Electronic Supplementary Material (ESI) for Analyst.

Evaluate Drug-Mediated Changes in Spontaneous Beating Cardiomyocytes by AFM

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Supporting Information

Video S1. Stack images of spontaneous beating human iPSC-CMs during AFM measurements.

Video S2. Stack images of spontaneous beating mouse iPSC-CMs during AFM measurements.

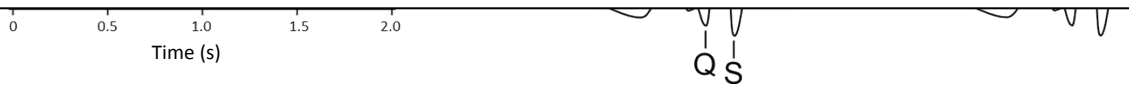

Figure S1. Representative AFM deflection vs. time plot (A) resembles a typical human ECG (B).

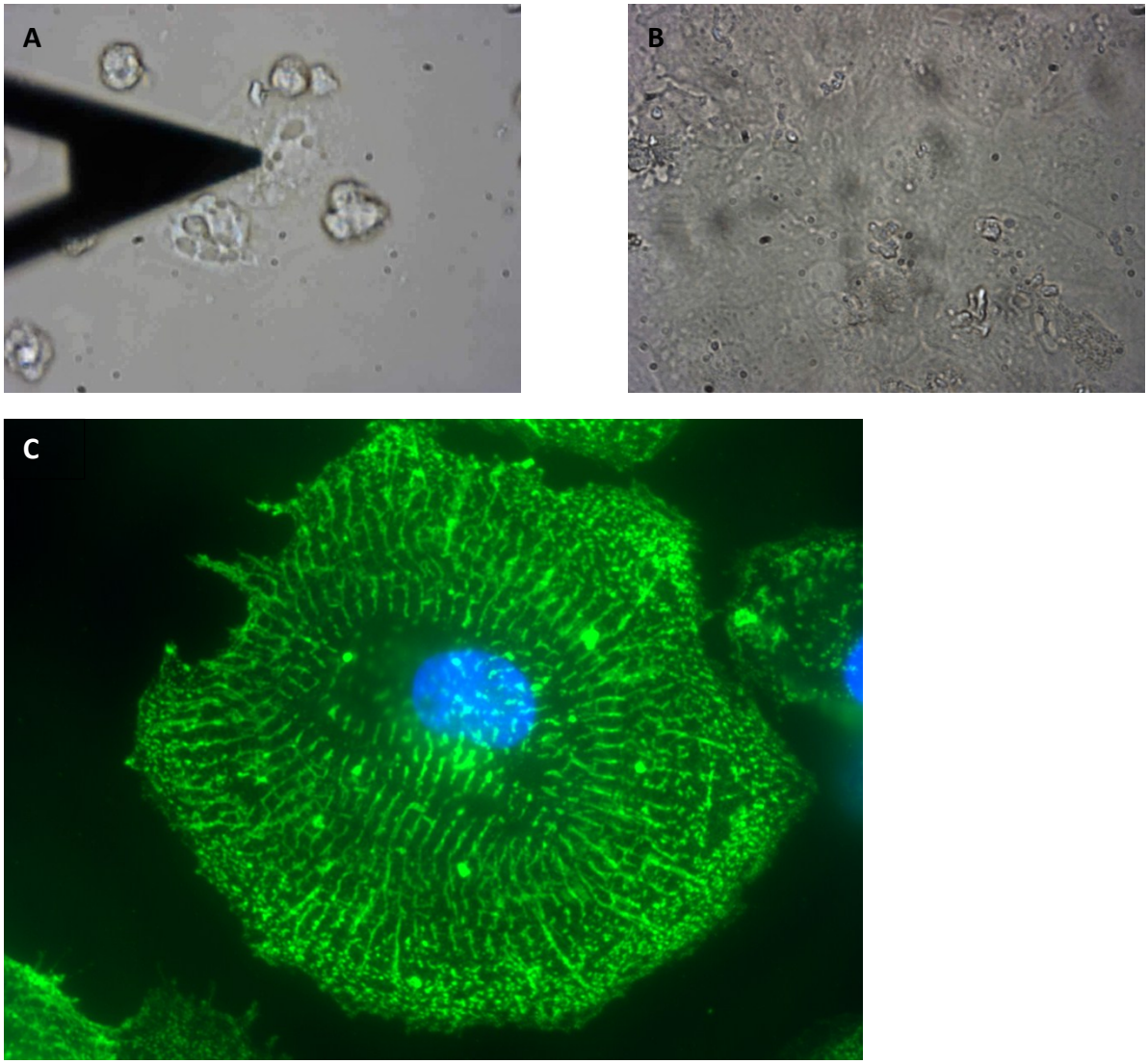

Figure S2. Bright field images of (A) individual hiPSC-CMs and (B) confluent miPSC-CMs; and Epi fluorescence image of hiPSC-CMs (C).

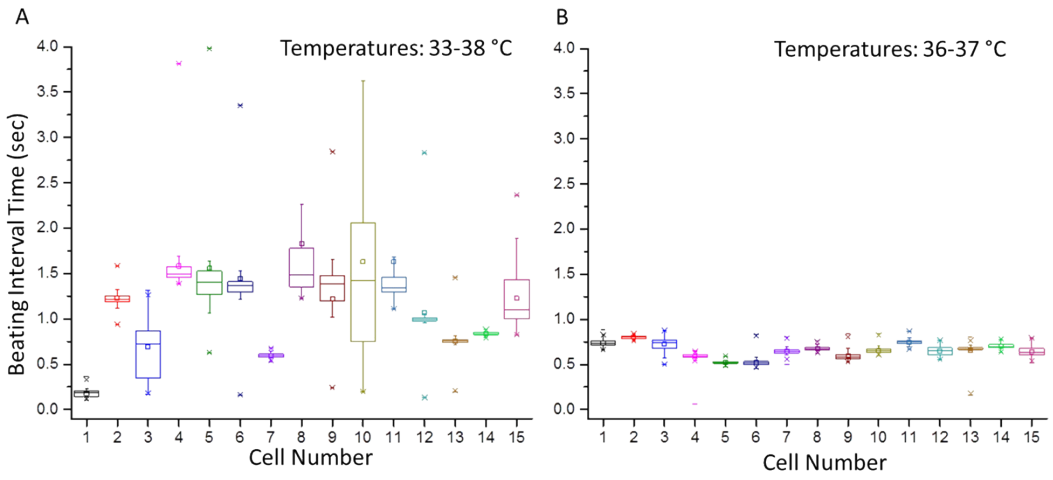

Figure S3. Box plots of beating intervals from 15 untreated murine cardiomyocytes either A) without or B) with rigorously temperature control.

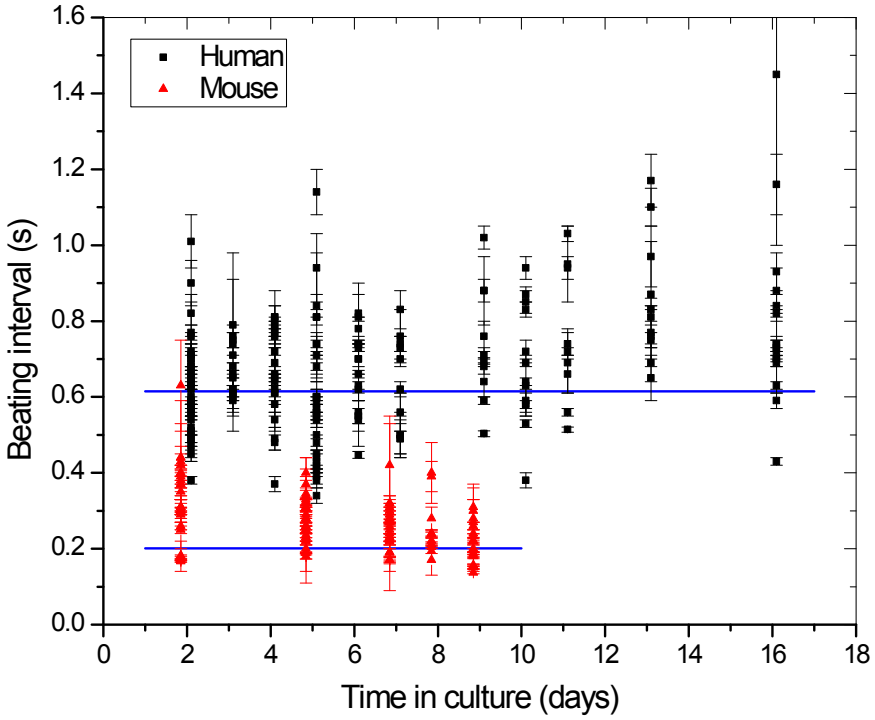

Figure S4. Weighted mean beating interval analysis.

The blue horizontal lines in Figure S4 indicated weighted mean beating interval values equal to 0.20 (0.02) and 0.61 (0.02) for the mouse and human cells, respectively, where the numbers in parentheses indicate 95% confidence intervals. Weights were $1/\sigma^2$, where σ is a standard deviation of intervals distribution for each cell. Therefore, cells with regular beating patterns thus, with narrow distribution of beating intervals, were given a higher weight. The beating intervals of mouse cells show at the 0.05 level no significantly different than zero trend as a function of time in culture. The beating intervals of human cells show at the 0.05 level a small trend significantly different than zero (slope equal to 0.0070 with the 95% confidence interval of (0.0031, 0.0108)). However, no trend significantly different than zero at the 0.05 level is detected for the human cells over the time range from day 2 through day 9, which is the time range the mouse cells were analyzed. The beating interval for human cells increases somewhat after 9 days in the culture.

Table S1. Complete rescue analysis of mESC/miPSC-CM and hiPSC-CM cells using hERG blocker E-4031. *Rescue Analysis Outline:* **Criteria I** cells exhibited wider beating interval distributions after E-4031 treatment than the untreated control, which returned to a narrower distribution (similar to the control's) after Nicorandil treatment; **Criteria II** exhibited "regular" beating patterns in the AFM deflection plots (ex. constant amplitude) during the untreated control, which became "irregular" (ex. large variation in amplitude) after E-4031 treatment, and was then returned back to a "regular" beating pattern (constant amplitude, similar to the control's) after Nicorandil treatment.

mESC/miPSC-CM E-4031 Complete Rescue Analysis			
"Full Rescue" (Criteria 1 and 2 met)	"Partial Rescue" (Criteria 1 met **only**)	"Partial Rescue" (Criteria 2 met **only**)	"No Rescue" (Criteria 1 and 2 **not** met)
2/9 (22.2%)	3/9 (24.7%)	3/9 (24.7%)	1/9 (11.1%)
hiPSC-CM E-4031 Complete Rescue Analysis			
"Full Rescue" (Criteria 1 and 2 met)	"Partial Rescue" (Criteria 1 met **only**)	"Partial Rescue" (Criteria 2 met **only**)	"No Rescue" (Criteria 1 and 2 **not** met)
5/9 (55.6%)	1/9 (11.1%)	1/9 (11.1%)	2/9 (22.2%)

Table S2. Limited rescue analysis of hiPSC-CM cells using hERG blocker E-4031, where analysis of criteria 2 (irregular deflection plot for the control) could not be performed.

hiPSC-CM E-4031 Limited Rescue Analysis (No Criteria 2 Analysis)	
"Partial Rescue" (Criteria 1 met **only**)	"No Rescue" (Criteria 1 **not** met)
5/12 (41.7%)	7/12 (58.3%)

Table S3. Limited rescue analysis of hiPSC-CM cells using hERG blocker E-4031, where the same cell before treatment could not be used (representative control was used instead).

hiPSC-CM E-4031 Limited Rescue Analysis (Representative Control)			
"Full Rescue" (Criteria 1 and 2 met)	"Partial Rescue" (Criteria 1 met **only**)	"Partial Rescue" (Criteria 2 met **only**)	"No Rescue" (Criteria 1 and 2 **not** met)
8/32 (25.0%)	13/32 (40.6%)	3/32 (9.4%)	8/32 (25.0%)

Table S4. Limited rescue analysis of hiPSC-CMs cells using hERG blocker Sotalol, where analysis of criteria 2 could not be performed.

hiPSC-CM Sotalol Limited Rescue Analysis (No Criteria 2 Analysis)			
Sotalol 10 μM		Sotalol 100 μM	
"Partial Rescue" (Criteria 1 met **only**)	"No Rescue" (Criteria 1 **not** met)	"Partial Rescue" (Criteria 1 met **only**)	"No Rescue" (Criteria 1 **not** met)
6/9 (66.7%)	3/9 (33.3%)	5/6 (83.3%)	1/6 (16.7%)