4	0.002	5	-9.2 ± 0.61	-3.5 ± 0.58	0.0002
IV-4 (Mildly affected)	10	212 ± 16	205 ± 16	0.76	151 ± 13	135 ± 15	0.40	6-7	3.9 ± 0.33	-3.4 ± 0.41	0.51

Supplemental Table 7. Summary data of action potential and I_{CaL} characteristics from patient specific iPSC-CMs - and + *REM2* siRNA from Supplemental Figure 4**.** I_{CaL}: L-type Calcium Current. * denotes significance as assessed by unpaired Student's t-test ($p < 0.05$ considered significant).

iPSC-CM Background	n	APD_{90} (ms)	p value	APD_{50} (ms)	p value	n	I_{CaL} (pA/pF)	p value
IV-3 (Severely Affected)	14	304 ± 20	2.13E-11	237 ± 19	4.46E-10	14	-8.17 ± 0.72	1.6E-6
CRISPR-Cas9 Corrected IV-3	37	171 ± 13		137 ± 11		24	-4.54 ± 0.67	

Supplemental Table 8. Summary data of action potential and I_{CaL} characteristics from IV-3 patient iPSC-CM - and + CRISPR-Cas9 genome editing from Figure 7**.** CRISPR-Cas9 corrected *REM2* on IV-3 background, I_{CaL}: L-type Calcium Current. p-value assessed by unpaired Student's t-test ($p < 0.05$ considered significant).