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Supplemental Information

Functional and Pharmacological Analysis of Cardiomyocytes Differentiated from Human Peripheral Blood Mononuclear-Derived Pluripotent Stem Cells

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Supplemental Data

Supplemental Figures

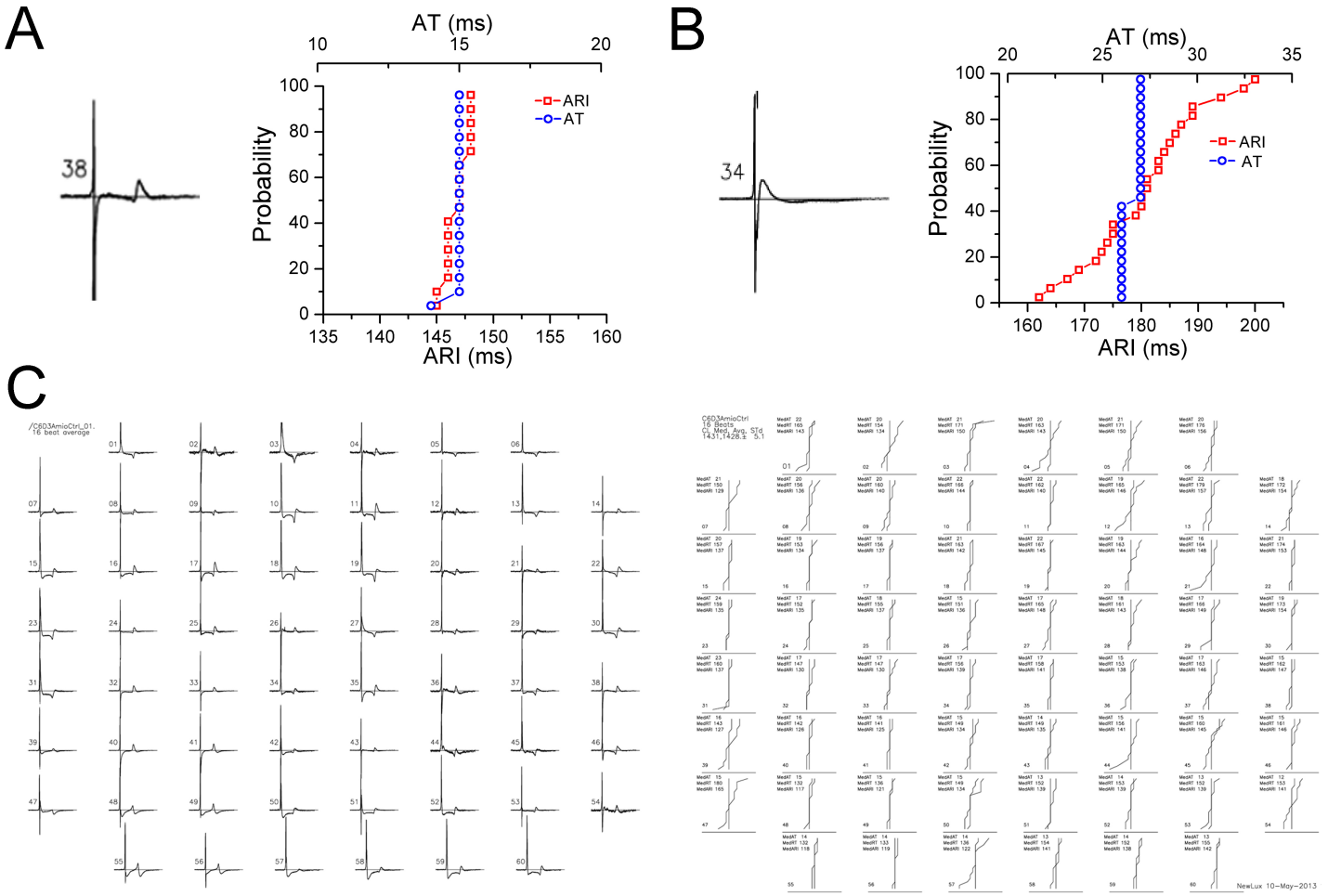

Figure S1. Methodology to assess the quality of MEA recordings, related to Figure 5.

Plots of the cumulative probability distribution functions (CPDFs) of activation times (AT) and activation-recovery intervals (ARI) for an electrogram displaying a well-defined T upstroke (**A**) and an electrogram with low amplitude repolarization waveform (**B**). Each plot shows all N measurements for each variable. The plots provide a means to reject outliers and thereby calculate average values while excluding 20% of the measurements at the extremes. **C**, Left panel shows the 60 individual MEA electrograms for a recording having excellent repolarization waveforms. The right panel shows all the CPDFs for ATs and ARIs from each electrogram. These measures were used to quality control the analyzed data and thereby improve precision.

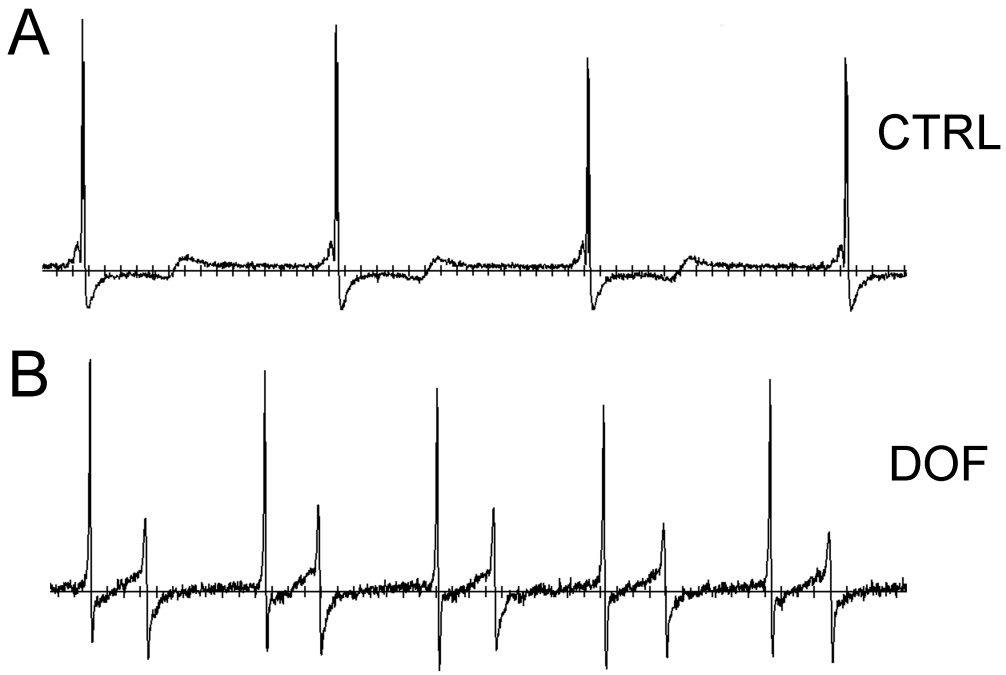

Figure S2. Early after-depolarizations induced by dofetilide, related to Figure 6. A, Baseline recording of local electrogram from the 60 MEA chamber in control solution. B, Same electrogram after perfusion with 10 nM dofetilide showing spontaneous depolarizations that emanate from repolarization time period. Each horizontal tick represents 50 ms. CTRL, control; DOF, dofetilide.

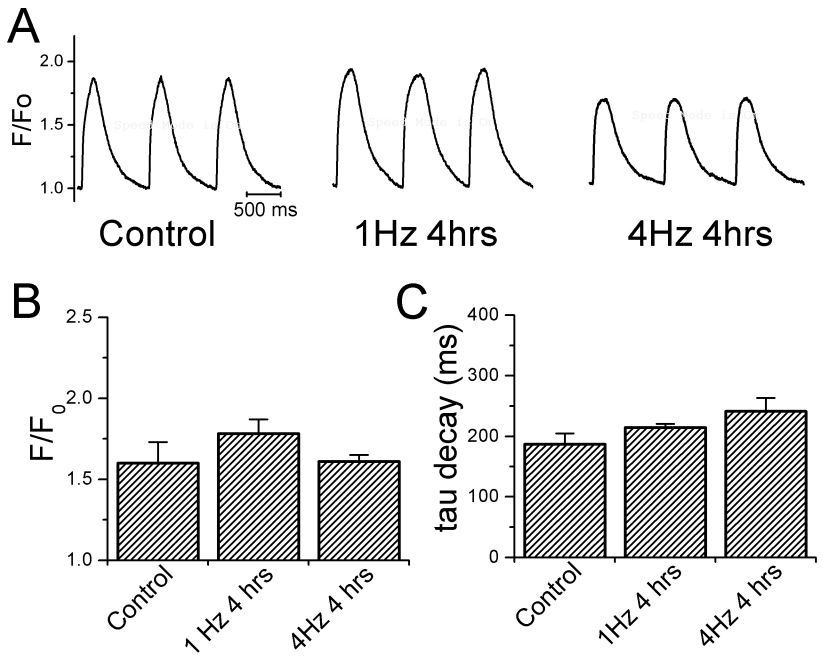

Figure S3. Rapid electrical stimulation for short periods of time (4 hrs) does not reduce Ca^{2+} transients, Related to Figure 7.

A, Representative Ca^{2+} transients recorded using fluo-4 labeled human iPSC-CM unpaced (control) and paced at 1Hz or 4 Hz for 4 hours. **B**, Bar graphs showing magnitude of Ca^{2+} transient expressed as F/F_0 and rate of transient decay (tau, ms). N=10 independent recordings, each group. Unlike rapid electrical stimulation for 8 hours, stimulation at 4 Hz for 4 hours did not alter the magnitude or rate of Ca^{2+} decay.

Supplemental Tables

Table S1. RT-qPCR primers for pluripotency gene characterizations

Hs_RRN18S_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00199367	human *18S rRNA*
Hs_SOX2_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00237601	human *SOX2*
Hs_NANOG_2_SG QuantiTect Primer Assay (200) Cat. No./ID: QT01844808	human *NANOG*
Hs_PODXL_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00005138	human *TRA-1-60*
Hs_POU5F1_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00210840	human *OCT4*

Table S2 . RT-qPCR primers for in vitro 3 germ layer characterizations

Hs_RRN18S_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00199367	human *18S rRNA*
Hs_AFP1_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00085183	human *AFP1*
Hs_GATA4_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00031997	human *GATA4*
Hs_MYH6_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00030807	human cardiac *MYH6*
Hs_T_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00062314	human *BRACHYURY*
Hs_TUBB3_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT0083713	human *β-III-TUBULIN*
Hs_VIM_1_SG QuantiTect Primer Assay (200) Cat. No./ID: QT00095795	human *VIMENTIN*

Table S3. Antibodies used in this study.

NANOG	abcam ab80892
SSEA4	abcam ab16287
OCT4	abcam ab27985
TRA-1-81	abcam ab16289
cardiac TROPONIN T	abcam ab45932
sarcomeric α-ACTININ	Sigma A7732

Supplemental Methods

Measurement of intracellular calcium

Calcium transients (CaTs) were detected in human iPSC-CMs with an epifluorescence system using the fluorescent indicator fluo-4 AM (Molecular Probes). Cells were bathed in a buffered physiological solution containing 7.5 mM fluo-4 AM for 3 minutes, then transferred to a recording chamber coated with poly-l-Lysine and continuously perfused with a physiological solution 37^{o}C. CaTs were recorded from spontaneously beating cell clusters or clusters paced at various cycle lengths using an external field stimulator. Fluorescence emission (535 ± 11 nm, band-pass filter) was collected with a photomultiplier tube via the ×40 oil objective (numerical aperture 1.3) during continuous excitation at 485 nm with a 150-W Xenon lamp. Fluorescence signals were background corrected and expressed as F/F_0 (the ratio of fluorescence during the CaT divided by diastolic fluorescence). The time course of CaT decay (tau decay) was meaured by fitting the decay phase to a monoexponential function. All measurements were performed in triplicate.