AWARD NUMBER: W81XWH-19-1-0206

TITLE: Exploring and Exploiting Novel Mammalian Regeneration Models

PRINCIPAL INVESTIGATOR: Guo Huang

CONTRACTING ORGANIZATION: The Regents of the University of California, San Francisco

REPORT DATE: SEPTEMBER 2020

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution is unlimited.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2020	Annual	1SEPT2019 - 31AUG2020

4. TITLE AND SUBTITLE

Exploring and Exploiting Novel Mammalian Heart Regeneration Models

5a. CONTRACT NUMBER
W81XWH-19-1-0206

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Guo Huang, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University
of California, San Francisco
1855 Folsom Street, Ste 425
San Francisco, CA 94143-4249

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Many lower vertebrate animals and even mammals at early developmental stages possess striking abilities to restore damaged and lost heart tissues. However, most adult mammals examined so far lack robust cardiac regenerative potential. It still remains enigmatic why such a seemingly beneficial trait is lost in animal evolution, and among 6,399 mammalian species whether there are any animals that still retain significant cardiac regenerative potentials. Here we present our pilot work in searching and charactering novel mammalian models with possible but previously unknown cardiac regenerative capability. Our preliminary analyses suggest the existence of unusual cardiac regenerative capability in certain mammals such as naked mole-rats. In this proposal, we will perform analysis of cardiomyocyte ploidy, a proxy of cardiac regenerative potential, in mammalian species, especially those with low metabolic rates and body temperatures. In addition, we will examine cardiomyocyte proliferative and regenerative potential in adult naked mole-rats and even mouse-naked mole-rat interspecies chimeras. Our strategy of exploring and exploiting new organisms for the study of regenerative biology is generalizable and can be applied to discovering animal and plant species with extraordinary yet unreported physiology and capability. We envision that novel mammalian regeneration models will provide new paradigms for investigation of tissue renewal capability of various organs and appendages, and may yield unprecedented insights into the fundamental principles governing tissue regeneration in animal development and evolution.

15. SUBJECT TERMS
Evolution, heart regeneration, naked mole-rats

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	16	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

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

This study aims to identify transition species in evolution that retain significant cardiac regenerative potential and investigate naked mole-rats as such a candidate. Our work will provide novel insights into the mechanism underlying the lack of cardiac regenerative capacity in adult human.

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

Evolution, heart regeneration, naked mole-rats

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

HYPOTHESIS Based on our preliminary data, we formulate the following central hypothesis: **mammalian species with low metabolic rates and body temperatures – such as naked mole rats (NMRs) – have abundant diploid cardiomyocytes and may retain significant cellular regenerative potential**.

Aim 1. Perform phylogenetic analysis of cardiomyocyte ploidy and decode the design principle in evolution.
Aim 2. Investigate cardiomyocyte proliferative and regenerative potential of NMRs and mouse-NMR interspecies chimeras.

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. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities

I have been invited to present my research related to this grant in the following events.

Conferences/symposiums/workshops

2020 Basic Cardiovascular Sciences 2020 Scientific Sessions, Chicago, IL
2020 Annual meeting for Society of Developmental Biology, Chicago, IL

Departmental/university seminars

2020 Cardiovascular Disease Program, National University of Singapore, Singapore
2020 Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX
2019 Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY
2019 The Heart Institute, Cincinnati Children's Medical Center, Cincinnati, OH
2019 Distinguished Lecture Series, University of Pennsylvania, Philadelphia, PA

Local seminars

2019 UCSF Annual TETRAD Graduate Program Research Conference, Lake Tahoe, CA
2019 Parnassus Faculty Talk, UCSF, San Francisco, CA

2) Specific objectives

Not applicable.

3) Significant results or key outcomes

Aim 1. Perform phylogenetic analysis of cardiomyocyte ploidy and decode the design principle.

We have collected the hearts from the proposed monotreme, edentate, and cetacean species. CM nucleation and ploidy have been analyzed (**Figure 1**).

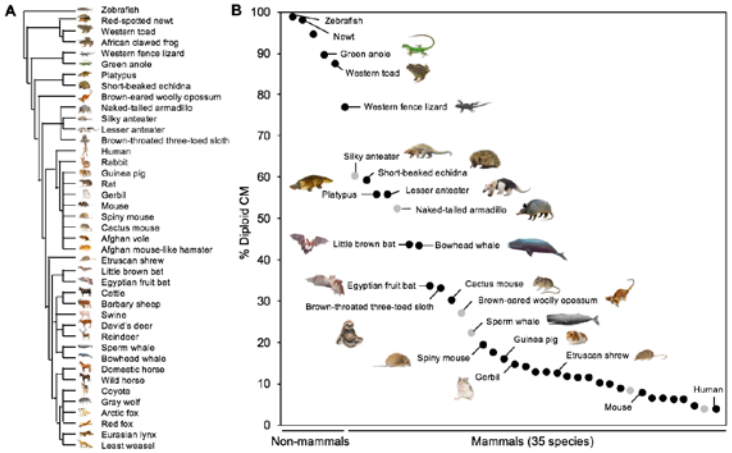

Figure 1. **Phylogenetic analysis of vertebrate cardiomyocyte (CM) nucleation and ploidy.** (A) Cladogram of species. (B) Percentages of mononucleated diploid CMs. Each dot represents the value from the adult heart(s) of one species (black: multiple samples are quantified; grey: only a single specimen is collected and analyzed). The data are listed from left to right based on the value of diploid CMs.

Consistent with our hypothesis, these animals have more diploid CMs than most mammals. In addition, we discovered that animals with more diploid CMs have lower metabolic rates (**Figure 2A**). Following Boltzmann distribution, we derived how CM ploidy is related to $e^{-1000/T}$, thermodynamic temperature T and body temperature t, and found that animals with more diploid CMs have lower body temperatures (**Figure 2B**). These data suggest that heart regenerative potential may become lost during poikilotherm-to-endotherm transition in both animal evolution and postnatal growth. The above findings contribute significantly to a recent major publication from my group (Hirose et al. Huang, ***Science*** 2019).

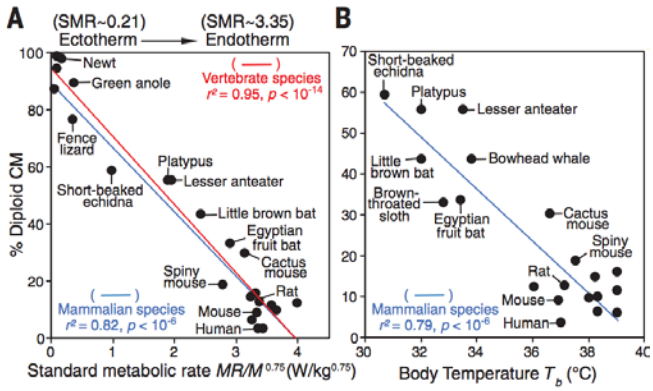

Figure 2. Diploid CM frequency inversely correlates with standard metabolic rate (A) and body temperature (B).

Our current analysis shows that the power expended by the heart (cardiac output *W*) in overcoming viscous forces is a size-independent fraction of metabolic rate (*BMR*), i.e. $W \propto BMR$.

Here *W* is a function of the volume rate of fluid flow (*Q*) and blood pressure (Δp) as $W = Q \Delta p$. It is known that BMR determines the volume rate of fluid flow (*Q*) that transports oxygen and nutrients for metabolism as $Q \propto BMR$. *BMR* scales to body mass (*M*) as $BMR \propto M^{3/4}$, thus $Q \propto M^{3/4}$. While blood pressure Δp is independent of body mass (both mathematically and experimentally supported), cardiac output $W = Q \Delta p \propto M^{3/4}$, which consequently means $W \propto BMR$. Thus, increases of basal metabolic rate in evolution and development would increase blood flow through the cardiovascular system as powered by increase of cardiac output.

<u>Aim 2. Investigate naked mole rats (NMR) and bats as putative cardiac regenerative models.</u>

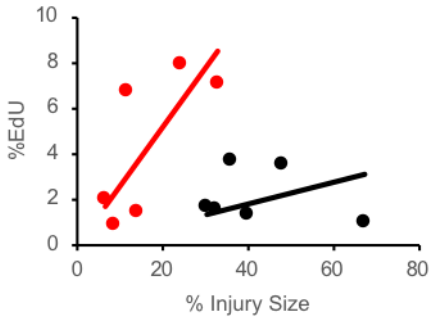

Figure 3. Adult cardiomyocytes of naked mole rats (red) are more proliferative than those of mice (black) after myocardial injury. Each dot represents one animal.

We have performed myocardial infarction surgery on a batch of NMRs and mice. Although we did not observe heart functional improvement, we did observe enhanced cardiomyocyte proliferation when compared with mice (**Figure 3**). There are at least two possible interpretation. First, even though adult NMR cardiomyocytes retain significant proliferative potential, the post-injury expansion of cardiomyocytes is not robust enough to yield functional restoration. Second, other factors such as the inability to form new blood vessels in the infarcted area in NMR may limit the extent of regeneration.

Due to COVID19, we have stopped NMR iPS culture because our transgenic core was closed for four months. Now the school is operated at a 25% density, and we have restarted NMR iPS culture. We will schedule for injection of NMR iPS cells into mouse blastocysts in the next several months.

4) Other achievements: none.

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."

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.

This grant provided opportunities for my trainees to attend and present in the following conferences.

Kentaro Hirose (Postdoc fellow) Oral presentation
2019 Weinstein Meeting in Cardiovascular Development and Regeneration, Indianapolis, IN

Alex Payumo (Postdoc fellow) Poster Presentation
2019 Gordon Conference on Tissue Regeneration and Repair, New London, NH

Stephen Cutie (Ph.D. student) Poster presentation
2019 Pan-American Evo-Devo Conference, Miami, FL

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 published four papers supported in part by this grant (see also in Product).

a. Hirose, K., Payumo, A. Y., Cutie, S., Hoang, A., Zhang, H., Lunn, D., Gu, L., Bigley, R. B., Yu, H., Wang, J., Smith, M., Gillett, E., Wilson, E., Field, K. A., Reeder, D. M., Maden, M., Yarsev, M. M., Grutzner, F., Scanlan, T. S., Flamant, F., Buffenstein, R., Hu, G., Olgin, J. E., & **Huang, G. N.** (2019) Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. ***Science*** 364:184-188. PMCID:PMC6541389.

b. Payumo, A. Y. & **Huang, G. N.** (2020) Lamin B2, guardian of cardiomyocyte nuclear division. ***Dev Cell.*** 53(1): 5-7.

c. Judd, J., Lovas, J., & **Huang, G. N.** (2019) Defined factors to reactivate cell cycle activity in adult mouse cardiomyocytes. ***Scientific Reports*** 9 (1): 18830. PMCID:PMC6906479

d. Cutie, S., Payumo. A. Y., Lunn, D., & **Huang, G. N.** (2020) *In vitro* and *in vivo* roles of glucocorticoid and vitamin D receptors in the control of neonatal cardiomyocyte proliferative potential. ***J Mol Cell Cardiol.*** 142:126-134. PMID:32289320

Our findings in the *Science* paper have been reported by 11 news stories from 11 outlets and disseminated by 279 tweeters. https://www.altmetric.com/details/56660658/news

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

In the next reporting period, we plan to (1) submit the paper describing the enhanced cardiomyocyte proliferative activity in NMR, and (2) generate mouse-NMR chimeric animals.

4. **IMPACT:** D*escribe 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).

We provide evidences for a new theory that our ability to regenerate the heart gets lost when we become warm-blooded, thus the loss of cardiac regenerative potential may be a tradeoff of our gain of the activity to generate body heat and maintain body temperature.

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.

Nothing to report.

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.*

This provocative idea has stimulated widespread interests in both scientific and general public community. These findings have been reported by 11 news stories from 11 outlets (including *Scientific American*) and disseminated by 279 tweeters. https://www.altmetric.com/details/56660658/news

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

Nothing 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.

We had a major delay due to COVID19 and complete shutdown of research activity. Now we are trying to catch up as much as we can.

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.

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

None.

Significant changes in use of biohazards and/or select agents

None.

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).*

Hirose, K., Payumo, A. Y., Cutie, S., Hoang, A., Zhang, H., Lunn, D., Gu, L., Bigley, R. B., Yu, H., Wang, J., Smith, M., Gillett, E., Wilson, E., Field, K. A., Reeder, D. M., Maden, M., Yarsev, M. M., Grutzner, F., Scanlan, T. S., Flamant, F., Buffenstein, R., Hu, G., Olgin, J. E., & **Huang, G. N.** (2019) Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. ***Science*** 364:184-188. PMCID:PMC6541389.

Payumo, A. Y. & **Huang, G. N**. (2020) Lamin B2, guardian of cardiomyocyte nuclear division. ***Dev Cell.*** 53(1): 5-7.

Judd, J., Lovas, J., & **Huang, G. N.** (2019) Defined factors to reactivate cell cycle activity in adult mouse cardiomyocytes. ***Scientific Reports*** 9 (1): 18830. PMCID:PMC6906479

Cutie, S., Payumo. A. Y., Lunn, D., & Huang, G. N. (2020) In vitro and in vivo roles of glucocorticoid and vitamin D receptors in the control of neonatal cardiomyocyte proliferative potential. J Mol Cell Cardiol. 142:126-134. PMID:32289320

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).

None.

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.*

Conferences/symposiums/workshops
2020 Basic Cardiovascular Sciences 2020 Scientific Sessions, Chicago, IL
2020 Annual meeting for Society of Developmental Biology, Chicago, IL

Departmental/university seminars
2020 Cardiovascular Disease Program, National University of Singapore, Singapore
2020 Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX
2019 Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY
2019 The Heart Institute, Cincinnati Children's Medical Center, Cincinnati, OH
2019 Distinguished Lecture Series, University of Pennsylvania, Philadelphia, PA

Local seminars
2019 UCSF Annual TETRAD Graduate Program Research Conference, Lake Tahoe, CA
2019 Parnassus Faculty Talk, UCSF, San Francisco, CA

Trainee presentation

Kentaro Hirose (Postdoc fellow) Oral presentation
2019 Weinstein Meeting in Cardiovascular Development and Regeneration, Indianapolis, IN

Alex Payumo (Postdoc fellow) Poster Presentation
2019 Gordon Conference on Tissue Regeneration and Repair, New London, NH

Stephen Cutie (Ph.D. student) Poster presentation
2019 Pan-American Evo-Devo Conference, Miami, FL

- **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.

Our findings have been reported by 11 news stories from 11 outlets. Websites and details can be found here. https://www.altmetric.com/details/56660658/news

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

None.

- **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;*
- *physical 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.*

None.

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Guo Huang, PhD – Principal Investigator: 0.6 calendar months
Xi Chen, PhD – Postdoctoral Researcher: 4.5 calendar months (as proposed)
Sheamin Khyeam – Junior Specialist: 3 calendar months. Shea has quantified naked mole-rat and mouse cardiomyocyte proliferation (Ki67, pHH3, EdU) after myocardial infarction, and helped maintain the naked mole-rat iPS cell culture. She also contributed to figure generation in our published papers.
Alison Hoang – Staff Research Associate: 3 calendar months. Alison has analyzed cardiomyocyte ploidy for echidnas, platypus, brown-eared woolly opossum, silk anteater, lesser anteater, naked-tailed armadillo, brown-throated three-toed sloth, bowhead whale, sperm whale and beluga whale, and contributed to the manuscript writing and figure making of our published papers.

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.

No change since JIT

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*

Organization Name: University of Ottawa
Location of Organization: (if foreign location list country): Ottawa, Canada
Partner's contribution to the project: Matthew Pamenter and Adam Shuhendler performed a myocardial infarction model in naked mole-rats, measured cardiac function post injury, and sent the hearts of naked mole-rats to the Huang lab for molecular and histological analyses.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.