

**JOURNAL:** *Scientific Reports-Nature Publishing Group*

**TITLE:** hiPSC-CM Monolayer Maturation State Determines Drug Responsiveness in High Throughput Pro-Arrhythmia Screen

**RUNNING TITLE:** Mature hiPSC-CMs for pre-clinical pro-arrhythmia screening

**AUTHOR INFORMATION**

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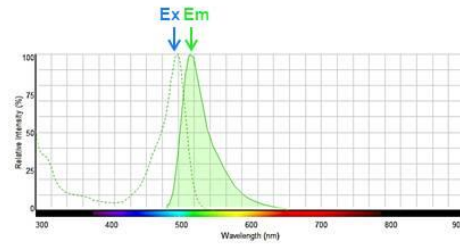
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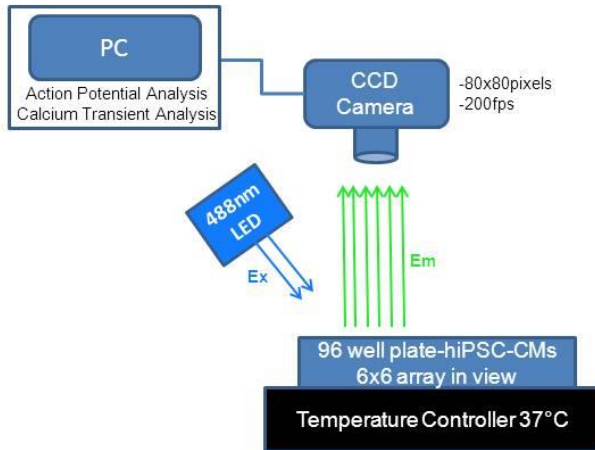
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## Supplemental figures and legends

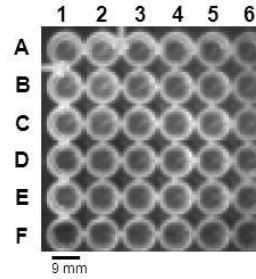
A, FluoVolt™ Membrane Potential Dye Spectra:



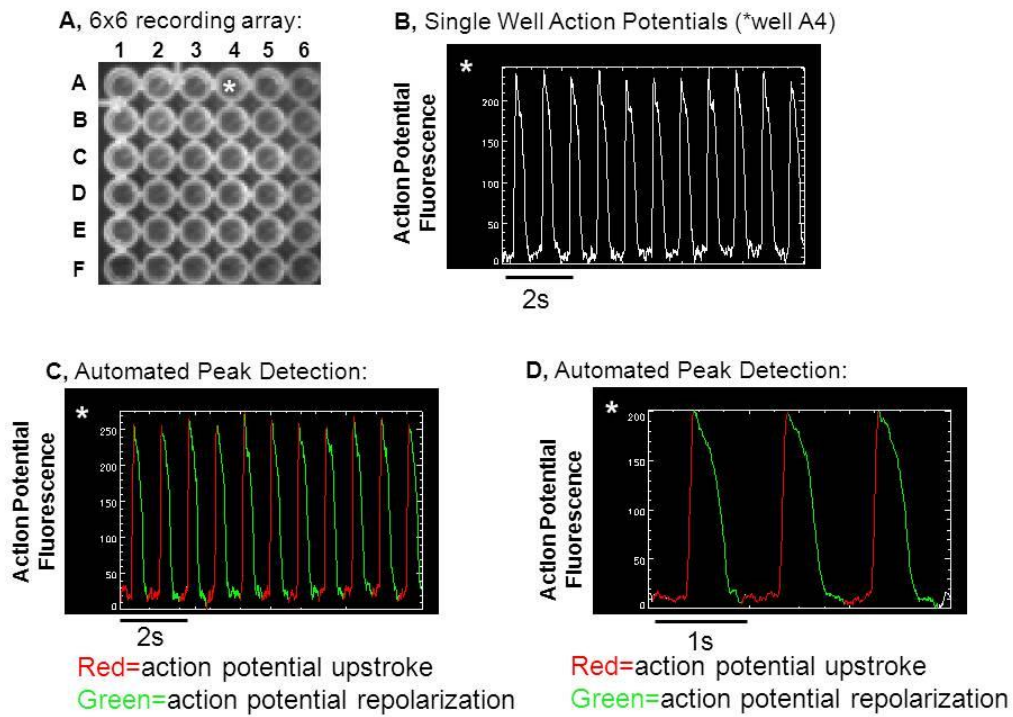
B, HTS Experimental set up:



C, 6x6 recording array:

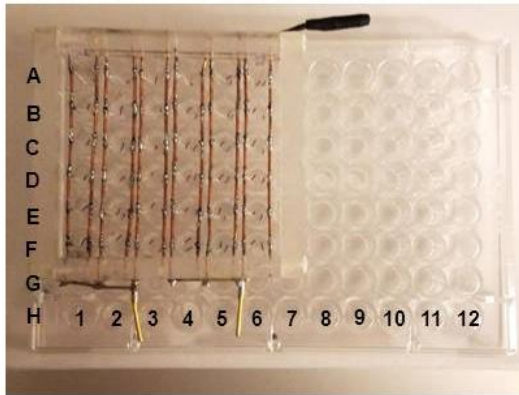


**Supplemental figure 1.** Configuration for 96 well plate pro-arrhythmia HTS. This platform was used for experiments to compare fetal hiPSC-CM monolayers to mature hiPSC-CM monolayers. Each plate was manually translated into the field of view to enable interrogation of the entire 96 well plate.

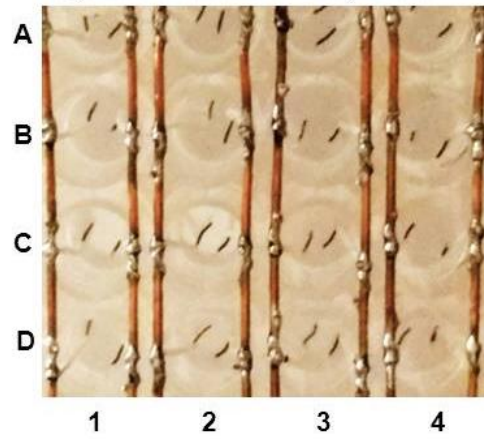


**Supplemental figure 2.** Examples of data acquired and analysis of action potential recordings. **A**, 6x6 array visualized. **B**, 10s recording of spontaneous action potentials from well A4. **C&D**, Automated peak detection using custom software.

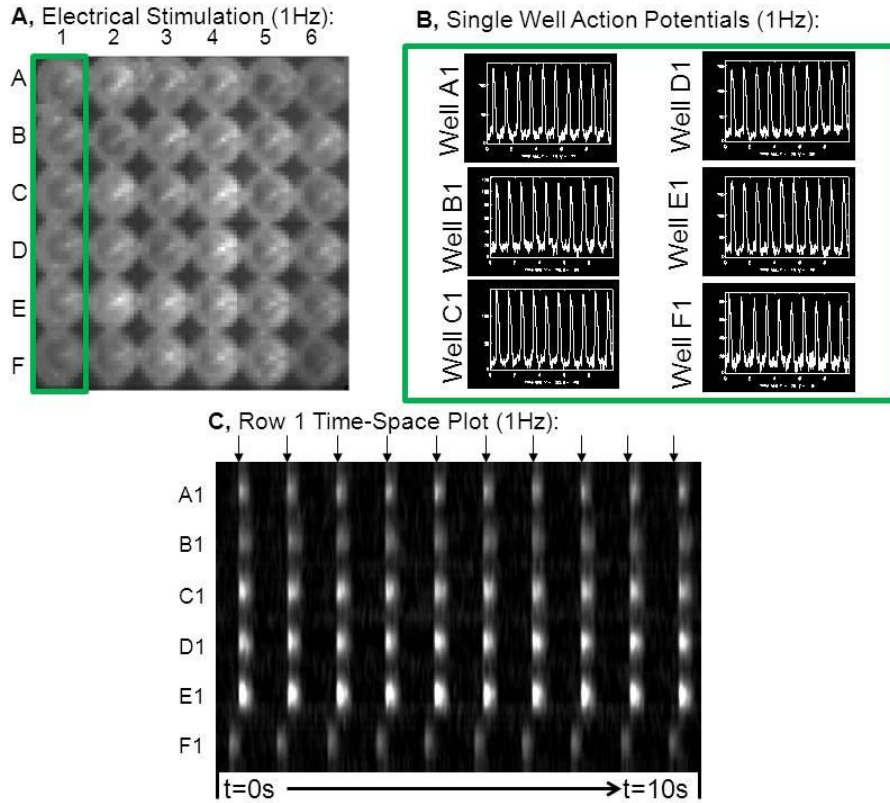
**A**, 96 well plate with 6x6 field stimulation array:



**B**, Field stimulation array zoom:

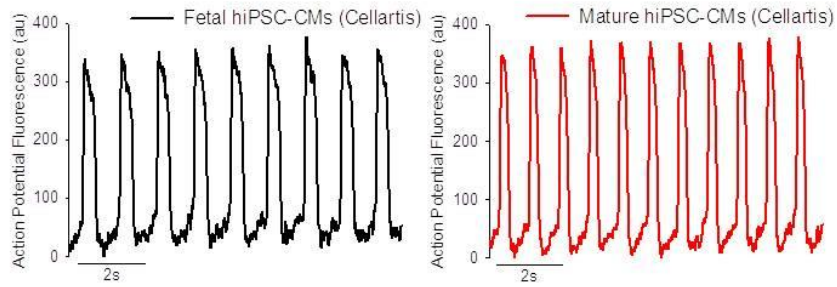


**Supplemental figure 3.** Custom made field stimulation frame for electrical pacing of 6x6 array of hiPSC-CM monolayers.

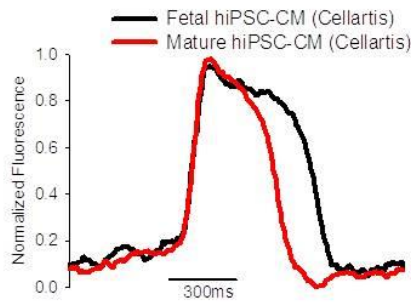


**Supplemental figure 4.** Example traces of synchronized (1Hz) hiPSC-CM monolayers using electrical pacing frame shown in supplemental figure 3. **A&B**, Representative traces collected for column 1 of this 6x6 array. Single pixel recordings of 10s movie (1Hz pacing) from each well of column 1 show synchronization of activation. **C**, Time space plots for column 1 also show synchronization of activations. arrows indicate electrical stimulation.

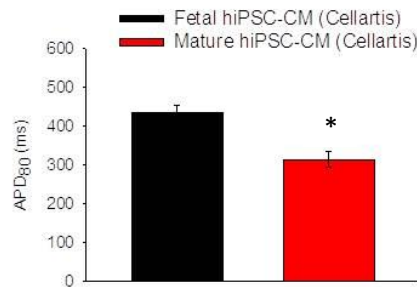
**A, Spontaneous Activations:**



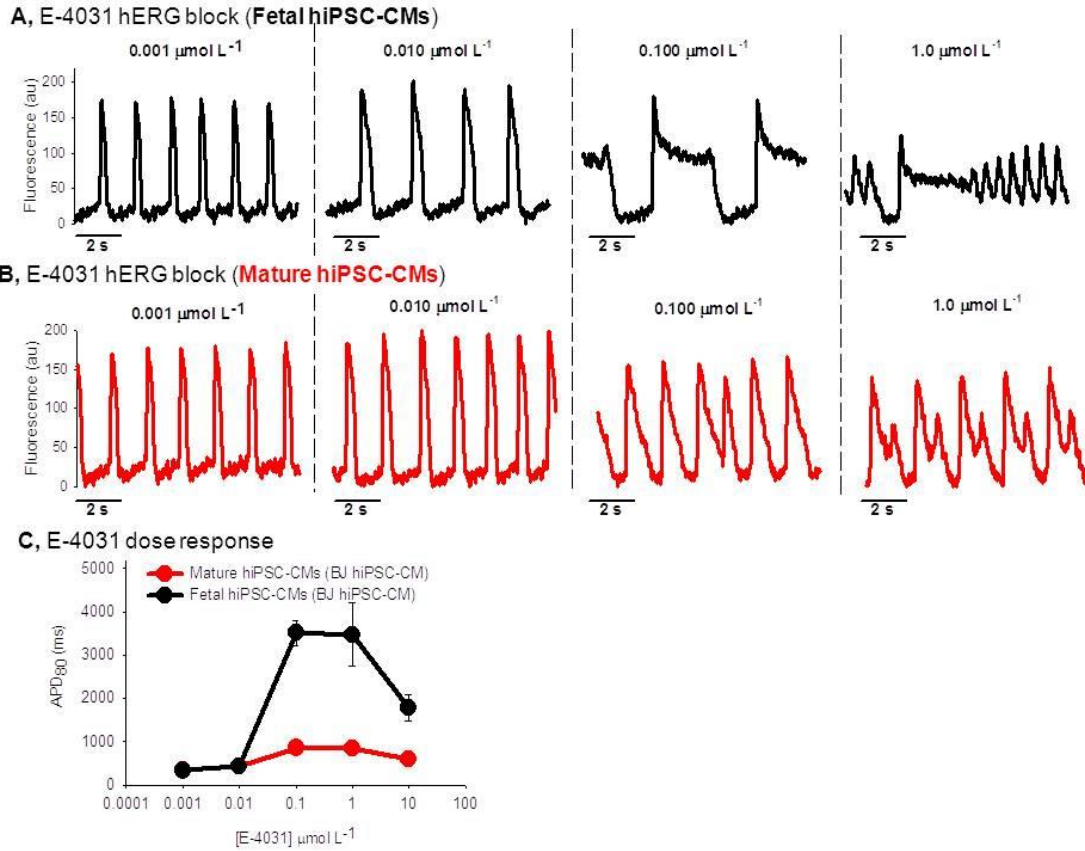
**B, Spontaneous Activation:**



**C, Action Potential Duration:**

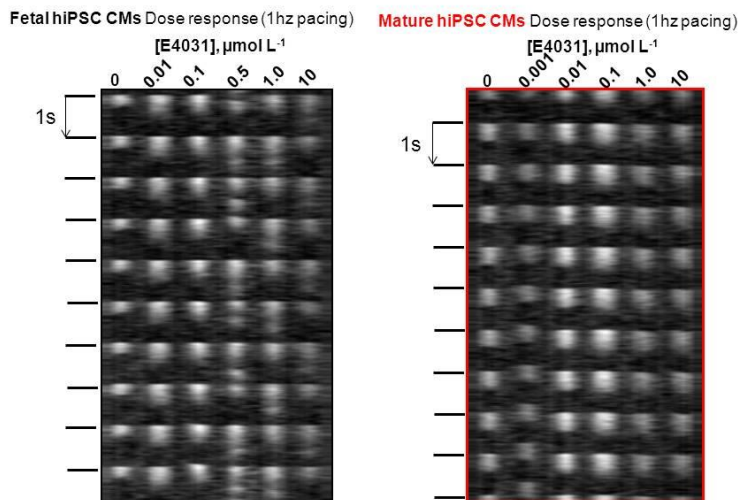


**Supplemental figure 5.** Maturation of Cellartis commercially available hiPSC-CMs. These are unpurified hiPSC-CM monolayers. **A**, Spontaneous activations of fetal and mature monolayers. **B**, Overlay of spontaneous action potentials shows difference of action potential duration. **C**, APD<sub>80</sub> is significantly abbreviated in mature hiPSC-CM monolayers; \* $P < 0.01$ , unpaired t-test.

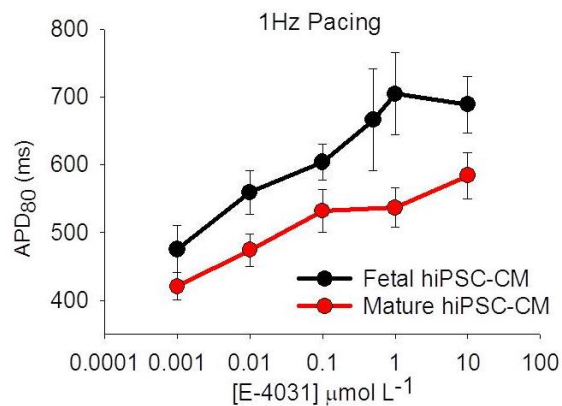


**Supplemental figure 6.** E-4031 effect in BJ hiPSC-CM monolayers generated using the small molecule approach with magnetic activated cell sorting (MACS) based cardiomyocyte purification. **A&B**, original recordings of action potentials over various doses in fetal (A, black) or mature hiPSC-CM monolayers (B, red). **C**, dose response shows divergent effects of E-4031 in fetal (black) compared to mature monolayers (red).

### A, Time space plots

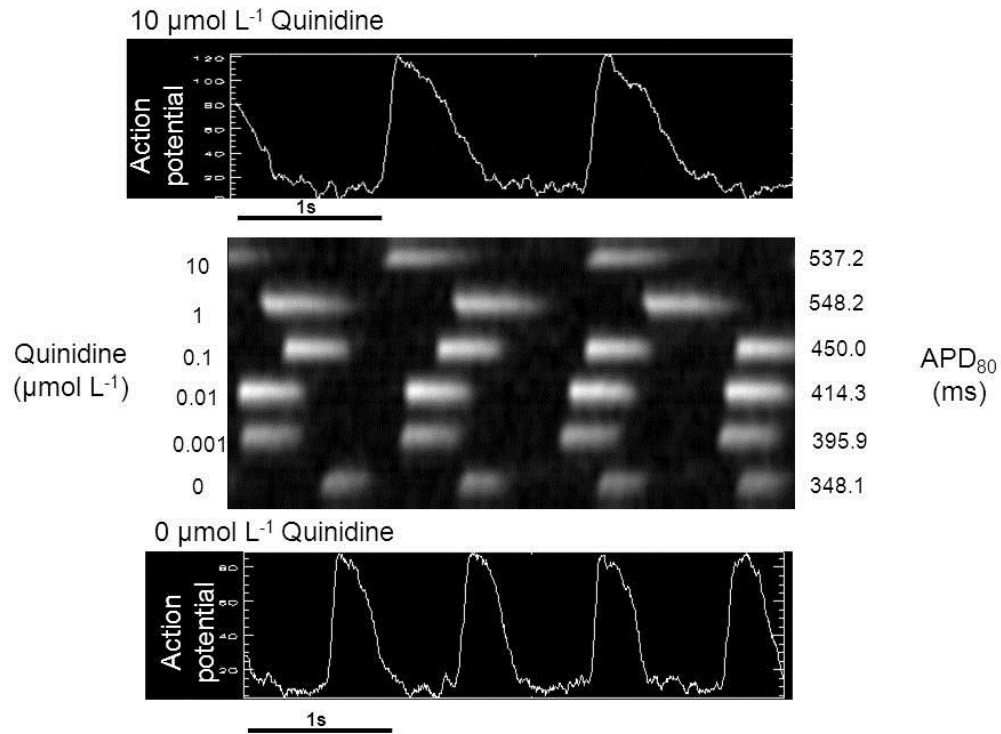


### B, Dose Response

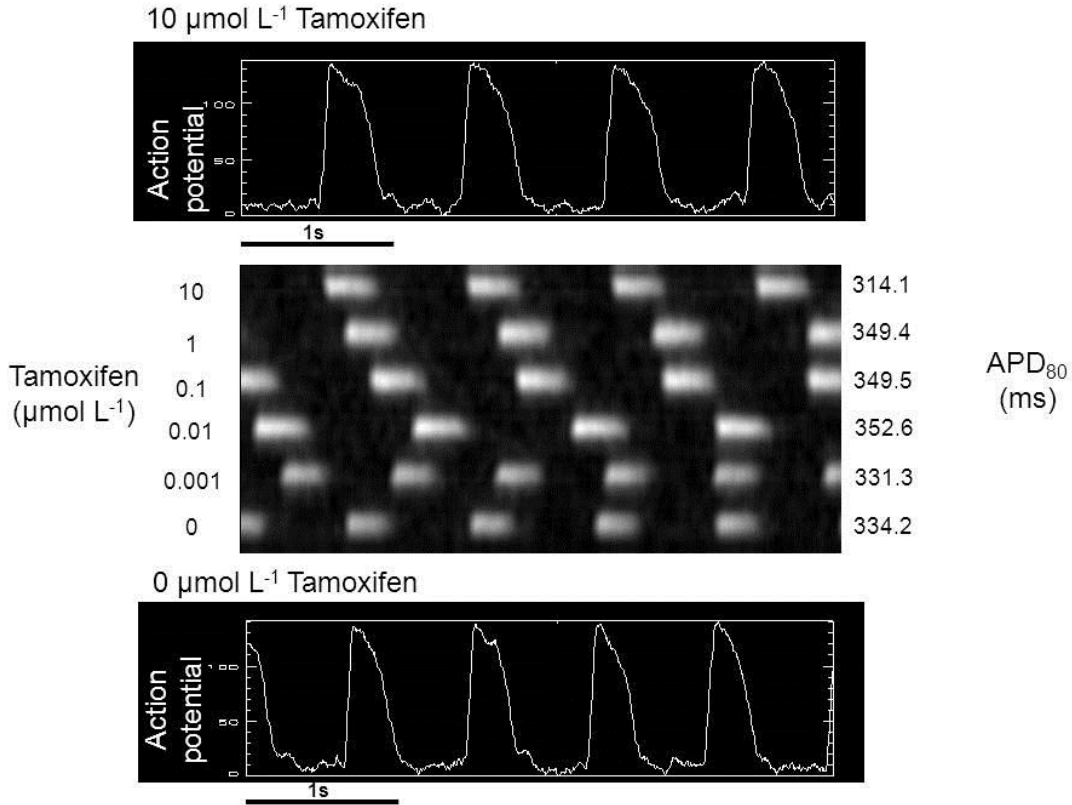


**Supplemental figure 7. E-4031 effects in electrically paced iCell hiPSC-CM monolayers. A,** Time space plots of a single row across the full range of E-4031 concentrations tested. **B,** Dose response shows that the fetal hiPSC-CM monolayers (black) have longer duration APD<sub>80</sub> compared to mature (red) monolayers when pacing frequency is matched (1Hz).

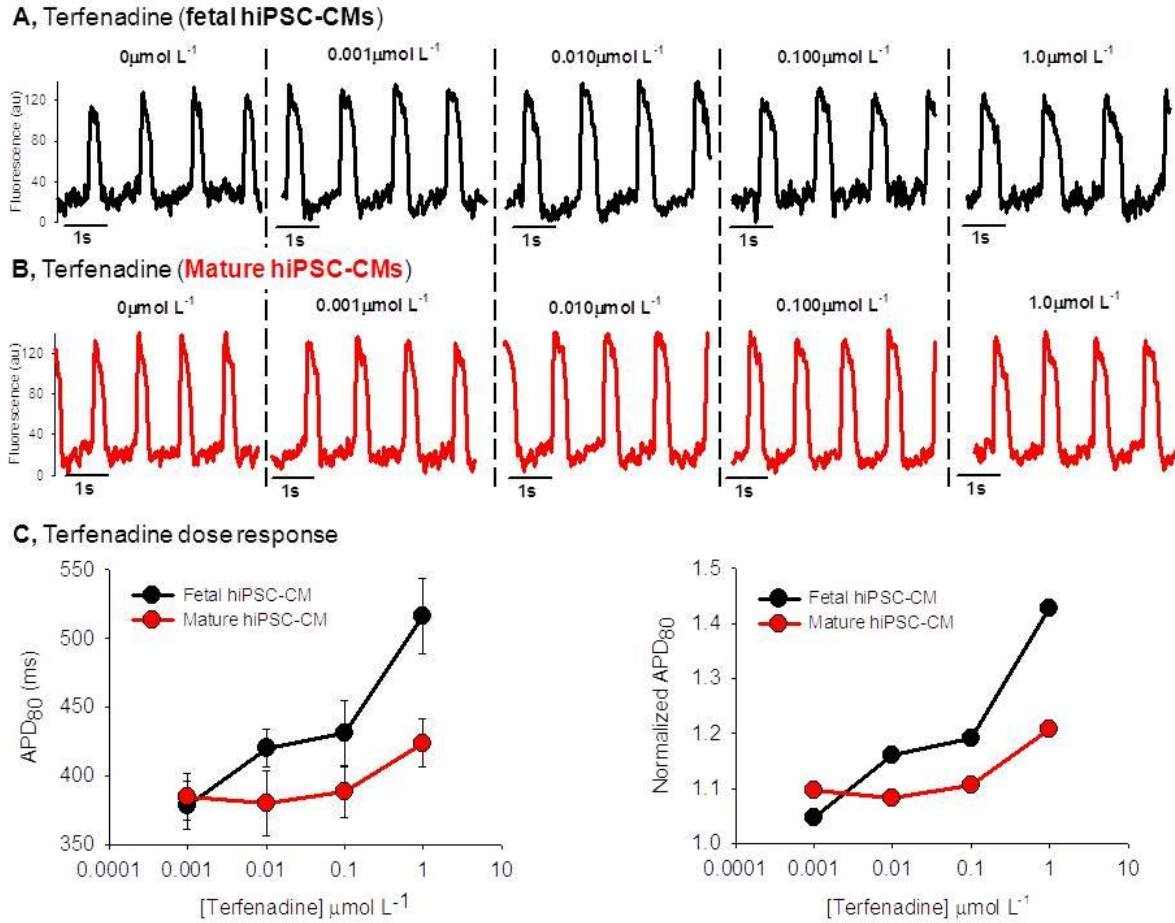




**Supplemental figure 8. Validation of platform using quinidine.** In mature hiPSC-CM monolayers quinidine (a high risk compound) caused prolongation of the APD in a dose dependent way-as expected. Time 0 is to the left and the single pixel recordings are time matched with the time space plots.



**Supplemental figure 9. Validation of platform using tamoxifen.** In mature hiPSC-CM monolayers tamoxifen (a low risk compound) did not prolong the APD at any concentration used. Time space plot shows spontaneous action potentials.



**Supplemental figure 10. Dose response for terfenadine.** A&B are original recordings of spontaneous action potentials. C, Dose response for fetal (black) and mature (red) hiPSC-CM monolayers.