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Supplemental Information

Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes as a Model to Study *Trypanosoma cruzi* Infection

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Supplemental Figures and Legends

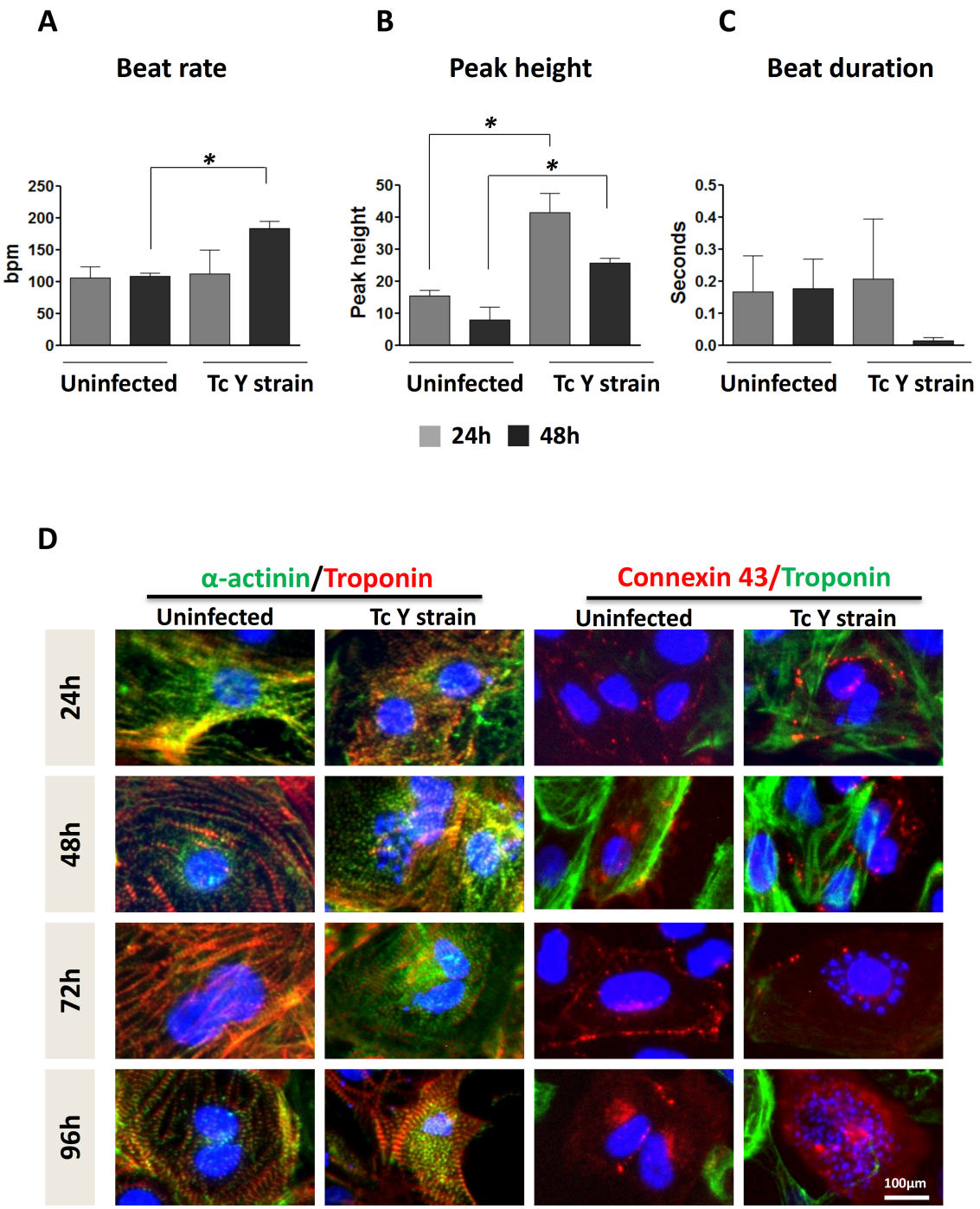

Figure S1. Related to Figure 2. Contractility and Sarcomeric Structure of iPSC-CMs after *T. cruzi* Infection. (A-C) Beat rate, peak height, and beat duration before and after *T. cruzi* infection at 24 and 48 hr. The average of biological triplicates is expressed in columns graphs. Significant statistical ($p<0.05$) differences are indicated between samples (*). **(D)** Immunofluorescence of iPSC-CMs uninfected and infected with Tc Y strain (1:5, 3 hr interaction) shows the distributions of α-actinin, troponin T, and Connexin 43 over the course of infection (24, 48, 72, and 96 hr).

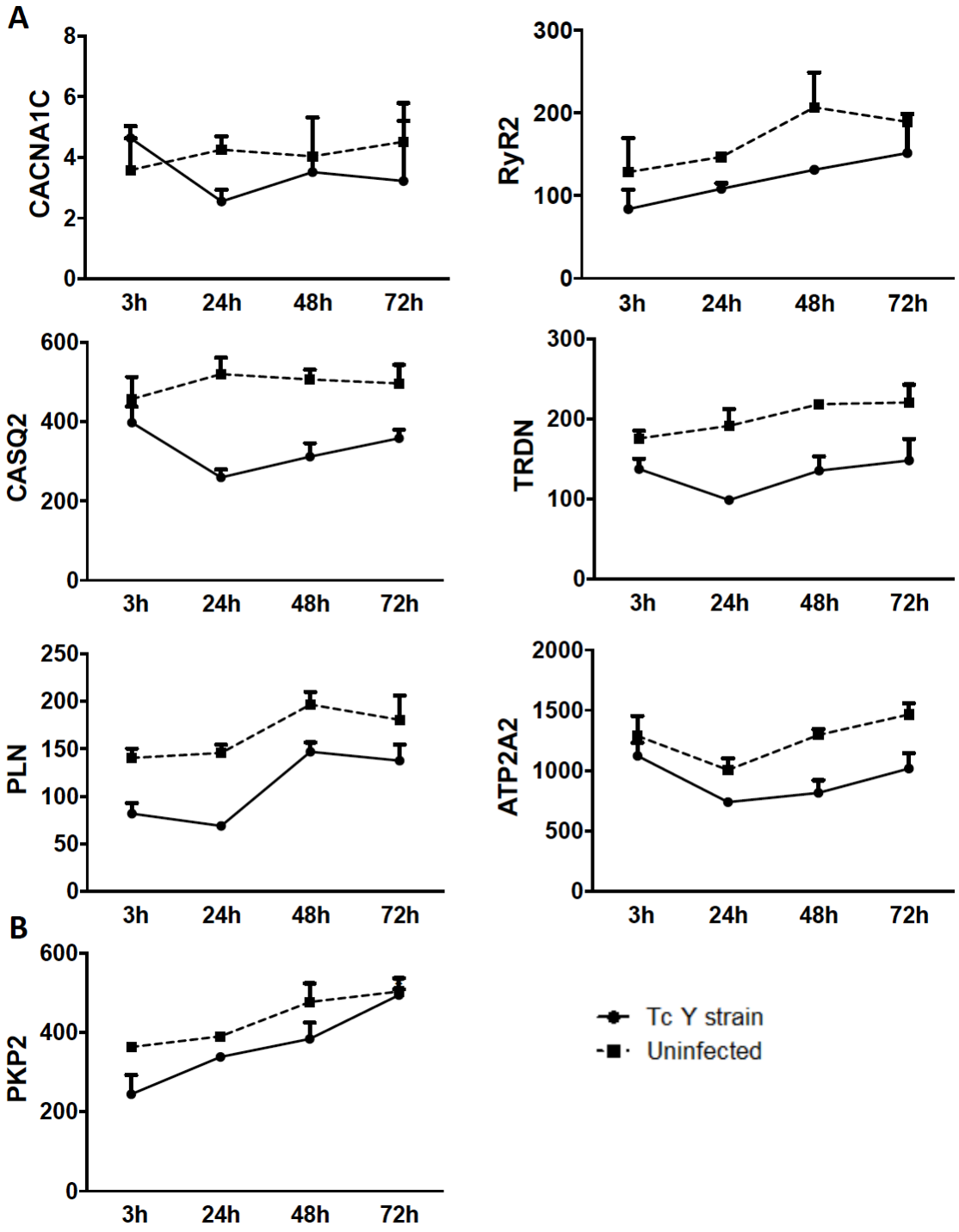

Figure S2. Related to Figure 4. Intracellular Calcium Cycling after *T. cruzi* Infection. (A) Gene expression for calcium handling genes *CACNA1C*, *RYR2*, *CASQ2*, *TRDN*, and *ATP2A*, and **(B)** for the desmosomal gene *PKP2* was analyzed in uninfected and infected (Tc Y strain) iPSC-CMs at different times. The average of biological duplicates is expressed in graphs.

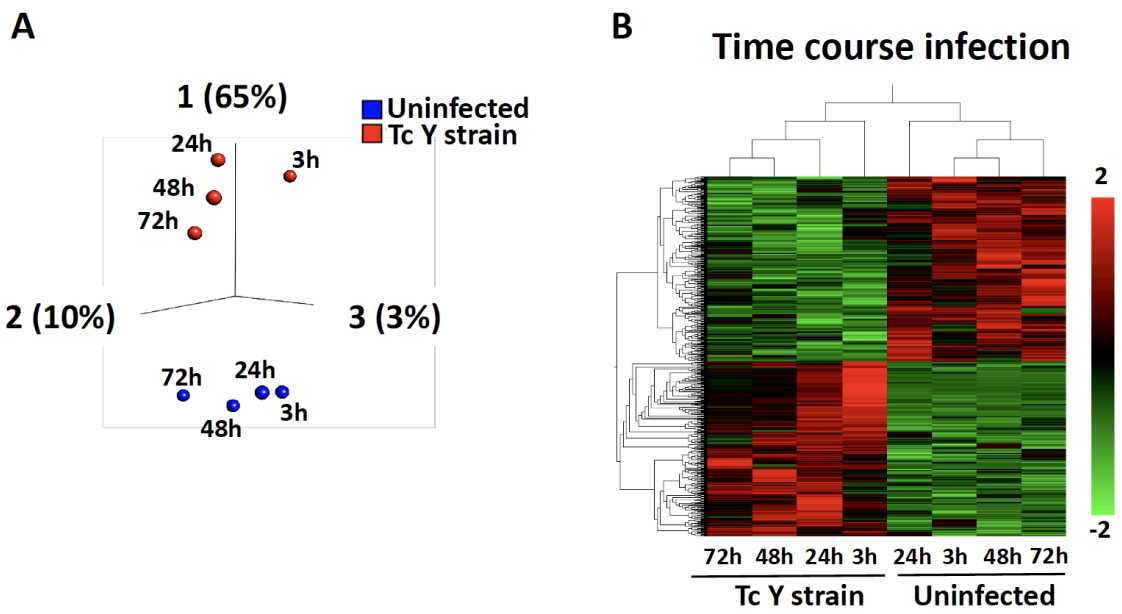

Figure S3. Related to Figure 4. RNA-seq of iPSC-CMs Infected with *Trypanosoma cruzi*. Correlations between uninfected and infected (Tc Y strain) iPSC-CMs at different times. **(A)** PCA with computation of closest neighboring samples. **(B)** Heat map shows the hierarchical clustering.

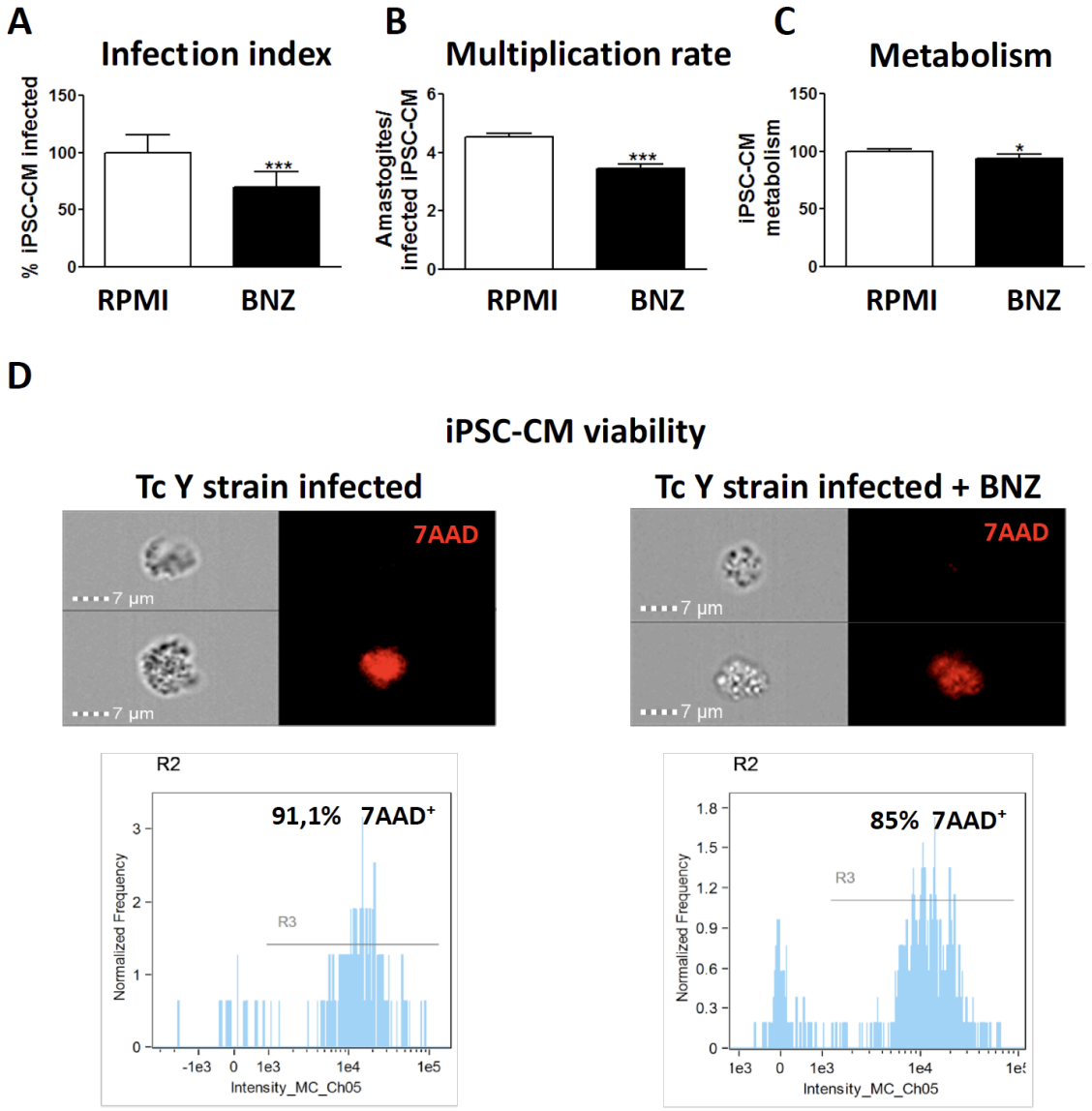

Figure S4. Effects of Benznidazole on *T. cruzi* Infected iPSC-CMs, Related to Discussion of iPSC-CM Model as a Platform to Screening New Drugs to ChD. Human iPSC-CMs infected with Tc Y strain *T. cruzi* show decrease in **(A)** infection index, **(B)** multiplication rate, and **(C)** metabolism when treated with 3.8 µM BNZ. The average of biological triplicates is expressed in graphs. Significant statistical ($p<0.05$) differences are indicated between samples. *$p<0.05$, ***$p<0.001$. **(D)** FACS analysis shows improvement in cell viability of iPSC-CMs infected with Tc Y strain *T. cruzi* when treated with 3.8 µM BNZ.

Supplemental Experimental Procedures

Generation of iPSCs. Reprogramming with the Sendai virus was used to generate three human iPSC lines from peripheral blood mononuclear cells (PBMCs) of three healthy individuals. iPSC colonies were maintained in an Essential 8 medium (E8) (Life Technologies, CA, USA) and dissociated with 0.5 mM EDTA into a single-cell suspension using E8 medium containing 10 μM ROCK inhibitor (Sigma, MO, USA).

Parasites. Culture-derived trypomastigotes (TCTs) of the Y strain were obtained from monolayers of Vero cells (ATCC® CCL-81TM, VA, USA), which were infected at a ratio of 5:1 (TCTs:Vero cells). Vero cells were incubated at 37°C in RPMI medium enriched with 5% inactivated FBS supplemented with antibiotics. Parasites were collected from the culture supernatants by centrifugation at 1000 x g for 10 min and the sediment was suspended in RPMI medium plus 5% FBS. Parasites thus obtained were counted in a Neubauer chamber and the number adjusted according to each assay.

Immunofluorescence. For indirect immunofluorescence, iPSC-CMs (1.5×10^5) grown on Matrigel (BD Biosciences, Franklin Lakes, New Jersey, USA)-coated coverslips of an 8-well chamber slide were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.2) for 15 min at room temperature (RT) and permeabilized with 0.1% Triton X-100 (Sigma, Chemical Company, St. Louis, MO, USA) for 15 min at RT. The cells were incubated overnight at 4ºC with rabbit anti-human alpha actin (Sigma, catalog # SAB5600071), mouse anti-human troponin T (Invitrogen, catalog # MA1-26935, Waltham, Massachusetts, USA), rabbit anti-human troponin T (Abcam, catalog # ab45932, Cambridge, United Kingdom), rabbit anti-human BDKRB1 (Abcam, catalog # ab75148), rabbit anti-human BDKRB2 (Abcam, catalog # ab236093), rabbit anti-human cortactin (Abcam, catalog # ab81208), or rabbit anti-human Connexin 43 (Abcam, catalog # ab217676, Cambridge, United Kingdom), diluted in 2% goat serum (GS) in PBS. After washing four times with 1% GS, the cells were incubated for 1 hr at room temperature with the secondary antibody, goat anti-mouse immunoglobulin G (IgG) Alexa Fluor 488, or goat anti-rabbit IgG Alexa Fluor 594 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The cells were washed four times and one drop of mounting medium (Slow Fade Gold Antifade; Thermo Fisher Scientific) with 4',6-diamidino-2-phenylindole (DAPI) was used for imaging, which was performed using a fluorescence hybrid microscope (Echo Laboratories, San Diego, CA, USA).

Giemsa staining. To quantitate the *T. cruzi* infection on iPSC-CMs, Giemsa staining was performed. Approximately 2×10^5 iPSC-CMs/well were seeded into each well of an 8-well chamber slide (Nunc, Waltham, Massachusetts, USA). Cells were allowed to settle for 48 hr before the invasion assay with 10^6 parasites, as described previously. After 24, 48, 72, and 96 hr, the iPSC-CM were stained with Giemsa (Sigma). The cells were fixed with Bouin's fixative solution (Sigma) for 10 min and washed with 0.15M PBS twice. Then cells were incubated overnight with Giemsa solution diluted 1:20 in water and buffered to pH 6.8. Chamber slides were rinsed with water and air-dried. The cells were analyzed using a light microscope (Leica Microsystems, Wetzlar, Germany) at 1000x magnification.

Viability assay. Viability of iPSC-CMs was analyzed using 7-Amino-Actinomycin D (7-AAD) (BD pharmingen, San Diego, California, USA) nucleic acid dye. 1×10^5 iPSC-CMs were seeded in a pre-coated 96-well plate in RPMI/B27 plus insulin medium. Following 24 hr at 37ºC at 5% CO_2, iPSC-CMs were infected with *T. cruzi* Y strain at a 1:5 (cells:parasites) ratio. After 3 hr interaction time, medium was replaced for another 72 hr. Cells were stained with 5 μL (0.25 μg) of 7-AAD for 10 min at 20ºC and washed with PBS. 7-AAD fluorescence was detected in the far-red range of the spectrum (650 nm long-pass filter) in the ImageStream®X Mark II Imaging Flow Cytometer (Burlington, Massachusetts, USA). The viability was estimated by taking the percentage of cells not stained with 7-AAD once this dye is efficiently excluded by intact cells.

iPSC-CM counting. DAPI reagent (Invitrogen) was used to determine the number of iPSC-CMs cultured with *T. cruzi*. The invasion assay was performed in 24-well plates with 2×10^5 iPSC-CM and 10^6 parasites per well, as described above. The number of iPSC-CMs was accessed at 24, 48, 72, and 96 hr after invasion. The cells were fixed with 4% paraformaldehyde in PBS (pH 7.2) for 15 min at room temperature and stained with DAPI. Using a fluorescence plate reader (Cytation 5 multi-mode reader - BioTek, Winooski, Vermont, USA), the iPSC-CMs' nuclei were measured, and the *Trypanosoma* nuclei were excluded by size.

Reactive oxygen and nitrogen species assay. To analyze oxidative stress following *T. cruzi* infection, ROS and reactive nitrogen species RNS were measured in cell culture supernatants of iPSC-CMs, using

the OxiSelect *in vitro* ROS/RNS assay kit (Cell Biolabs, Inc, San Diego, CA, USA) according to the manufacturer's instructions. Using a plate reader (Promega, Madison, Wisconsin, USA), fluorescence was measured at 480 nm excitation / 530 nm emission. The data are shown as the average of duplicate and triplicate measurements.

Contractility analysis; related to iPSC-CM contractility following *T. cruzi* infection result. iPSC-CM contractility was analyzed before and after infection with Y strain TCTs. The invasion assay was performed in a 6-well plate with 2 x 10^6 iPSC-CMs/well plus 10^7 parasites. Movies were recorded at 24, 48, and 72 hr of the infection using an inverted microscope (Leica) and Leica LMD software. iPSC-CM contractility was analyzed online using the Cellogy service (www.pulseservice.cellogy.com).

Cytokine and chemokine analysis. The human 63-plex kit (eBiosciences/Affymetrix, MA, USA) was used according to the manufacturer's recommendations, with modifications as described below. Briefly, beads were added to a 96-well plate and washed in a Biotek ELx405 washer. Filtered supernatant (0.25 μm) of iPSC-CMs cultured with TCTs for 24 or 48 hr was added to plates containing mixed antibody-linked beads and incubated at room temperature (RT) for 1 hr, followed by overnight incubation at 4°C with shaking. Cold and RT incubation steps were performed on an orbital shaker at 500-600 rpm. Following overnight incubation, plates were washed in a Biotek ELx405 washer and the biotinylated detection antibody was added for 75 min at RT with shaking. The plate was washed and streptavidin-PE (Luminex, TX, USA) was added. After incubation for 30 min at RT, a wash was performed and reading buffer was added. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower boundary of 50 beads per sample per cytokine. Custom assay control beads by Radix Biosolutions (TX, USA) were then added to all wells.

RNA-sequencing. RNA was isolated from frozen pellets of iPSC-CMs using the Qiagen RNeasy kit (Qiagen, Germany) according to the manufacturer's instructions. Libraries for RNA-seq were prepared from 10 ng RNA using the AmpliSeq Transcriptome Human Gene Expression kit (Life Technologies). Briefly, reverse transcribed RNA (cDNA) was prepared, and targets were amplified with Ion AmpliSeq Transcriptome Human Gene Expression Core Panel. Primer sequences were partially digested, linked to adapters and Ion XpressTM Barcode X, and purified using AMPure XP reagent. The library was quantified by PCR and diluted to 50 pM. Samples were sequenced in the Ion Torrent Platform (Thermo Fischer, MA, USA) according to the manufacturer's instruction.

Drug assay. Drug assay was performed with benznidazole (BNZ) to analyze infection index, multiplication rate of parasite, metabolism, and viability of iPSC-CMs. For infection index and multiplication rate analyzes, monolayer of iPSC-CMs was prepared on 8-well chamber slides at a density of 2 x 10^5 cells/well and further cultivated for 48 hours at 37°C in a 5% CO_2 atmosphere. The iPSC-CMs was cultured in chamber slides pre-coated with Matrigel, and RPMI 1640 plus B27 with insulin. The infection was accessed in a target effector ratio of 1:5 (cell:parasite) at 24 hr in the presence of Benznidazole (BNZ) 3.8μM. Then, cells were washed with PBS 0.15M, fixed with Bouin's fixative solution, and stained with Giemsa, as described previously. Infected cells were defined as at least 1 amastigote/cell. The number of infected cells (infection index) as well as the number of amastigotes per infected cells (multiplication) was determined for each vision field by microscopy. For comparison to BNZ-treated wells, control infections (without BNZ) were regarded as 100%. Cell metabolism of iPSC-CMs uninfected was determined by 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt (XTT) (Sigma-Aldrich, St. Louis, MO) metabolic assay. Into a 96 well plate 10^5 cells were seeded, and allowed to settle for 24 hr at 37ºC at 5% CO_2 and 80% humidity. After medium change, BNZ were added, and cells were incubated for 24 hr at 37ºC at 5% CO_2 and 80% humidity. XTT with menadione (Sigma-Aldrich) (200 μg/ml, 40 μM in a volume of 100 μl) in RPMI with 5% FBS (containing B27 for cardiomyocyte tests) were added to each well and incubated at 37ºC. Tests were evaluated using a plate reader (Opsys MR, DYNEX Technologies, Chantilly, VA).