SUPPLEMENTAL MATERIAL

Thyroid and Glucocorticoid Hormones Promote Functional T-tubule Development in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

Short Title: T3+Dex generates functional t-tubules in hiPSC-CM

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Detailed Methods

HiPSC Culture and Maintenance

Human induced pluripotent stem cells (hiPSC) were generated using fibroblasts isolated from dermal punch biopsies. These cells were acquired from two consenting healthy individuals (one male, one female) and reprogrammed to hiPSC using a non-integrating episomal based reprogramming approach. In brief, Epi5™ episomal oriP/EBNA1 vectors (Thermo) containing 5 reprograming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc) were electroporated into fibroblasts using the Thermo NEON® Transfection System as per manufacturer's guidelines. Single colonies were manually picked and clonally expanded. Clones were subjected to a battery of analyses (data not shown) for verification of stemness and genomic stability as per including karyotyping, embryoid body differentiation, and immunostaining (Tra1-60, SSEA4, SSEA5, and Oct4). HiPSCs were maintained on growth factor reduced Matrigel (Corning) coated plates in mTeSR1 medium (Stem Cell Technologies). Plates were coated with 1:200 Matrigel diluted in DMEM/F12 for at least 1 hour at 37 °C prior to use. HiPSC were passaged every four days. Cells were washed with 1xPBS without $CaCl_2$ or $MgCl_2$ and dissociated with 0.5 mmol/L EDTA in PBS without calcium or magnesium for 7 minutes at room temperature (RT) followed by resuspension in mTeSR1 with 10 µmol/L RHO kinase inhibitor Y-27632 (Calbiochem) and immediately seeded on Matrigel coated plates. Cells received fresh mTeSR1 daily and were maintained at 37 °C with 5% CO_2 and 5% O_2. The male iPSC line was used as the primary source of cardiomyocytes (CM) for imaging and functional studies. The female iPSC line was used to confirm the concordant effect of the hormone treatment on CM morphology.

Differentiation and Maturation of iPSC to CM

HiPSC-cardiomyocytes (hiPSC-CM) were generated using a small molecule based cardiac differentiation protocol[1, 2]. HiPSCs were cultured until they reached 65-85% confluence, at which point the chemical differentiation protocol was initiated (D0) using the media listed in Table 1.

Table 1: Differentiation Media Components

Media Name	Basal Media	Supplement
M1	RPMI 1640 with glucose (11875 Life Technologies)	B27 minus insulin (A1895601, Life Technologies)
M2	RPMI 1640 no glucose (11879 Life Technologies)	B27 minus insulin (A1895601, Life Technologies)
M3	RPMI 1640 with glucose (11875 Life Technologies)	B27 (17504044, Life Technologies) With 1% Pen-Strep (Life Technologies)

At D0 the hiPSC transition from mTeSR1 to M1 was supplemented with 6 µmol/L GSK3 inhibitor CHIR99021 (Selleck Chemicals). On day 2, the media was changed to fresh M1. On day 3, the media was changed to M1 with 5 µmol/L IWR-1 (Sigma). On days 5 to 9 the media was changed every other day with M1. Metabolic selection was started with M2 media on day 10 and cells received fresh M2 media on days 12 and 14. On day 16, cells were transitioned to 3 mL M3 media. For generation of hormone treated cells, M3 was supplemented with the combination of hormones or their vehicles as in Table 2. All solutions were generated, stored at -20 °C, and used within 3 months of constitution. Media supplemented with the final concentrations of hormones was prepared fresh daily.

Table 2: Hormone Treatments

Experimental Group	Supplement	Concentration of Stock	Final Concentration
Vehicle	DMSO and NaOH	-	0.01% DMSO + 0.325 mmol/L NaOH
T3	Triiodo-L-thyronine hormone (Sigma T2877)	30.73 mmol/L in 100 mmol/L NaOH	0.0001 mmol/L in M3
Dexamethasone	Dexamethasone (Cayman 11015)	63.7 mmol/L in DMSO	0.001 mmol/L in M3
T3 + Dexamethasone	Both	-	0.0001 mmol/L T3 and 0.001 mmol/L Dex in M3

hiPSC-CM Dissociation and Plating on Matrigel Mattress

At D30 of the chemical differentiation process, hiPSC-CM were dissociated and plated as previously described[1, 2]. Primarily, freshly differentiated cells were utilized for experiments. Freshly differentiated hiPSC-CM were washed with 1x DPBS without calcium and magnesium and then incubated in 1 mL of TrypLE Express (Life Technologies) for 10 minutes at 37 ^{0}C. A 10 mL serological pipet was then used to add equal volumes of M3 to neutralize TrypLE and for resuspension. Cells were centrifuged at 200 x *g* for 5 minutes, re-suspended in 1-3 mL of M3, and filtered through a 40 μM filter (Falcon). The filtered cells were counted and seeded on Matrigel mattress as previously described[1]. Cells were allowed to attach for at least 10 minutes at RT before adding an additional 1 mL of normal M3 media and returned to the incubator for experimental use at days 4-6. M3 media was refreshed on a daily basis until experimentation.

Cell Selection for Imaging and Functional Measurements

The Matrigel mattress method generates single, elongated CM located in a glass bottom culture dish that can be used for imaging, Ca fluorescence, and patch-clamp studies without the need for cell transfer[1]. Since not all iPSC-CM achieve a rod shape on mattress, iPSC-CM were selected for experimental studies using the same morphological and functional selection criteria that are widely used in studies of CM acutely-isolated from adult hearts: (1) rod-shape, (2) no contact with other cells, and (3) robust contractile response to field stimulation (for Ca and patch-clamp studies). In all cases, the experimenter that selected cells for studies was blinded to the treatment group.

T- Tubule Staining and Analysis

HiPSC-CM were seeded at a density of 10-15,000 cells on Delta TPG Culture Dish (Fisher) using the Matrigel-mattress. Cells were loaded (as per manufacture's recommendations) with CellMask Orange for 20 minutes followed by three 5 minute washes at RT in Tyrodes solution consisting of (mmol/L): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES (pH = 7.4). Images were collected as Z-stacks using a Zeiss LSM510 inverted laser scanning confocal microscope. Images were first deconvolved, and thresholded to the mean fluorescence intensity of the entire cell, and t-tubule density calculated by normalizing the supra-threshold signal within the cell interior to cross-sectional cell area[3]. Cross sectional area was determined using ImageJ. Presented t-tubule densities correspond to images taken within the central plane of the cell. 3D reconstruction of the t-tubular network was performed as described previously[3]. For rendering of the surface sarcolemma, the membrane was identified manually across entire Z-stacks using ImageJ, and smoothed by dilation and re-erosion. Sarcolemma Z-stacks were then merged with the t-tubule stacks and rendered in 3D.

T-tubule development in hiPSC-CMs was compared with t-tubule density and organization in the left ventricles and right atria of healthy individuals. This tissue was obtained using protocols approved by Norwegian South-Eastern Regional Committee for Medical Research Ethics (Permit numbers s-07482a and

2010/2226), in agreement with guidelines outlined in the Declaration of Helsinki. Tissue from the left ventricular free wall was obtained from non-diseased hearts considered for transplantation, but deemed unsuitable due to surgical reasons (n=4). These hearts were kept in a cardioplegic solution at 4 ℃ for 1-4 hours prior to tissue collection and freezing. Cause of death in these patients was cerebrovascular accident, and none had a history of heart disease. Approval for study inclusion was obtained from next of kin.

Tissue from the right atrial appendage was obtained during open-heart surgery in patients undergoing coronary artery bypass surgery. Atrial tissue was rapidly excised and frozen in liquid nitrogen until further use[4]. All patients (n=3) provided informed written consent.

Using a cryostat, human tissue was sliced into 10 μm thick sections at -20 ℃, collected on Poly-prep Slides (Sigma-Aldrich, St. Louis, MO), and fixed in 4% paraformaldehyde. T-tubules were stained with wheat germ agglutinin (WGA) conjugated to Alexa Fluor 480 prepared in PBS. An LSM 710 (Zeiss, GmbH, Jena, Germany) confocal scanning system with a 40× objective was used to visualize t-tubules and cellular membranes. T-tubule density was analyzed as described above for hiPSC-CMs. Organization of t-tubules was compared by calculating the amount of transverse and longitudinal elements in the representative 3D cells.

Paced Calcium Transients

HiPSC-CM were incubated for 30 min at RT in 5 μM Fluo-4 AM (Invitrogen) with 0.02% pluronic (Invitrogen) followed by three 5-minute washes in Tyrodes solution consisting of (mmol/L): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES (pH = 7.4). Single-cell Ca^{2+} transients were recorded at RT using a Zeiss LSM 510 inverted laser scanning confocal microscope in line scan mode (521 lines per second) with a 40X / 1.30 NA planar oil immersion objective. The pinhole settings corresponded to a measured optical slice of 0.85 μm. HiPSC-CM were stimulated by an electric field of 40 mV with a 5 ms bipolar pulse duration at 0.2 Hz using a MyoPacer (IonOptix). The focal plane was positioned in the middle of the Z-dimension from the top and bottom edges of the cell and transverse lines were drawn across the widest part of the cell, avoiding nuclei. Fluo-4 was excited at 488 nm with an argon laser and the emission was passed through a 505 nm long pass filter to photomultiplier tube detectors. Images were normalized to the intrinsic background fluorescence and ImageJ was used to measure the calcium transient amplitude and rate based parameters at the peripheral and central regions of the cell. Time-zero for time to peak measurements were defined as the point of calcium rise.

Immunostaining

HiPSC-CM were cultured on Matrigel mattress-coated cell culture chambers (Falcon 354104) and fixed with 4% paraformaldehyde (Thermo #28906) for 10 minutes at RT. Cells were washed three times with PBS for 10 minutes at RT and permeabilized with 0.4% Triton X-100 in PBS for 1 hour, after which they were blocked for 1 hour (5% Goat serum, 0.4% Triton X-100 in PBS). Cells were incubated with primary antibody overnight at 4 ^{0}C as per Table 3. Then cells were washed 3 x 5 minutes with 0.4% Triton X-100 in PBS and incubated with secondary antibody for 1 hour. After washing cells with 0.4% Triton X-100 in PBS 3 x 5 minutes followed by PBS another 3 x 5 minutes each, the cells were counterstained with ProLong Diamond Antifade with DAPI-containing Mountant (Invitrogen #P36962) and mounted on a cover slide for imaging. Imaging of t-tubule markers was completed using the Zeiss LSM 510 followed by calculation of percent stained area using ImageJ. For co-labeling of t-tubule markers and α-actinin, imaging was performed using the LSM 880 inverted laser scanning confocal microscope with a 63X / 1.40 Plan-APOCHROMAT oil immersion objective. Each channel was sequentially scanned using photomultiplier tube detectors. Alexa Fluor 488 was excited using a 488 nm Argon laser and the emitted light was collected from 499 – 570 nm; Alexa Fluor 568 was excited using a diode-pumped solid state 561-10 laser and collected from 570 – 712 nm; DAPI was excited using a 405-30 diode laser and collected from 409 – 497 nm. Using ImageJ, immunostaining images were background corrected by subtracting the mean value of background next to the cell and the contrast was linearly adjusted. Each image from a particular

immunostain (e.g. BIN-1) was adjusted equally and all images were cropped and scaled equally. Regularity of RyR2, Cav3, BIN1, α-actinin and JP2 localization along z-lines was assessed using fast Fourier transforms (FFT), computed by the FFT tool in Matlab (MathWorks). The power spectrum was plotted as a function of 1 / axial spatial frequency, and the amplitude of the first peak measured. Co-localization analysis was then completed using the Manders split coefficients in ImageJ.

Table 3: Antibodies for Immunostaining

Antibody	Manufacturer	Host	Catalogue number	Concentration
Bin1	Abcam	Rabbit	Ab185950	1:100
Cav3	Abcam	Rabbit	Ab2912	1:100
Sarcomeric α-actinin	Sigma	Mouse	A7811	1:100
Sarcomeric α-actinin	Abcam	Rabbit	Ab137346	1:100
Junctophillin2	Abcam	Mouse	Ab180362	1:100
RyR2	Thermo	Mouse	MA3-916	1:50
anti-mouse 488	Invitrogen	Goat	A11029	1:250
anti-rabbit 568	Invitrogen	Donkey	A10042	1:250

Sparks Measurements in Permeabilized Myocytes

Isolated hiPSC-CM were permeabilized with saponin (20 µg/mL) for 30 seconds and then bathed for 5 minutes in a freshly-made internal solution (pH = 7.2) containing (mmol/L): K-aspartate (120), KCl (15), K_2HPO_4 (5), $MgCl_2$ (5.6), HEPES (10), dextran (4% w/v), MgATP (5), Phosphocreatine-Na_2 (10), creatine phosphokinase (10 U/mL), reduced L-glutathione (10), EGTA (0.5), $CaCl_2$ (0.12), and Fluo-4 pentapotassium salt (0.03). Free [Ca] was 100 nmol/L (calculated with MaxChelator). Confocal imaging was performed on a Zeiss LSM 510 inverted laser scanning confocal microscope equipped with a 40X / 1.30 NA planar oil immersion objective. Fluo-4 was excited by an argon laser line at 488 nm and the fluorescence emission was passed through a 505 nm long pass filter to PMT detectors. Cell sparks were imaged in line-scan mode in isolated cells that had no contact with other cells. Lines were positioned longitudinally near the center of the cell. SR Ca load was measured as the Ca transient amplitude induced by application of 10 mmol/L caffeine. Image analysis was performed in ImageJ with the SparkMaster plugin[5] using a background setting of 5 and criteria of 3.8. Spark mass was calculated from the equation: spark mass = 1.206 * Amplitude * FWHM^3.

Measurement of Intracellular Calcium and Contractility

HiPSC-CM were seeded 10-15,000 cells on Matrigel mattress on Delta TPG Culture Dish (Fisher). After 5 days of acclimation on mattress with daily M3 media changes, cells were loaded with Fura-2 AM (Molecular Probes Inc, Eugene, OR) as previously described[1, 2]. Fura-2 AM was reconstituted in DMSO at 2 mmol/L in a light-protected vessel. HiPSC-CM were incubated at a final concentration of 2 µmol/L Fura-2 AM in M3 medium for 8 minutes at RT and then washed twice with 1.2 mmol/L calcium containing Tyrode's solution with 250 µmol/L probenecid. Measurements were made with a 40x LUCPlanFLN Ph2 (NA 0.6, 0-2/FN22, WD 2.7-4 mm) objective using a Nikon Eclipse T5100 fitted with an IonOptix video microscopy system (Ionoptix, Milton, MA). Tyrode's solution contained in mmol/L: $CaCl_2$ as indicated below, NaCl 134, KCl 5.4, $MgCl_2$ 1, glucose 10, and HEPES 10, with the pH adjusted to 7.4 with NaOH.
A. Calcium Kinetics: Calcium transients were recorded from cells which elicited a response during **0.2 Hz electrical field stimulation** in Tyrode's solution with 2 mmol/L Ca (2Cal) for 20 seconds at RT. Stimulation was then stopped and immediate puffer based application of 10 mmol/L caffeine in 2Cal for 5 seconds was initiated followed by 5 seconds of recording with 2Cal alone to assess for sarcoplasmic

reticulum (SR) calcium content and SERCA-independent calcium extrusion. The recording was then paused for 1 minute while cells were continuously perfused with 2Cal solution. Cells were then exposed to 0 mmol/L Na 0 mmol/L calcium solution (0Na0Ca) for 10 seconds followed by puffer-based application of 10 mmol/L caffeine containing 0Na0Ca solution for 30 seconds in addition to 10 seconds of recording in 0Na0Ca solution to estimate non-NCX mediated calcium extrusion. For examination of SR calcium contribution to the calcium flux, hiPSC-CM were pretreated for 5 minutes with 10 µmol/L thapsigargin (Sigma) and 50 µmol/L ryanodine (Sigma) in 2Cal after the last wash. For each cell, the following parameters were determined: baseline and peak amplitude of transient, time to peak and baseline, and tau (τ) of A) field stimulated calcium transients, B) 2Cal Caffeine transient, and C) 0Na0Ca Caffeine transient. Analyses of calcium transients and calculation of calcium flux balance was completed as previously[2]. Time zero for time to peak measurements were defined as the time of electrical stimulation.

B. Contractility: Video based edge detection was used to simultaneously assess cellular shortening of the hiPSC-CM during the first 20 seconds of field stimulation. In brief, during acquisition of calcium transients on single HiPSC-CM, edge detectors were placed to define the ends of the cells using IonWizzard™ v6.5 acquisition software (Ionoptix, Milton, MA).
Analysis of traces was completed using the instrument packaged IonWizard™ v6.5 data analysis software. Cell selection was limited to cells which were amenable to 0.2 Hz field stimulation and captured by edge detection.

Morphometric Measurements
HiPSC-CM were seeded on Matrigel mattress. Cellular volume measurements were taken as previously described[1, 2]. Briefly, Z-stacks of single D35 hiPSC-CM were obtained using a Zeiss LSM 510 inverted laser scanning confocal (40x oil immersion 1.30 Plan-NEOFLUAR lens). HiPSC-CM were loaded with 5 µmol/L calcein AM (Molecular Probes, Eugene, OR) in Tyrode's solution for 30 minutes at RT followed by three five-minute washes at RT to remove any residual extracellular dye. Calcein AM was exited at 488 nm with an argon laser. Z-stack images were collected in 0.44 µm intervals and reconstructed using IMARIS (Bitplane, South Windsor, CT) to allow for the measurement of volume and surface area. Manual measurements for length and width were completed using ImageJ measurement tools. Image acquisition and analysis was completed with the user blinded to the treatment groups.

Measurement of Excitation Contraction Coupling (ECC) Gain and L-type Ca current inactivation
To measure ECC gain, hiPSC-CM from both groups (30 days after cardiac induction, 3-5 days after plating) were studied using patch clamp technique in whole-cell mode. External solution contained (in mmol/L): NaCl 134, CsCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 10, adjusted to pH 7.4 with NaOH. The pipette solution contained (in mmol/L): CsCl 110, $MgCl_2$ 1, MgATP 5, cAMP 0.2; EGTA 1; Hepes 20; adjusted to pH = 7.25 with CsOH. For Ca fluorescence measurements, 100 µmol/L Fluo-4 pentapotassium salt was added to the pipette solution. Currents were elicited by 50 ms depolarizing steps ranging from -40 mV to +40 mV (in 10 mV increments), from the holding potential of -70 mV. A 10 ms conditioning pulse to -45 mV was applied prior to the test pulse in order to inactivate Na currents. To assess inactivation kinetics of ICa, a single 500 ms depolarizing step to 0 mV (from the holding potential of -70 mV, with 10 ms conditioning pulse to -45 mV) was applied. Inactivation of ICa was fit with a double exponential curve. All experiments were carried out at RT.

Statistics
GraphPad Prism v7 was utilized for testing statistical differences with Student's t-tests or, when applicable, ANOVA followed by post hoc analysis. P-values of <0.05 were considered statistically significant. All data are presented as mean±SEM. Image acquisition and analyses were conducted in a blinded fashion.

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Online Videos

3-D rendering of t-tubules in Cell Mask Orange stained hiPSC-CM. Red delineates border of cellular area assessed. 1. Vehicle treated hiPSC-CM on Matrigel mattress, 2. T3+Dex treated hiPSC-CM on Matrigel mattress, 3. Human Atrial-CM, and 4. Human Ventricular-CM.

Online Table I: Calcium Handling

Parameter	Vehicle	T3+Dex	p-value
Diastolic Ca (F_{Ratio})	1.3 ± 0.029	1.27 ± 0.027	0.22
Ca transient amplitude (F_{Ratio})	0.94 ± 0.044	0.88 ± 0.034	0.23
Time to peak (s)	0.59 ± 0.025	0.49 ± 0.022	0.0036
Time to peak 50% (s)	0.21 ± 0.026	0.17 ± 0.023	0.036
Time to baseline 50% (s)	0.82 ± 0.03	0.60 ± 0.017	1.10E-09
SR Ca content (F_{Ratio})	1.1 ± 0.054	0.97 ± 0.045	0.093
Ca transient decay (t_{twitch}, s)	1.21 ± 0.053	0.84 ± 0.033	1.33E-08
Caff decay (t_{caff}, s)	2.46 ± 0.086	2.12 ± 0.098	0.011
0Na0Ca Caff decay ($t_{Caff0Na}$, s)	12.82 ± 0.77	10.67 ± 0.85	0.065
K_{SERCA}	0.43 ± 0.036	0.65 ± 0.057	0.02
K_{NCX}	0.36 ± 0.021	0.44 ± 0.03	0.03
$K_{non\text{-}NCX}$	0.089 ± 0.0054	0.10 ± 0.0062	0.089

Online Table I: Ca kinetic measurements of T3+Dex treated vs vehicle treated hiPSC-CM paced at 0.2 Hz. Data presented are mean±SEM (n=68 for control and 79 for T3+Dex). Unpaired, two tailed Student's t-test, p-value as listed.

Online Table II: Contractile Kinetics

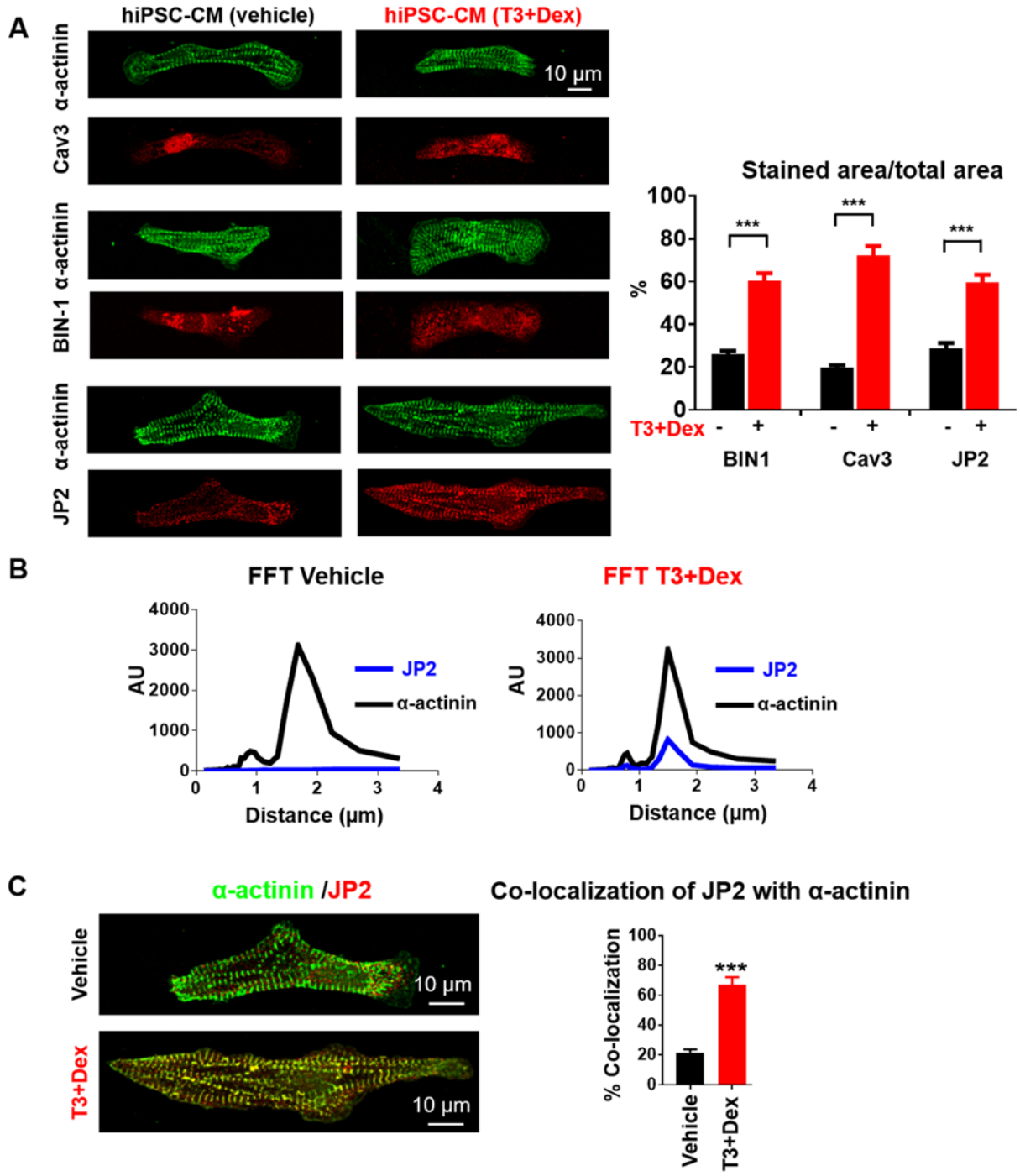
Parameter	Vehicle	T3+Dex	p-value
Cell shortening (% of resting length)	5.55 ± 0.50	7.53 ± 0.70	0.02
Time to peak (s)	1.01 ± 0.04	0.71 ± 0.02	1.88E-07
Time to peak 50%	0.48 ± 0.03	0.37 ± 0.02	0.0028
Time to peak 90%	0.78 ± 0.03	0.56 ± 0.02	7.03E-06
Time to baseline 50%	0.58 ± 0.04	0.41 ± 0.09	2.07E-07
Time to baseline 90%	1.87 ± 0.02	1.20 ± 0.09	4.60E-06

Online Table II: Contractile kinetic measurements of T3+Dex treated vs vehicle treated hiPSC-CM paced at 0.2 Hz. Data presented are mean±SEM (n=68 for control and 79 for T3+Dex). Unpaired, two tailed Student's t-test, p-value as listed.

Online Table III: Calcium Spark Parameters

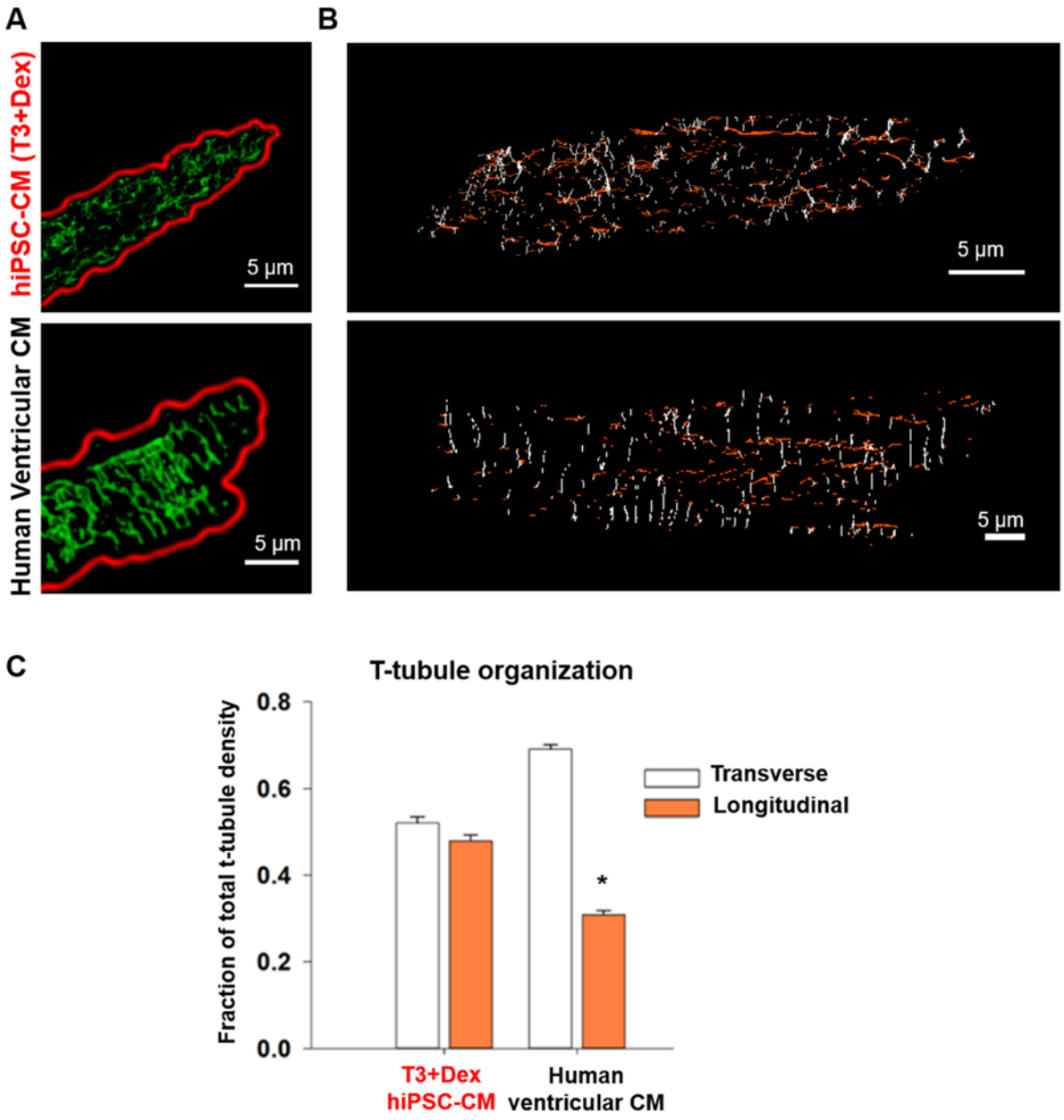
Parameter	Vehicle (Mattress)	T3 (Mattress)	Dex (Mattress)	T3+Dex (Mattress)	T3+Dex (Non-mattress)
Spark frequency (sparks/100µm/s)	4.02 ± 0.51	5.82 ± 1.06	4.09 ± 0.78	8.31 ± 0.67***	4.95 ± 0.94
Spark amplitude ($\Delta F/F_0$)	0.29 ± 0.02	0.26 ± 0.01	0.25 ± 0.01	0.46 ± 0.03**	0.31 ± 0.03
Spark mass ($\Delta F/F_0$*µm^3)	6.62 ± 0.99	7.36 ± 1.64	7.53 ± 1.61	33.36 ± 8.37***	12.76 ± 4.0
FWHM (µm)	2.52 ± 0.06	2.59 ± 0.07	2.56 ± 0.07	2.84 ± 0.05***	2.91 ± 0.07**
FDHM (ms)	27.84 ± 0.86	25.3 ± 0.63	25.42 ± 0.88	36.49 ± 0.86***	27.9 ± 1.21
Time to peak (ms)	16.24 ± 0.61	15.0 ± 0.61	16.73 ± 0.98	21.69 ± 0.78***	16.03 ± 0.82
Caffeine transient amplitude ($\Delta F/F_0$)	2.79 ± 0.15	2.54 ± 0.13	2.46 ± 0.2	2.92 ± 0.27	2.72 ± 0.13

Online Table III: Full width half max (FWHM), Full duration half max (FDHM). Data are reported as mean±SEM. One-way ANOVA with Tukey's post hoc test; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs vehicle.

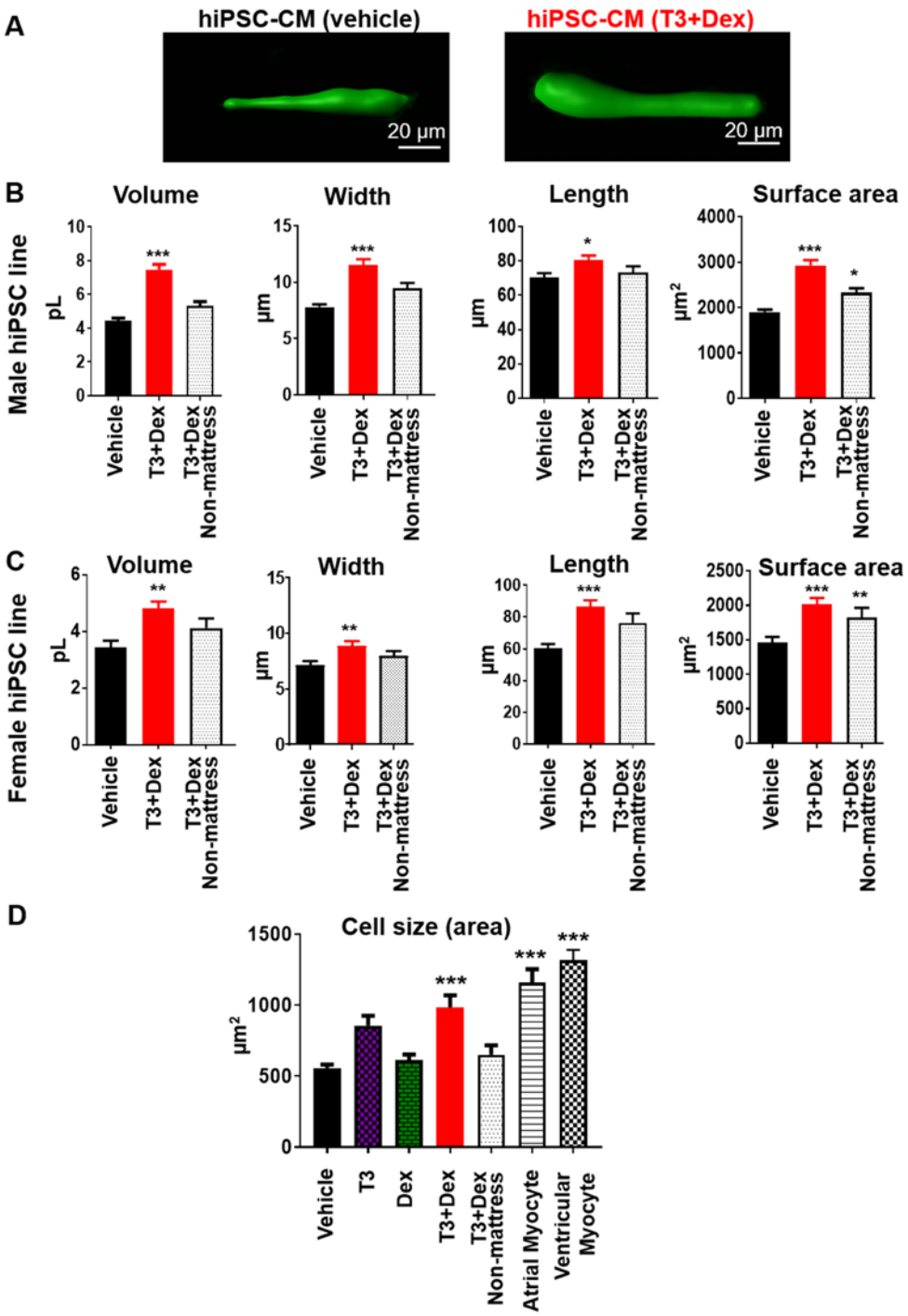


Online Figure I: T3+Dex treated cells exhibit increased subcellular distribution of t-tubule related proteins.

A) Representative cell images after co-labeling for α-actinin and the indicated t-tubule related proteins Cav3, BIN1, or JP2 in vehicle and T3+Dex treated cells (scale bar = 10 μm for all cells). Summary data show percent stained area of indicated protein of total cell area. Data reported as mean±SEM (n=17-23 cells). **B)** Fast Fourier transform for JP2 and α-actinin in vehicle and T3+Dex treated cells. **C)** Representative merge of α-actinin and JP2 with percent co-localization of JP2 with α-actinin (n=5 cells/group). Unpaired, two tailed Student's t-test: ***p<0.001.

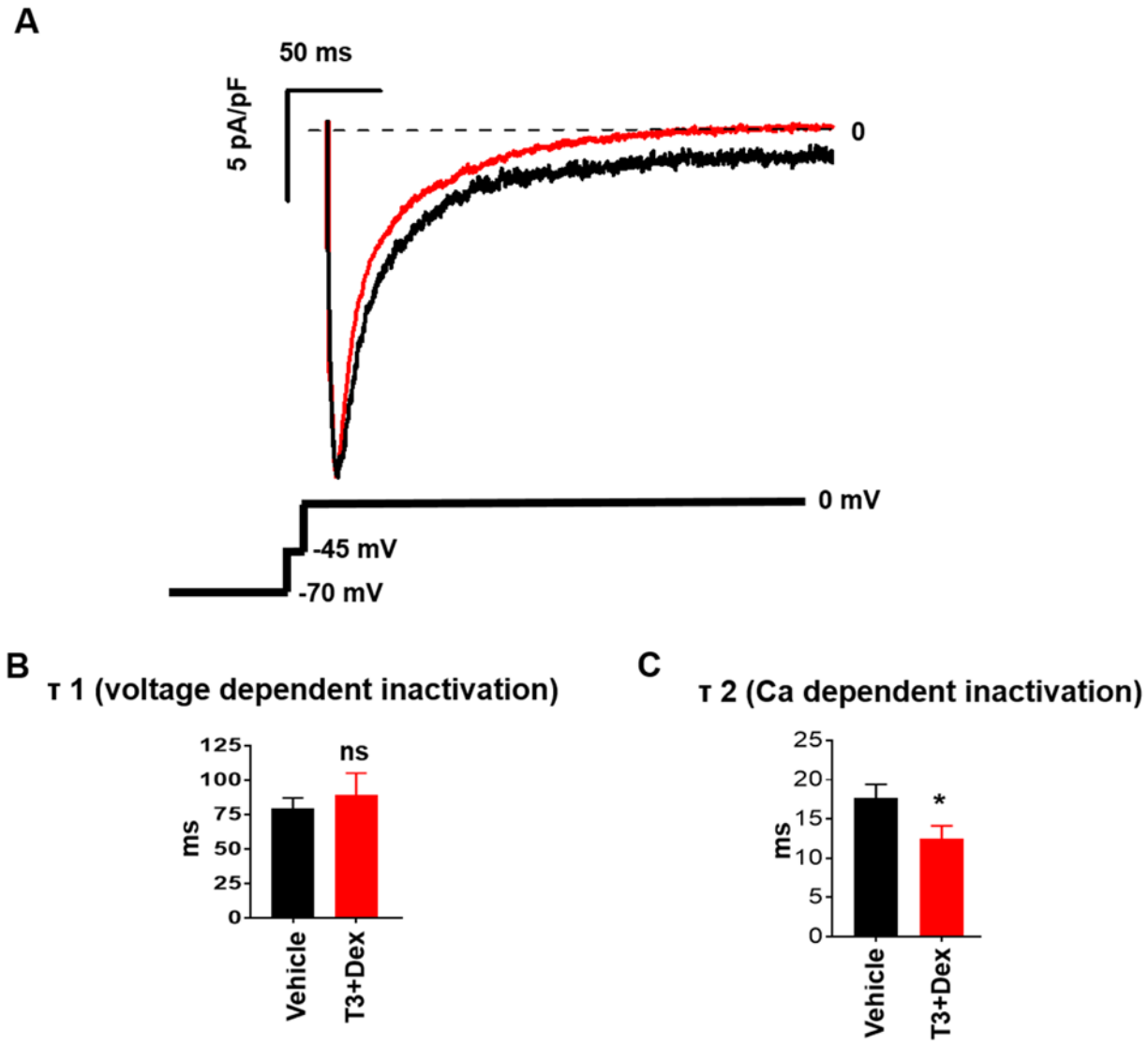


Online Figure II: T-tubule organization: comparison of longitudinal vs transverse t-tubules
A) Representative examples of planar projection after 3D reconstruction of t-tubules in T3+Dex vs human ventricular myocardium demonstrate intracellular location of t-tubule membrane structures. **B)** Comparison of longitudinal vs transverse t-tubules in 3D reconstructed T3+Dex CM vs human ventricular CM, (white represents transverse t-tubules, red represents longitudinal t-tubules) **C)** Summary data showing the fraction of transverse vs longitudinal t-tubule of total t-tubule density. Data are mean±SEM (n=18-19 cells). Unpaired, two tailed Student's t-test: *p<0.05 vs transverse.



Online Figure III: T3+Dex treated cells exhibit increased cell size

A) Representative examples T3+Dex vs control treated hiPSC-CM. Images are planar projections of 3D reconstructions using Calcein-AM. **B) and C)** Summary data: morphometric measurements obtained from hiPSC-CM derived from two independently-generated iPSC lines (male and female). Data reported as mean±SEM (n=51-108 for male line from 4 independent differentiations and 24-35 for female line). **D**. Summary data for cross sectional cell area used for calculating t-tubule density for indicated groups. Note atrial and ventricular are of human origin (n=19-59). One-way ANOVA with Tukey's post hoc test: *p<0.05, **p<0.01, and ***p<0.001 vs vehicle.



Online Figure IV: Ca-dependent inactivation of L-type Ca current is accelerated in the T3 +Dex treated cells.
A) Representative traces of I_{CaL} from a conditioning step of -45mV to 0mV in T3+Dex versus vehicle treated cells. Double exponential fit yields estimates of voltage dependent inactivation (tau 1) and Ca dependent inactivation (tau 2). **B-C)** Summary data for tau 1 and tau 2. Data reported as mean±SEM (n=9-12). Unpaired, two tailed Student's t-test: *p<0.05 vs vehicle, ns – non significant.