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TITLE: Human Cardiac Microtissues to Study Sex-Dependent Genetic Determinants of Heart Failure

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Farmington, CT 06030-5335

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14. ABSTRACT
Heart failure with preserved ejection fraction (HFPEF) is a disorder with high morbidity and mortality. Unique among cardiovascular conditions, HFPEF affects women predominately more than men, but mechanisms for this sex dimorphism in HFPEF prevalence are incompletely understood. Sex-based dimorphisms have been studied mostly in non-human model systems, but are limited by incomplete recapitulation of human sex-based gene regulation and gene-environment interactions. Thus, there is a critical unmet need to develop in vitro human model systems to interrogate how sex regulates HFPEF pathogenesis and cardiac function broadly. The overarching goal of this project is to apply a cardiac microtissue assay to study sex dimorphisms in cardiac function. In Aim 1, we have generated cardiac microtissues from male and female induced cardiomyocytes (iCMs) differentiated from induced pluripotent stem cells (iPSCs) to study sex-based regulation of cardiac function. We quantified contractile function and gene expression analyses in these models. In Aim 2, we have produced the first FHL1 knockout iPS model using CRISPR/Cas9. We characterized the role of FHL1 in cardiomyocyte and cardiac tissue function using single cell and cardiac microtissues assays. Insights from this study have illuminated potential sex-dependent genetic mechanisms of HFPEF.

15. SUBJECT TERMS
Heart failure; cardiomyopathy; X chromosome; sex dimorphism; contractility; engineered heart tissue; cardiology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Heart failure with preserved ejection fraction (HFPEF) is a disorder with high morbidity and mortality. Unique among cardiovascular conditions, HFPEF affects women predominately more than men, but mechanisms for this sex dimorphism in HFPEF prevalence are incompletely understood. Sex-based dimorphisms have been studied mostly in non-human model systems, but are limited by incomplete recapitulation of human sex-based gene regulation and gene-environment interactions. Thus, there is a critical unmet need to develop in vitro human model systems to interrogate how sex regulates HFPEF pathogenesis and cardiac function broadly. The overarching goal of this project is to apply a cardiac microtissue assay to study sex dimorphisms in cardiac function. In Aim 1, we will study cardiac microtissues generated from male and female induced cardiomyocytes (iCMs) differentiated from induced pluripotent stem cells (iPSCs) to study sex-based regulation of cardiac function. In Aim 2, we will study the role of FHL1 by generating an FHL1 knockout human induced pluripotent stem cell model and characterize using cardiac microtissues assays.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Heart failure; cardiomyopathy; X chromosome; sex dimorphism; contractility; engineered heart tissue; cardiology

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goals of the project:
Aim 1: Create HFPEF cardiac microtissue (CMT) models using sex-specific human iPS-CMs and three conditions that induce HFPEF in patients (isoproterenol, angiotensin and increased mechanical load)

1A: Create CMTs from fro three pairs of male and female iPS-CMs, and measure contractility parameters in HFPEF conditions.
i. Create CMTs from pair 1 of male (M1) and female (F1) iPS-CMs – We have collected and validated set 1 of male and female human iPSCs and differentiated to iCMs. We have generated cardiac microtissues and measured contractility parameters in seven F1 and ten M1 samples.
ii. Create CMTs from pair 2 of male (M2) and female (F2) iPS-CMs- We have collected and validated set 2 of male and female human iPSCs and differentiated to iCMs. We have generated cardiac microtissues and measured contractility parameters in eight F2 samples.
iii. Create CMTs from pair 3 of male (M3) and female (F3) iPS-CMs- We have collected and validated set 3 of male and female human iPSCs and differentiated to iCMs.
iv. Computational analysis of CMT contractility assays – We have imaged and computationally analyzed all generated cardiac microtissue assays from i-iii.
Milestone (8 months): Goal analysis of ~120 CMTs analyzed for contractility parameters
Achieved: 50%

1B: Structural analysis of sex-specific CMTs using immunostaining
i. Immunostain CMTs for cardiomyocyte, fibroblast and extracellular composition – We have fixed and immunostained 30 cardiac microtissues from Aim 1A. We have performed confocal imaging.
ii. Computational analysis of imaging results-we processed 20 confocal images for tissue structure.

Milestone (12 months): Structural analysis of ~120 CMTs
Achieved: 20%

1C: Expression analysis of heart failure-associated genes by quantitative PCR. We have collected biological triplicates of male and female cardiomyocyte RNA samples. We have assessed RNA quality and have generated cDNA libraries.
Milestone (anticipated, month 14): qPCR analysis of seven gene transcripts across six iCM lines
Achieved: 10%

Aim 2: Determine the role of sex dimorphisms in FHL1 expression in HFPEF CMT assays.
2A: Create iPS lines with allelic series of FHL1 mutations using CRISPR/Cas9
i. Genome editing of iPS lines to achieve FHL1 allelic series of clones. We have generated six control and nine FHL knockout human iPSC lines using CRISPR/Cas9. We have differentiated these iPSC lines to iCMs and validated FHL1 expression analysis.
ii. Sequencing confirmation of mutations- We have Sanger sequenced 96 iPSC lines to assess FHL1 genotypes.
Milestone (8 months): Create isogenic iPS model of FHL1 deficiency
Achieved: 100%

2B: CMT assays of allelic series of FHL1 mutations
i. Measure contractility parameters in three HFPEF conditions for FHL1 allelic series of CMTs- We have measured CMT function in 15 FHL1 deficient and 15 control cardiac microtissues assays.
ii. Structural analysis of CMTs by immunostaining cardiomyocyte, fibroblast and extracellular components- We have fixed and immunostained 15 FHL1 deficient and 15 control cardiac microtissues assays.
iii. Gene expression analysis by quantitative PCR for heart-failure associated genes. We have collected biological triplicates of three control and six FHL1 deficient iCMs for transcript analysis.
Milestone (16 months): Functional analysis of FHL1 dosage in CMT function and structure.
Achieved: 75%

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met.

1) *Major activities*: To date, we have collected seven iPSc lines (3 male and 4 female) and confirmed differentiation to the cardiomyocyte lineage with high efficiency (Figure 1a). We have generated cardiac microtissue models of three lines and quantified contractility parameters (Figure 1b, c). We have engineered nine iPSC clones with FHL1 knockout mutations and six clones for isogenic controls using clustered regularly interspaced short palindromic repeat (CRISPR) technology. We have differentiated FHL1 knockout and control iPSCs to iCMs with high efficiency and characterized cellular and microtissue contractility function. We have also performed additional computational analyses of sex-based gene expression changes in human cardiac samples from GTEX data and from iCM data generated by our lab.

a

iPSC lines	Sex	Passage
M1	M	45
M2	M	23
M3	M	34
F1	F	25
F2	F	28
F3	F	13
F4	F	13

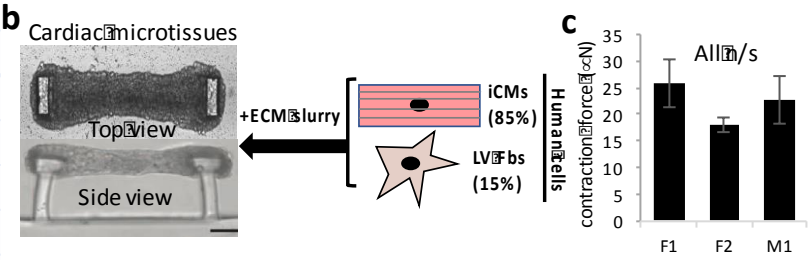


Figure 1. Sex-based differences in CMT contractility. (a) Summary table of sex-specific human iPScs collected for this study (M-male and F-female). **(b)** General outline of cardiac microtissue production (scale bar=150μm) **(c)** CMT contractility quantified by cantilever displacement and assessed for three lines (n>5 CMTs each)

2) *Specific objectives*: The specific objective in this reporting period was to assess sex-based differences in cardiac microtissue function, identify sex-based differences in gene expression and determine the role of FHL1 in iCM and cardiac microtissue function as FHL1 exhibits sex dimorphism in gene expression levels.

3) *Significant results:* To date, we have collected seven iPSC lines from three males and four females to identify lines that have high propensity for iCM differentiation. We have next generated cardiac microtissues from three of these iPSC lines to study sex-based differences in cardiac tissue function. We have also completed additional computational analysis of gene expression data sets from human heart samples obtained as part of GTEX consortium and in our series of iCM models. Through additional validation, we have a more comprehensive understanding of sex-based dimorphisms in gene expression, especially those genes with conserved expression between iCMs and in vivo cardiac samples. We also have identified limitations in the utilization of human iPSCs for studying sex dimorphisms. Finally, we have also utilized CRISPR technology to study the role of FHL1 in iCM and cardiac microtissue function. We have generated the first FHL1 knockout iPSC models nad have identified phenotypes associated with FHL1 loss. Significant and preliminary results from this project period are as follows:

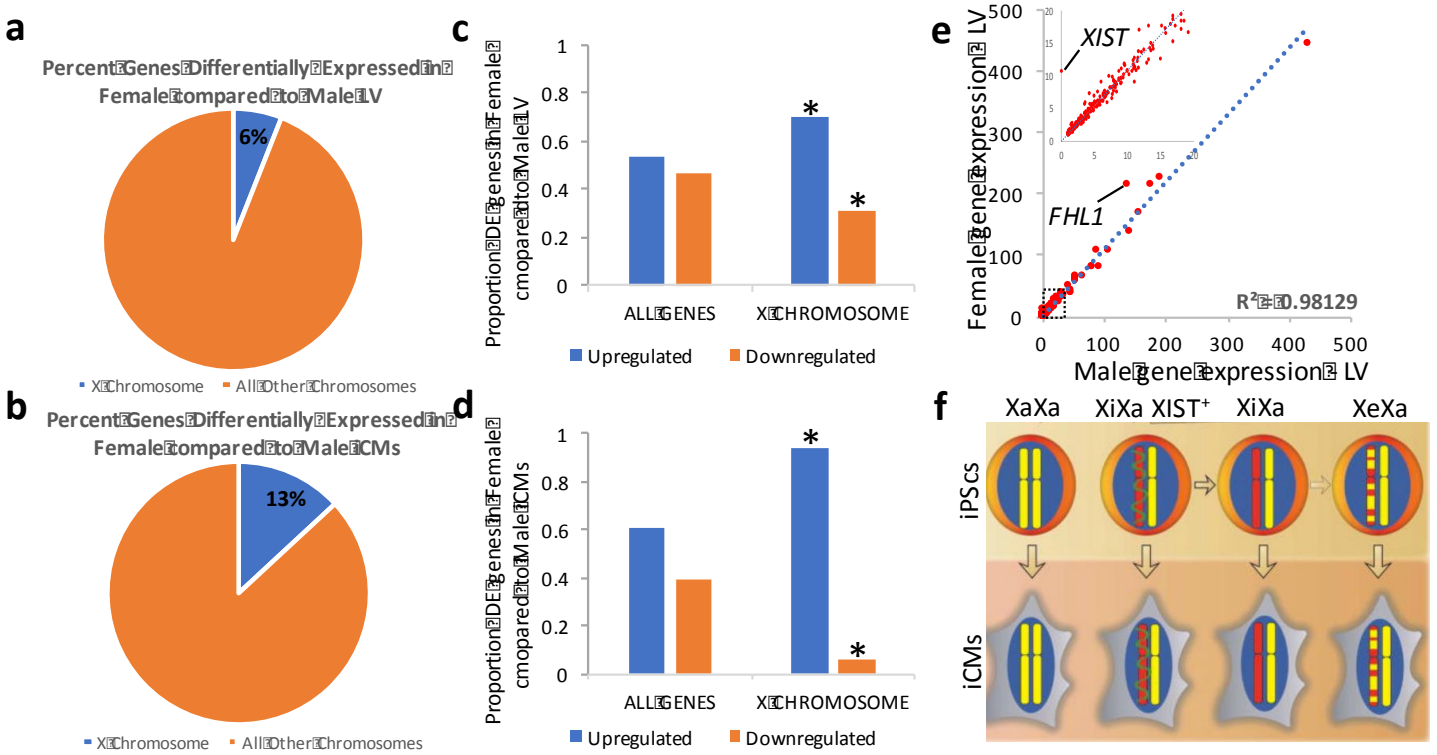


Figure 2. Summary of sex-based gene expression analysis in GTex and iCM data. (a) Sex-based differential gene expression analysis (FDR<0.1) of transcripts located on the X chromosome (blue) compared to autosomes (orange) and expressed in human cardiac left ventricle (LV) and **(b)** iCM lines. **(c)** Upregulated and downregulated gene expression based on chromosome location from LV and **(d)** iCMs (*p<0.001). **(e)** Female compared to male gene expression levels from LV tissue (note: *XIST* and *FHL1* are more highly expressed in females). **(f)** Human iPScs have four states of X chromosome activation (Xa-active (yellow), Xi-inactive (red), XIST+-coated with XIST (green), Xe-eroded)(adapted from Patel *et al*. PMID#27989715).

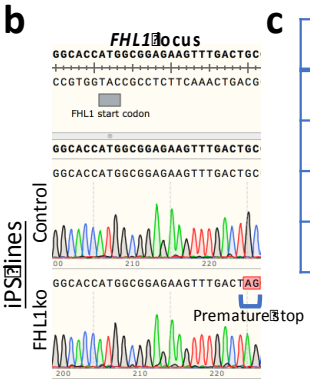
1. **Generation and phenotyping of sex-specific iCM and cardiac microtissue models.** As shown in Figure 1, we have collected seven iPSC lines from healthy males and females. We have differentiated iPSCs to iCMs, and generated cardiac microtissues from two females and one male. After generating cardiac microtissues, we found non-significant differences in contractile function at baseline. Because of low cardiomyocyte differentiation rates, we could not produce additional lines for cardiac microtissue testing to this point. We are currently optimizing cardiomyocyte differentiation methods to address this challenge prior to the next reporting period.

2. **Sex-based dimorphisms in iCM gene expression are confounded by iPSC X chromosome erosion.** As shown in Figure 2, we have analyzed differential gene expression in females compared to males from iCM and human heart datasets (left ventricle (LV) expression from GTEX). To our surprise, we identified that a disproportionate fraction of genes encoded on the X chromosome are upregulated in female iCMs compared to male iCMs when compared to LV samples (Figure 2a-d). We were surprised to identify that many of the differentially-expressed iCM genes were not shared in LV samples. However, FHL1 (focus of Aim 2) is upregulated in both datasets (Figure 2e), which further supports the role of FHL1 dosage in sex-based changes in cardiac function. During the last year, several research studies have reported that both human embryonic stem cells and iPScs are prone to unpredictable X chromosome leak, which could explain the increased expression of X chromosome genes in our female iCM lines. Several mechanisms including loss of XIST expression and epigenetic erosion have been reported (Figure 2f). Because of this confounding variable, we have paused additional experiments in Aim 1 until we can verify the status of our iPSC models.

a

Gene editing summary	FHL1 gRNA1	FHL1 gRNA2
Total number of clones analyzed	48	48
Low DNA content	4	1
Polyclonality	10	13
Overall editing efficiency	79.4%	44.1%
HR premature TAG	3.7%	26.7%
Start codon deletion	25.9%	0%
Insertion/deletion frameshift	70.4%	73.3%

b



c

Genotype	Sex	#lines
Control 1	M	3
FHL1ko 1	M	5
Control 2	M	3
FHL1ko 2	M	4

Figure 3. Summary of sex-based gene expression analysis in GTex and iCM data (a) Summary table of sex-specific human iPScs collected for this study (M-male and F-female). **(b)** General outline of cardiac microtissue production (scale bar=150μm) **(c)** CMT contractility quantified by cantilever displacement and assessed for three

3. **Generation of FHL1 knockout iPSC models to study the role of FHL1 in cardiac microtissue function.** As shown in Figure 3a, we have generated an FHL1 knockout iPSC model using CRISPR genome engineering technology. In total, we produced 96 iPSC clones, of which we obtained nine FHL1 knockout alleles that are sequence-verified and do not express FHL1 protein (Figure 3b, c). We have generated iCMs from these iPSC lines and studied iCM structure and cardiac microtissue contractility. We are excited to report that FHL1 knockout iCMs have dramatically reduced cell size and contractile function in cardiac microtissue assays (Figure 4). In sum, we studied 100 FHL1 knockout iCMs and 100 controls to assess changes in cell structure, and we generated 30 cardiac microtissues. Our leading hypothesis related to the deficit in contractility is due to diminished hypertrophic signaling, which is downstream of FHL1 function.

Stated goals not met: We have successfully collected and generated a series of male and female iPSCs that we differentiated to iCMs for further investigation. We successfully generated cardiac microtissues from male and female iCMs and studied contractile parameters by these assays. Through additional computational analysis by our group of iCM and LV (GTEX) gene expression and through new insights from published studies on X chromosome regulation in iPSCs and ESCs, we have halted using iPSC models to study sex dimorphisms in gene expression (Aim 1). This is because the X chromosome in female iPSCs is not reliably silenced in accord with in vivo X chromosome regulation. In Aim 2, we have successfully generated FHL1 knockout iPSC models and generated cardiac microtissues to study contractile function. We are meeting our goals for all components of Aim 2. Indeed, FHL1 is one of the X chromosome genes that is increased in females compared to males in both in vitro and in vivo LV samples (Figure 2e), and therefore we will continue our efforts to understand its role in sex dimorphism in HFPEF.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

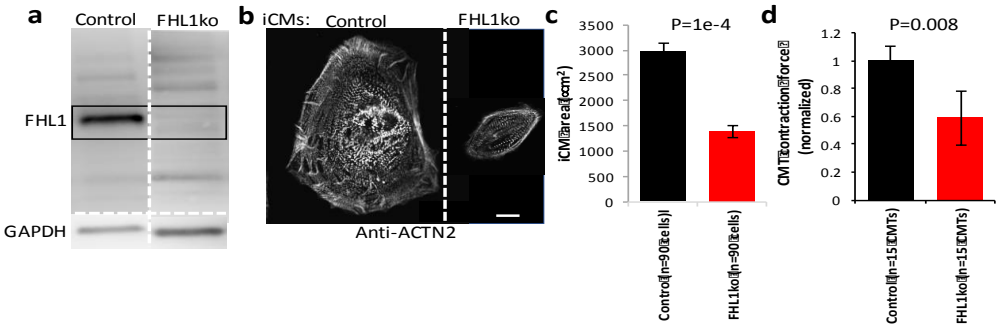


Figure 4. Characterization of FHL1 knockout iPSC models. (a) Immunoblot of iCM lysates from FHL1 knockout and controls probed with antibodies to FHL1 and GAPDH. **(b)** Representative confocal image of control and FHL1 knockout iCMs stained with antibodies to cardiac alpha actinin (ACTN2)(scale=10μm) **(c)** iCM cell area analysis of control and FHL1 knockout iCMs. **(d)** iCM cell area analysis of control

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Professional Development:
Yu-Sheng Chen and I meet every week for one-on-one mentoring sessions to discuss experimental results, progress and data interpretation.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We have presented this data to the UConn Health Department of Cell Biology quarterly meetings. We have also presented this to the UConn Health Department of Medicine Grand Rounds in May 2018. We are presenting these data to "Work-In-Progress" meetings at The Jackson Laboratory.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are happy to report on our progress towards generating male and female iCMs and cardiac microtissues for investigation of sex dimorphism in HFPEF pathogenesis. Our ongoing comprehensive computational analyses of gene expression in iCMs and LV have demonstrated that the iPSC model is an unreliable model system to study human sex dimorphisms in gene expression because of incomplete and unpredictable X chromosome activation status. Therefore, further development of Aim 1 experiments would not be warranted. Instead, we propose to continue to study the role of FHL1 (Aim 2) as this gene is expressed more highly in iCMs and LV. The study of FHL1 by using FHL1 knockout iPSC models would not be affected by the X chromosome status. We will accomplish the goals of the study by the following:

2b: CMT assays of allelic series of FHL1 mutations
Milestone (anticipated, month 18): Comprehensive analysis of FHL1 knockout and control iCMs and cardiac microtissues including transcriptome analysis.

Plans moving forward: We will continue to generate FHL1 knockout iCMs and cardiac tissues for ongoing comprehensive functional interrogation including effects on cell structure, hypertrophic signaling and gene expression. We will perform transcriptome analysis to identify differential gene expression, gene expression networks and pathways perturbed by FHL1 loss of function.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

In accord with recent scientific studies, our study has confirmed that the X chromosome activation status in human iPSCs is unreliable, and the use of sex-specific iPSCs for studying heart disease is challenging. This is because unlike human cells within an individual, human cells in cell culture have increased expression especially from the X chromosome because of "expression leak." Despite this limitation, our study does confirm that the FHL1 gene is more highly expressed in female hearts compared to males, and we have generated the first FHL1 knockout human stem cell model that can be used to identify the role of FHL1 in heart function broadly. This is a unique resource to the biomedical field, and will inform how FHL1 dosage affects heart function as female hearts have higher FHL1 levels compared to males.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Our sex-specific iPSC study has led to new collaborations with Dr. Peter Robinson's group. The Robinson lab studies splicing, and has identified changes in gene splicing that are different in female hearts compared to male hearts by GTEX data analysis similar to our analyses for gene expression levels. Many of these isoforms are critical for cardiac contractility and relaxation, and we are collaborating to begin to develop functional assays to study the relevance of these splicing changes that are unique to female hearts. We are collaborating on a manuscript that will be submitted later this year.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

None to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

None to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Not applicable; project does not involve the use of vertebrate animals.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

None to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	*Dr. Travis Hinson*
Project Role:	*PD/PI*
Research Identifier:	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Hinson has managed the project, directing both the experiments and analyses completed by Chen.*

Name:	*Dr. Yu-Sheng Chen*
Project Role:	*Fellow*
Research Identifier:	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Chen has generated data, interpreted results and compiled figures for this proposal.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Since the commencement of this award, Dr. Hinson's grant titled; "Myosin Activators in IPS Models of Dilated Cardiomyopathy" expired on 6/15/18. .24 calendar months of effort was maintained throughout the life of the award. Also, his pending NIH/NHLBI application titled; "Comprehensive Analysis of Allelic, Cellular and Molecular Heterogeneity in Human 3-Dimensional Cardiac Microtissues with MYH7 Mutations" was awarded and began on 7/1/18. Dr. Hinson's effort is 3.0 calendar months on this award. Neither of these changes impacts the effort on the project that is the subject of this report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: None

QUAD CHARTS: Nione

9. APPENDICES: None