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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

Given the absence of prior data on human tissue-engineered ventricles, a statistical determination of sample size was not possible. Instead, we chose a sample size (n=4) compatible with existing datasets relative to contractility assays powered by the same commercially available line of hiPS-CMs. We confirmed this choice by power analysis, which indicated that a sample size of N=4 was sufficient, assuming a 30% increase in beat rate across all groups and a 10% variance.

2. Data exclusions

Describe any data exclusions.

Data was included for analysis if pressure or volume recordings were discernible at each isoproterenol dose (total experiment duration, ~20-30 minutes). Pressure–volume loops and stroke-work analysis required that both pressure and volume recordings were discernible at each isoproterenol dose. Approximately 50% of cultured ventricles (rat or human) produced pressure or volume differences that were measurable by catheterization. Of these, approximately 50% contracted with sufficient stability to conduct isoproterenol dose–response experiments.
In total, we built 20 rat (neonatal rat ventricular myocyte) and 10 human (Cor.4U) model ventricles, from which we obtained pressure–volume data as a function of isoproterenol from 8 rat and 4 human model ventricles.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We observed the expected biological difference in baseline recordings from the ventricles, that is, a distinct spontaneous beating rate. However, the pharmacological response was repeatable. Following isoproterenol exposure, seven out of eight rat ventricles showed positive chronotropy, whereas one ventricle showed a negligible response. For human ventricles, three out of four showed positive chronotropy, whereas the fourth showed mild negative chronotropy.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

We built a total of 20 rat (neonatal rat ventricular myocyte) and 10 human (Cor.4U) model ventricles. Of these, 12 rat and 6 human ventricles were selected for catheterization on the basis of the synchrony and amplitude of contraction observed in vitro by eye and microscope. We report data for 8 rat and 4 human ventricles, for which pressure or volume recordings were discernible at each of 7 isoproterenol doses (0.1 nM to 0.1 mM; total experiment duration, ~20-30 minutes). These data include 3 human and 4 rat ventricles, for which both pressure and volume were recorded at each isoproterenol dose.

For arrhythmia injury models, we built 4 NRVM model ventricles: 2 healthy and 2 pre-injured. Calcium mapping of pre-injured ventricles was done on day 11 but tissue had grown into the injury site, preventing stable-rotor pinning. We repeated calcium mapping experiments on day 12 using the two uninjured ventricles. Both showed evidence of spontaneously generated calcium plane-wave propagation but one had significantly greater homogeneity; this favorable sample is reported in our revised manuscript. Injuries were applied to this model ventricle following pre-injury recordings.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not performed because experiments were performed in a single setting, and data analysis was automated using commercial software.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Statistical analysis was performed using SigmaPlot (v13.0, Systat Software Inc.). Pressure–volume data was acquired using manufacturer-supplied acquisition software (LabChart v7.3, ADInstruments) and was exported for post-processing with Matlab import routines (Matlab R2016a, MathWorks). We used InspectX and CT Pro 3D (Nikon metrology) for X-ray imaging, CT acquisition and volume reconstruction. We used VG Studio MAX 2.2 (Volume Graphics) and Amira 6.0 (ThermoFisher Scientific) for 3D-volume visualization, rendering and analysis. Calcium imaging and analysis was done using MiCAM imaging software (BV_Ana, SciMedia) and a custom MATLAB-based optical mapping analysis package (Rhythm2014b), publicly available at https://code.google.com/archive/p/rhythm-analysis-software

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

We used standard antibodies validated by the manufacturers. Monoclonal anti-α-actinin (sarcomeric) antibodies produced in mouse were obtained from Sigma-Aldrich (clone EA-53, catalog number A7811-100UL). For secondary antibodies, we used goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (ThermoFisher Cat#A32723) and Alexa Fluor Plus 647 (ThermoFisher Cat#A32728).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Neonatal rat ventricular myocytes were isolated from 2-day old Sprague-Dawley using published methods. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) were acquired commercially (Cell-type: Cor.4U, Lot numbers CB169CL_V1_1M, CB301_CL_v1_1M, CB319CL_V1_1M, CB324CL_V1_1M, CB331CL_V1_4M; Axiogenesis, Cologne, Germany). For preliminary in situ hiPSC-CM differentiation, we used cell-type PGP1-iPSC (GM23338; Church lab/Coriell Institute GM23338).

b. Describe the method of cell line authentication used.

Cell viability, sterility, and behavior were authenticated by the vendor.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells tested negative for mycoplasma contamination as specified by the provider.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Neonatal rat ventricular myocytes were isolated from 2-day old Sprague-Dawley using published methods. For histology, we obtained hearts from adult female Sprague-Dawley rats. All procedures were approved by the Harvard Animal Care and Use Committee.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.