Supplementary Material

Table S1
List of reagents used in the study.

Reagent	Company	Product code
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher Scientific [GIBCO]	A14700
Matrigel® Corning® hESC-Qualified Matrix, LDEV-free	Corning	354277
Fibronectin bovine plasma	Merck [Sigma-Aldrich]	F1141
Essential 8™ Flex Medium Kit	Thermo Fisher Scientific [GIBCO]	A2858501
RPMI 1640 w/ L-Glutamine	Euroclone	ECB2000
RPMI 1640 Medium, no glucose	Thermo Fisher Scientific [GIBCO]	11879020
DMEM/F-12	Thermo Fisher Scientific [GIBCO]	11320033
B-27™ Supplement	Thermo Fisher Scientific [GIBCO]	17504044
B-27™ Supplement, minus insulin	Thermo Fisher Scientific [GIBCO]	A1895601
DPBS, no calcium, no magnesium	Thermo Fisher Scientific [GIBCO]	14190094
EDTA (0.5 M), pH 8.0, RNase-free	Thermo Fisher Scientific [Invitrogen]	AM9262
TrypLE™ Select Enzyme	Thermo Fisher Scientific [GIBCO]	A1217701
RevitaCell™ Supplement	Thermo Fisher Scientific [GIBCO]	A2644501
CryoStor® CS10	StemCell Technologies	7930
CHIR-99021 HCl	Selleckchem	S2924
IWR-1	Merck [Sigma-Aldrich]	I0161
24-well Plate with Gold Electrodes on FR4	Multichannel Systems	24W700/100F-288
96-well Plate with Gold Electrodes on FR4	Multichannel Systems	96W700/100F-288
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Hydroxychloroquine Sulfate	Selleckchem	S4430

Table S2
Baseline MEA parameters for all the lines used in the study.

Cell Line	Mean FPD (ms)	FPD sem (ms)	Mean RR (ms)	RR sem (ms)	FPD-RR Fitting Coefficient Pr(>|t|)	Mean cFPD (ms)	cFPD sem (ms)	cFPD-RR Fitting Coefficient Pr(>|t|)	N of MEAs
WT	500.4	15	1639.8	50.5	9.72e-05	393.9	9.9	0.729	58
WT2	286.1	5.1	890.5	12.3	0.875	304.1	5.7	0.0314	48

LQT1	174.9	4.9	599.6	17.3	2.14e-12	227.5	5.0	0.193	106
JLNS	225.5	13.6	546.4	37.1	<2e-16	303.9	11.7	0.000203	62
CALM-LQTS	308.5	17.7	1359.9	62.6	4.31e-05	263.7	13.1	0.0864	56

Patch Clamp

hiPSC-CMs were dissociated with TrypLE Select 10X (Thermo Fisher Scientific) and plated sparsely on Matrigel-coated glass coverslips (10 mm Ø). Isolated hiPSC-CMs were patched 3-10 days after dissociation. Tyrode's solution contained (mM): NaCl 154, KCl 5.4, $CaCl_2$ 1.8, HEPES-NaOH 5, D-Glucose 5.5. pH was set to 7.35 with NaOH. Intracellular solution contained (mM): K-Aspartate 125, KCl 20, NaCl 10, Na_2-ATP 5, HEPES 10. pH was set to 7.3 with KOH. Amphotericin B 0.22 mM in DMSO was added to the intracellular solution to record APs.

APs were recorded with a Molecular Devices digidata 1440A and a Molecular Devices Axopatch 200B amplifier at physiological temperature (~37 °C). No holding current injection was used to hyperpolarize the resting membrane potential (E_{diast}).

The duration and amplitude of the current pulses used to elicit the APs were in a range of 2-3 ms and 0.5-1.5 nA. Signals were digitized at 5 kHz and filtered at 2 kHz with a low pass Bessel filter.

Liquid junction potential was calculated according to the stationary Nernst-Planck equation using LJPcalc (Harden, SW and Brogioli, D (2020). LJPcalc [Online]. Available: https://swharden.com/software/LJPcalc, Accessed on 03/08/2021). The calculated LJP was 13.922 mV. The measured LJP was -11.2 +- 0.9 mV. Patch clamp data were obtained from multiple independent differentiations for each line.

Supplementary Figures

Figure S1

A) Percentage of hiPSC-CMs which had measurable AP parameters after 5 minutes in 10 μM HCQ during 1 Hz pacing (grey) against those which were completely depolarized and could not be further stimulated (red).
B) Average AP data from WT2, JLNS and CALM-LQTS at baseline (grey) and after acute stimulation with 10 μM HCQ. N = 16, 22, 9. * indicates $p < 0.05$ vs Baseline.
C) Representative AP traces (top), magnification of phase 0 (middle) and first derivative of the middle panel (bottom) from the WT2 (left), the the JLNS line (middle) and the CALM-LQTS line (right) at baseline (black) and after acute stimulation with 10 μM HCQ (red).

Figure S2

Time-course of an isolated hiPSC-CM from the JLNS line paced at 1 Hz during 10 μM HCQ exposure. The insets indicate pre-exposure (left), EAD and arrhythmias during exposure (middle) and washout (right).

Figure S3

A) Raw data for HCQ effect on FPD.
B) Raw data for HCQ effect on RR.
C) Raw data for HCQ effect on cFPD. In each plot, * indicates $p < 0.05$ vs Baseline.

Figure S4

A) Arrhythmogenic events detected in JLNS hiPSC-CMs.
B) Arrhythmogenic events detected in CALM-LQTS hiPSC-CMs.