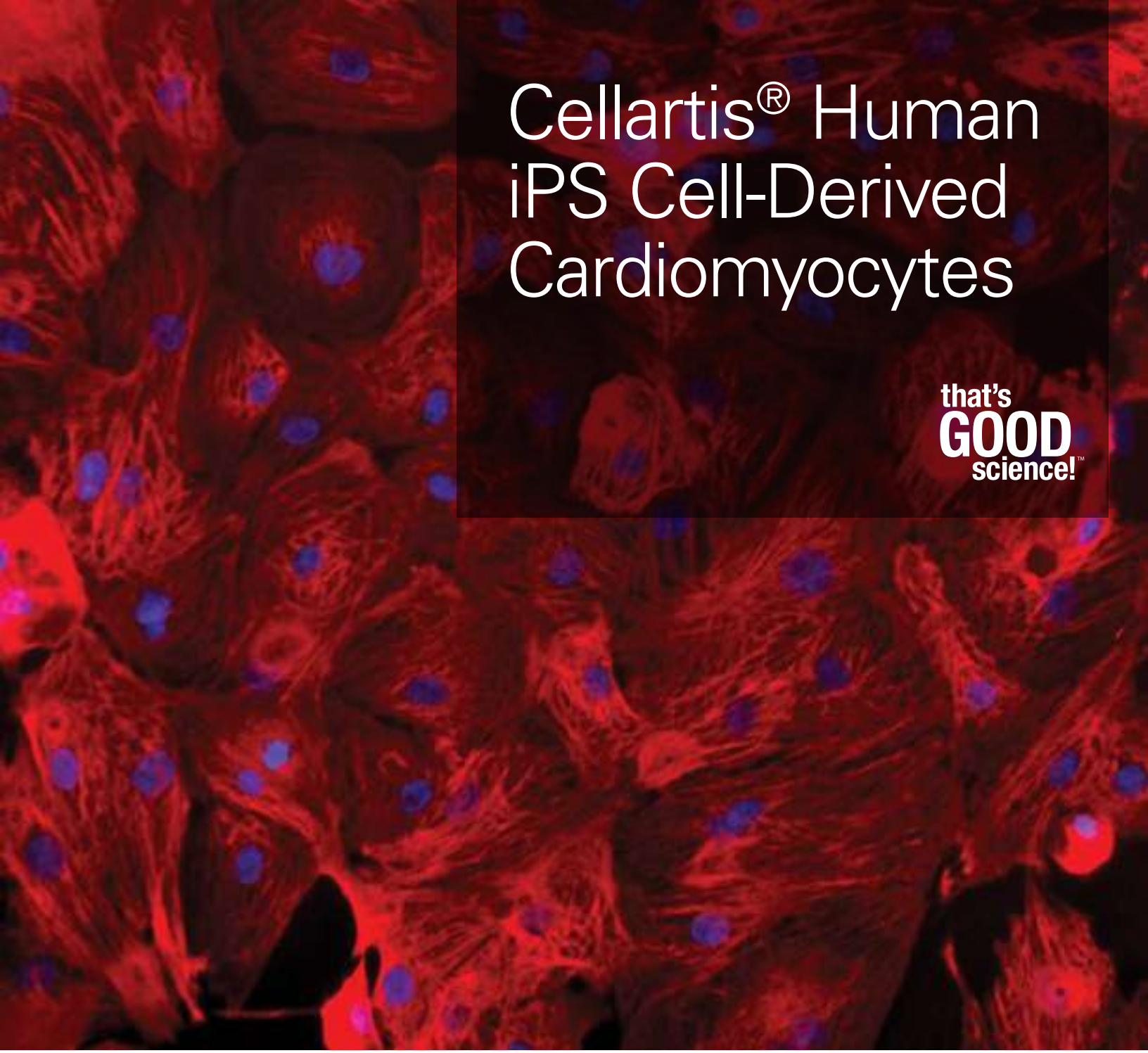


Cellartis® Human iPS Cell-Derived Cardiomyocytes

that's GOOD science!™

TaKaRa

Clontech TaKaRa cellartis

About us

With more than 15 years of experience in stem cell generation and differentiation, spanning the development of this field, Takara Bio has developed the dedicated portfolio of Cellartis stem cell products. The company was first in the world to bring to the market human embryonic stem cell-derived cardiomyocytes, which were launched in 2008.

Human cardiomyocytes derived from induced pluripotent stem cells

An effective tool for *in vitro* evaluation of cardiotoxicity, drug discovery, and basic research

Cellartis cardiomyocytes are spontaneously beating cardiomyocytes derived from human induced pluripotent stem cells. The cells express the major cardiac markers and ion channels, are functionally similar to adult human cardiomyocytes, and exhibit the expected responses to cardiac stimuli, making them excellent *in vitro* tools for studies of human cardiomyocyte function and for cardiac safety pharmacology assays. The cardiomyocytes have been dissociated into a single-cell suspension and frozen in vials for convenient use in downstream applications, providing accurate and reproducible data acquisition.

Features

- Expression of major cardiac markers and ion channels
- Physiological response to cardiac stimuli
- Generated using a standardized protocol that mimics human cardiac developmental pathways
    - No genetic engineering or purification/selection procedure
    - High yield (>80% cardiomyocytes)
    - About 20% supporting cells contributing to more physiological relevance
- Produced in consistent, quality-controlled batches
- 3D compatible
- Large proportion of the cells express calcium and sodium currents

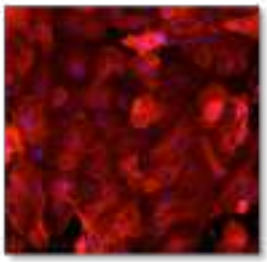


Figure 1. ICC staining of Cellartis cardiomyocytes (from ChiPSC22).

Red = cTroponin, Blue = DAPI (20x magnification).

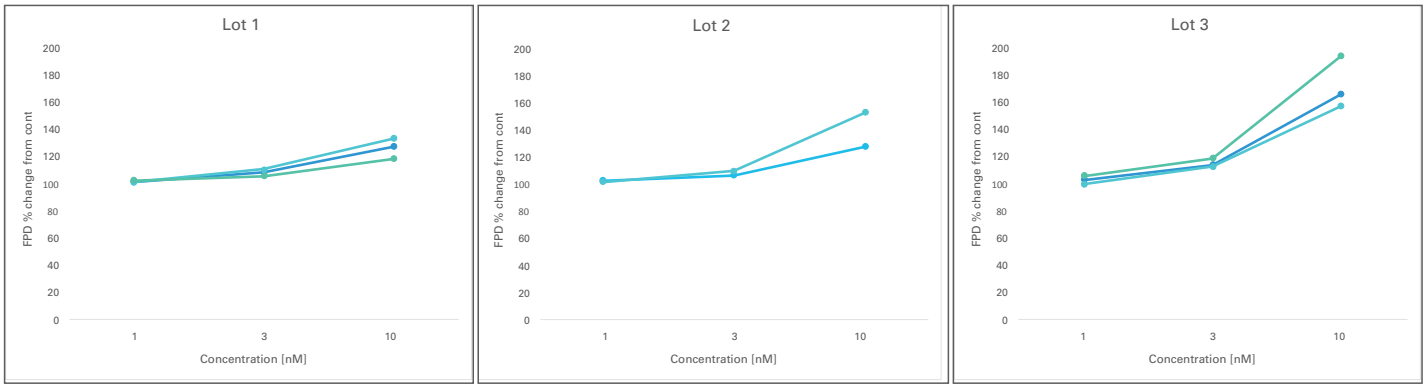
Cardiac markers and ion channels

Cellartis cardiomyocytes express major cardiac markers and ion channels.

Characteristic	Assay	Analyte
Protein markers	ICC	e.g. c-Troponin, Actin, Myomesin
Gene Expression	qPCR	e.g. MLC2V, ACTC1, TNNT2, MYH6, Connexin 43, HCN4, SERCA2, KCNa5, SCN5A, KCNIP2, KCNE2

Produced in consistent, quality-controlled batches

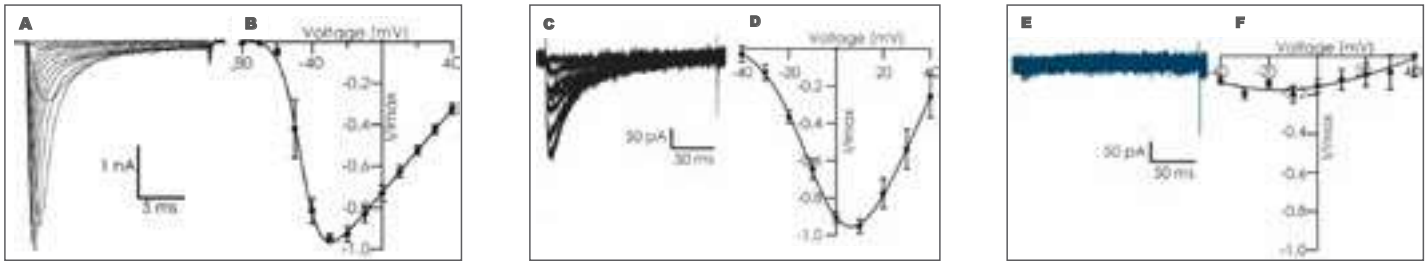
Reproducible behavior of three lots of Cellartis cardiomyocytes in response to E-4031(MED64 system; Alpha MED Scientific)



Field potential duration (FPD) was increased in a dose-dependent manner with E-4031 administration. EADs, typically, preceding TdP, were observed at an E-4031 concentration of 30 nM and higher. These results were reproducible across all lots tested. Each color indicates a measurement from a different channel of the MEA probe.

Physiologically relevant NaIV and CaIV

Evaluation of NaIV and CaIV in Cellartis cardiomyocytes (Patchliner, Nanion)



NaIV (A) and IV plot (B) in control, n = 7. The NaIV was fit using a Boltzmann equation which revealed a Vhalf of activation of –46 mV. This is in excellent agreement with the cardiac NaV channel, NaV1.5 (also known as h1), expressed in tsA-201 cells (see Catterall *et al*, 2005, Pharmacological Reviews, Vol. 57, No.4 pp. 397–409; Li *et al*, 2002, Molecular Pharmacology, Vol. 61, No. 1, pp. 136–141).

CaIV (C) and IV plot (D) in control, n = 18. The CaIV was also fit using a Boltzmann equation which revealed a Vhalf of activation of –5.8 mV. This value agrees well with e.g. Benitah *et al*, 1992, Pflügers Arch, Vol. 421, pp. 176–187 (–7.8 mV) recording human ventricular cells and Li *et al*, 1999, Am. J. Physiol. Heart. Circ. Physiol. Vol 276:H98–H106 (–4.8 mV) from human ventricular myocytes.

CaIV (E) and IV plot (F) in 10 µM nifedipine, n = 5. Complete block.

Large proportion of the cells express calcium and sodium currents

Statistics of NaV and CaV distribution in Cellartis cardiomyocytes (Patchliner, Nanion)

Table 1	Capture rate (%)	% cells with NaV (>–50 pA)	% cells with CaV (>–50 pA)
	58 (28/48)	71 (20/28)	68 (19/28)

Table 2	RSeal (MOhm)	Cm (pF)	Rs (MOhm)	INa at –30 mV (nA)	ICa at 10 mV (pA)
	976 ± 144 (28)	37 ± 6 (28)	6.0 ± 0.9 (28)	–5.4 ± 1.5 (7)	–157 ± 24 (18)

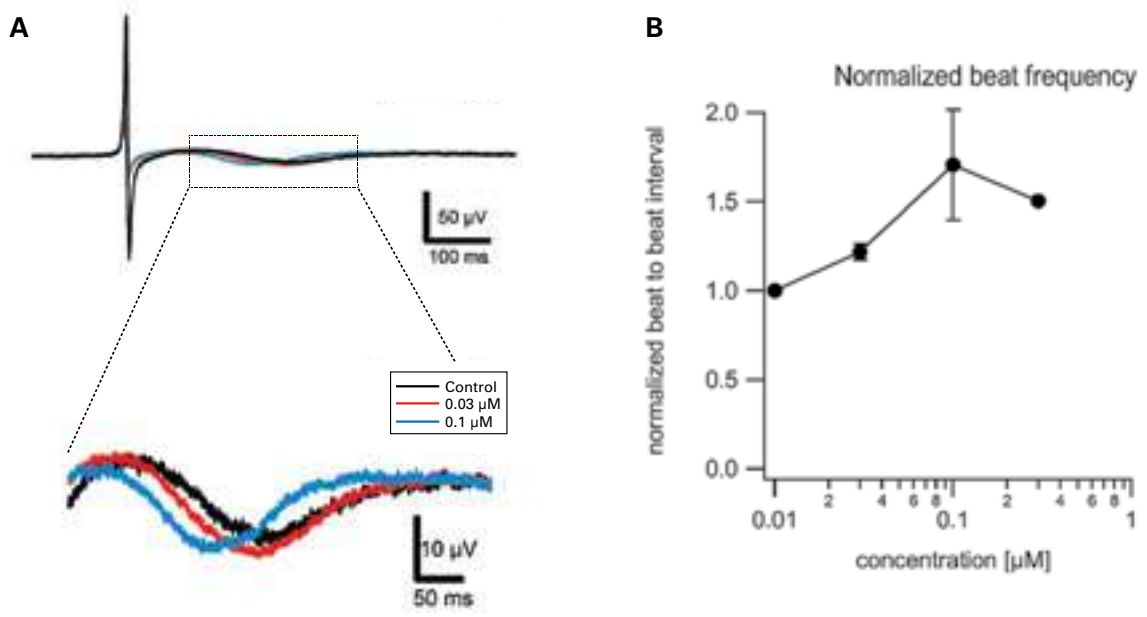
Table 1: Success rates for cell capture and cells expressing NaV and CaV currents. 6 experiments were performed using a total of 3 chips, therefore 48 potential sites on the chip were available and 28 cells were captured (with seal resistance >150 MOhm) resulting in a success rate for 58% for capture. Of the cells captured, 71% showed NaV current >–50 pA and 68% showed CaV current >–50 pA.

Table 2: Average values for seal resistance (RSeal), cell capacitance (Cm) and series resistance (Rs) captured with seal resistance >150 MOhm. NaV current at –30 mV and CaV current at 10 mV is also shown. Number of cells shown in brackets.

Physiologically relevant responses to compounds in various systems

Multiwell MEA System (Multi Channel System)

Cellartis cardiomyocytes display expected response to the L-type Ca^{2+} channel blocker Nifedipine.

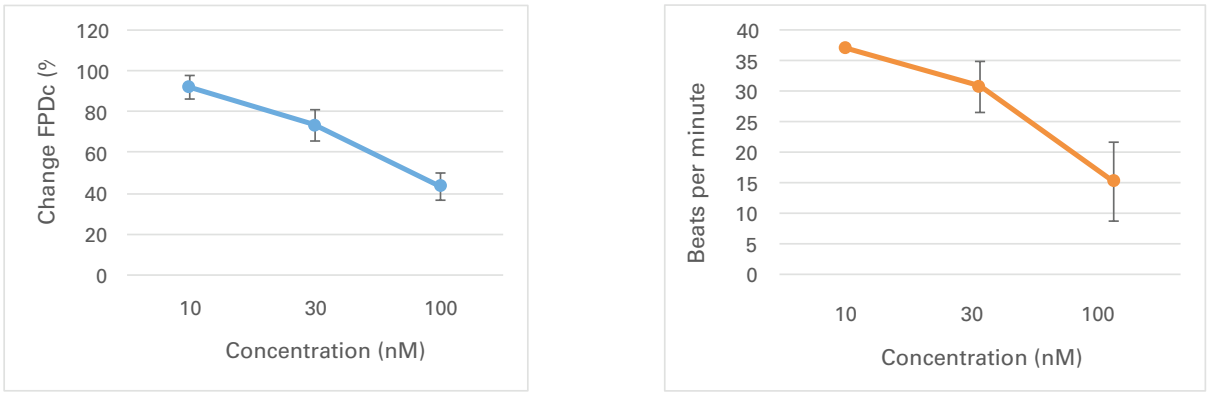


Panel A. Superposition of averaged fAP traces at different concentrations of Nifedipine with magnification of the repolarization component at the end of the fAP. Note the concentration-dependent left shift of the repolarization resulting in a shortening of the fAP.

Panel B. Normalized beat frequency. The beat to beat interval was prolonged with increasing concentrations of Nifedipine resulting in an expected decreased beat frequency. Each data point represents the average of n = 8 data points. Error bars indicate SEM.

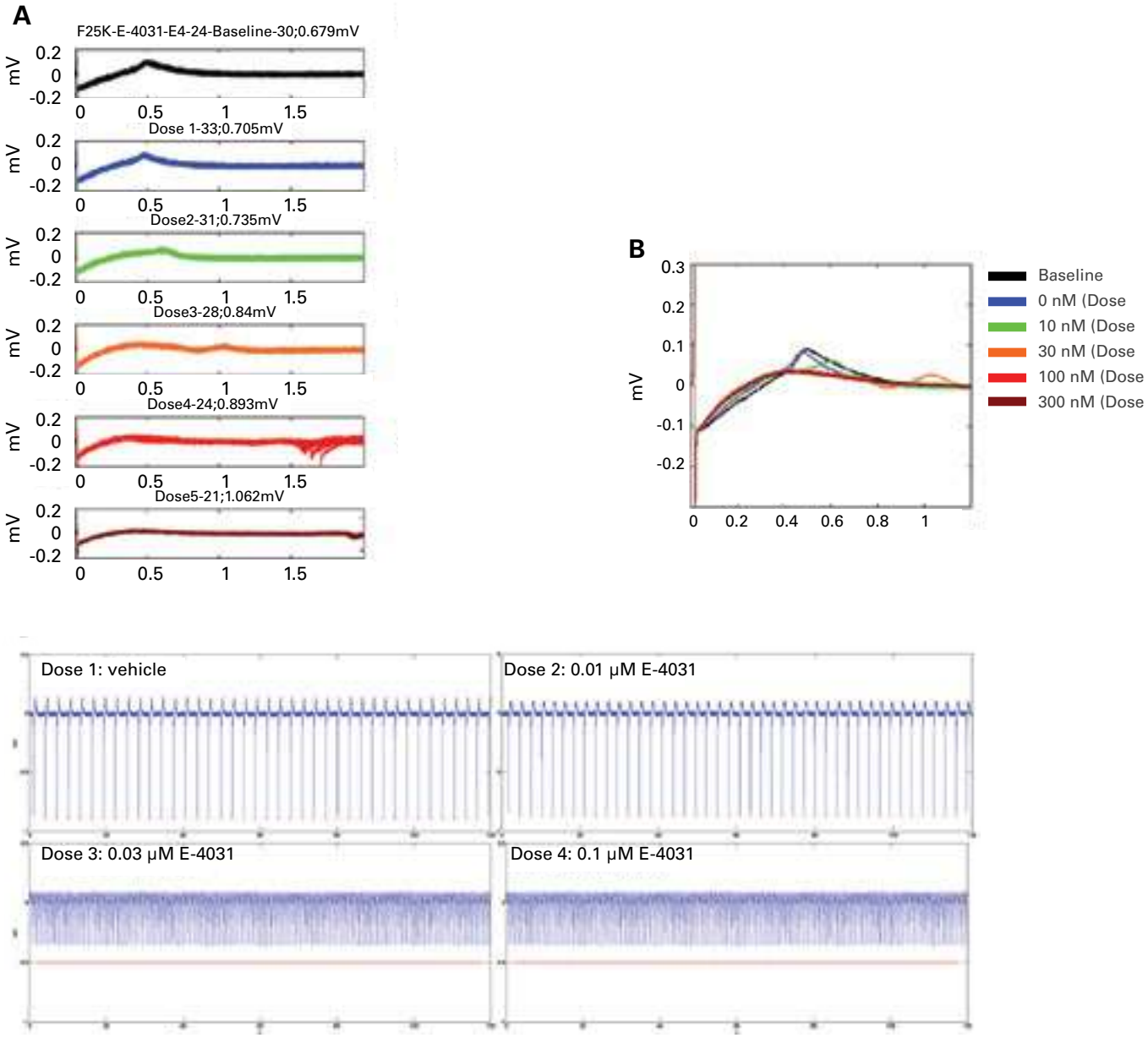
MED64 system (Alpha MED Scientific)

Physiological FPD shortening and beat rate decreases with Verapamil.



FPD (left) and beat rate (right) at different concentrations of Verapamil. Verapamil inhibits hERG and L-type Ca^{2+} channels at overlapping concentrations and is known to shorten FPD and decrease beat rate *in vivo*. These expected effects of Verapamil were reproduced *in vitro* in dose-dependent manner, suggesting high physiological relevance of the cells. n = 3; error bars indicate SEM.

Electrical activity of Cellartis cardiomyocytes exposed to hERG blocker E-4031



Panel A. Sodium spikes. Overlay of all detected sodium spikes during a two-minute recording from a single electrode. Sodium spikes after were temporally aligned at time zero to emphasize the subsequent electrical activity. Asterisk indicate triggered activity defined as sodium spike activity of low amplitude that follows the repolarizing T wave.

Panel B. Average of the overlaid records. Average of the overlaid records shown in A (same color code) to emphasize the changes on the field potential duration.

Panel C. Tachycardic response. Example of high-frequency tachycardic response in Matlab generated figures. Red dots indicate detection events.

xCELLigence RTCA CardioECR instrument (ACEA)

Impedance and Field Potential recordings of Cellartis cardiomyocytes exposed to Moxifloxacin

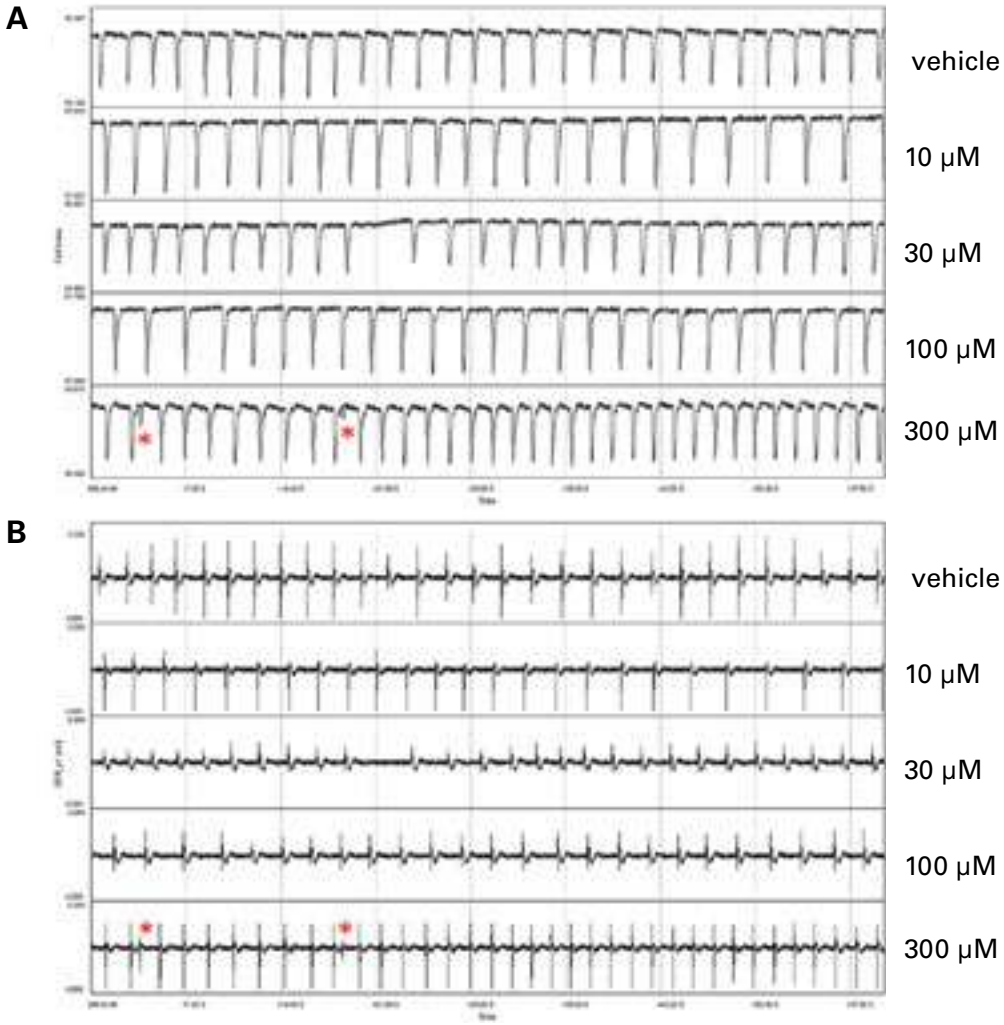
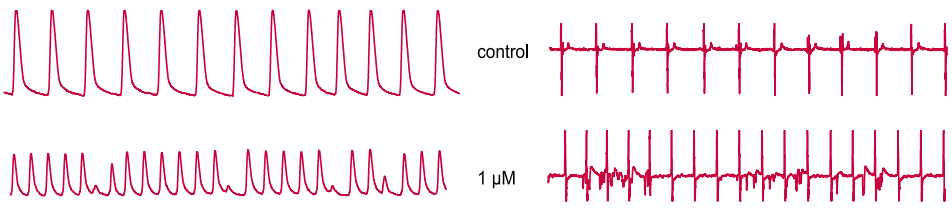


Table 1:

Moxifloxacin treatment	Time of exposure			
	Baseline	2 hours	5 hours	24 hours
10 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
30 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
100 µM	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)
300 µM	0 (0/4)	0 (0/4)	1.3 (1/4)	1.3 (1/4)

Impedance (A), Field Potential (B) and Averaged Irregular Beat Rate (Table 1) at different concentrations of Moxifloxacin. Impedance and Field Potential recordings after 5 hours' exposure to vehicle and increased moxifloxacin concentrations. Asterisks indicate ectopic beats due to triggered activity. Averaged Irregular Beat rate was calculated as [Number of irregular beats/(Number of irregular beats + number or regular beats) x 100]. Number in parenthesis indicate the number of wells with proarrhythmic activity to the total number of wells in that condition. Cellartis cardiomyocytes respond to moxifloxacin with prolongation of the FPD and proarrhythmic markers only at the highest concentration tested consistent with the clinical observations.

Detection of EADs after Sotalol administration



Representative Impedance (left) and Electrical Field Potential (right) traces after treatment of Cellartis cardiomyocytes with hERG blocker Sotalol. The cells showed sensitivity to Sotalol and produced EADs at 1 µM.

Takara Bio and the CiPA initiative

The objective of the Comprehensive *in vitro* Proarrhythmic Assay (CiPA) initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of hERG block and not at all by QT prolongation. The new CIPA paradigm will be driven by a suite of mechanistically based *in vitro* assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes.

Takara Bio is actively supporting the CiPA initiative to improve the current regulatory guidance for preclinical cardiac safety assessment. Takara Bio is participating in the CiPA Myocyte Subteam and has played an important role in establishing the minimum criteria to which stem cell cardiomyocyte providers and cardiomyocytes must adhere in order to be considered for use in the CIPA validation study.

Takara Bio is a reliable provider of clinically relevant human iPS cell-derived cardiomyocytes. Cellartis cardiomyocytes are currently supplied to participating sites globally for use in the CiPA validation study.

PRODUCTS

Cat. #	Product	Size
Y10075	Cellartis Cardiomyocytes (from ChiPSC22) Kit	1 kit (>3M viable cells/vial)
Y10062	Cellartis CM Thawing Base	32 ml
Y10063	Cellartis CM Culture Base	90 ml

Partnering for Your Success

We are committed to developing and providing optimal tools that are tailored to your projects. To ensure your success, our Cellartis Human Pluripotent Stem Cell Services team delivers professional expertise through close collaboration.

that's GOOD science!

Generating clinical-grade human pluripotent stem cell lines? We can help!

Derivation of proprietary, clinical-grade hES cell lines from blastocysts, or hiPS cell lines from blood samples includes:

- Project report including all relevant documents, QA and QC package
- Seed Bank(s) of up to three customer-proprietary donor hPS cell lines
- Master Cell Bank of one customer-proprietary hPS cell line with a project-specific QC

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