APPLICATION FOR FEDERAL ASSISTANCE

SF 424 R&R

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION

❍Pre-application ●Application ❍Changed/Corrected Application

2. DATE SUBMITTED

Applicant Identifier

4. a. Federal Identifier

b. Agency Routing Identifier

c. Previous Grants.gov Tracking ID

5. APPLICANT INFORMATION **UEI:** H1HYA8Z1NTM5

Legal Name: University of Kentucky Research Foundation
Department:
Division:
Street1: 500 South Limestone
Street2: 109 Kinkead Hall
City: Lexington
County/Parish: Fayette
State: KY: Kentucky
Province:
Country: USA: UNITED STATES
ZIP / Postal Code: 40526-0001

Person to be contacted on matters involving this application

Prefix:	First Name:	Middle Name:	Last Name:	Suffix:
	Cynthia	J	Curvin	

Position/Title: Research Administrator Sr
Department:
Division:
Street1: 500 South Limestone
Street2: 109 Kinkead Hall
City: Lexington
County/Parish:
State: KY: Kentucky
Province:
Country: USA: UNITED STATES
ZIP / Postal Code: 40526-0001
Phone Number: (859) 257-9420
Fax Number: (859) 323-1060
Email: ospa@uky.edu

6. EMPLOYER IDENTIFICATION NUMBER*(EIN) or (TIN)*: 1-616033693-C4

7. TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education
Other (Specify):
Small Business Organization Type ❍Women Owned ❍Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

●New ❍Resubmission
❍Renewal ❍Continuation ❍Revision

If Revision, mark appropriate box(es).

❍A. Increase Award ❍B. Decrease Award ❍C. Increase Duration
❍D. Decrease Duration ❍E. Other*(specify)*:

Is this application being submitted to other agencies? ❍ Yes ● No What other Agencies?

9. NAME OF FEDERAL AGENCY:
National Institutes of Health

10. ASSISTANCE LISTING NUMBER:
ASSISTANCE LISTING TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Contribution of Titin-Truncating Variants to Human Non-Ischemic Cardiomyopathy

12. PROPOSED PROJECT:

Start Date	Ending Date
12/01/2027	11/30/2028

13. CONGRESSIONAL DISTRICT OF THE APPLICANT:
KY-006

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	First Name:	Middle Name:	Last Name:	Suffix:
	Austin	Tyler	Minton	

Position/Title: Graduate Student
Organization Name: University of Kentucky Research Foundation
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Division: Medicine
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State: KY: Kentucky
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Country: USA: UNITED STATES
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15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	$108,955.00
b. Total Non-Federal Funds	$0.00
c. Total Federal & Non-Federal Funds	$108,955.00
d. Estimated Program Income	$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

●I agree

The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL (Disclosure of Lobbying Activities) or other Explanatory Documentation. File Name: Mime Type:

19. Authorized Representative

Prefix:	First Name:	Middle Name:	Last Name:	Suffix:
	Kim	C	Carter	

Position/Title: Associate Director
Organization Name: University of Kentucky Research Foundation
Department: Sponsored Projects Administrat
Division: Research
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City: Lexington
County/Parish: Fayette
State: KY: Kentucky
Province:
Country: USA: UNITED STATES
ZIP / Postal Code: 40526-0001
Phone Number: 8592579420
Fax Number: 8593231060
Email: ospa@uky.edu

Signature of Authorized Representative **Date Signed**

20. Pre-application File Name: Mime Type:

21. Cover Letter Attachment File Name: cover_letter1025621155.pdf Mime Type: application/pdf

COVER LETTER

Application title: Contribution of Titin-Truncating Variants to Human Non-Ischemic Cardiomyopathy

FOA Number: PA-23-272

List of referees (including name, departmental affiliation, and institution):

1. **Kerry McDonald, PhD**
   Bolm Distinguished Professor
   George L. and Melna A. Bolm Distinguished Chair in Cardiovascular Health
   Chair of Medical Pharmacology and Physiology
   Department of Medical Pharmacology and Physiology
   School of Medicine, University of Missouri

2. **Vedant Gupta, MD, FACC, FSCCT**
   Associate Professor of Medicine
   Associate Program Director, Cardiology Fellowship
   Associate Chief, Division of Cardiovascular Medicine
   Director, Ambulatory Services
   Department of Internal Medicine
   College of Medicine, University of Kentucky

3. **Esther Dupont-Versteegden, PhD**
   Professor, Physical Therapy
   Director of the Rehabilitation Sciences Doctoral Program
   Director of Graduate Studies, Rehabilitation and Health Sciences
   Director, Center for Muscle Biology
   Department of Physical Therapy
   College of Health Sciences, University of Kentucky

4. **Rachel Pritchard, PhD**
   Assistant Professor, Biological and Physical Sciences
   Department of Biological and Physical Sciences
   Department of Newark
   Central Ohio Technical College

Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: University of Kentucky Research Foundation

* Street1: 500 South Limestone		Street2: 109 Kinkead Hall
* City: Lexington	County: Fayette	* State: KY: Kentucky
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 40526-0001
UEI: H1HYA8Z1NTM5	* Project/Performance Site Congressional District: KY-006	

File Name	Mime Type

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ● Yes ❍ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If yes, check appropriate exemption number

Exemption Number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If no, is the IRB review Pending? ● Yes ❍ No

IRB Approval Date:

Human Subject Assurance Number 00005295

2. * Are Vertebrate Animals Used? ❍ Yes ● No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ❍ Yes ● No

4.a. * Does the Project have an Actual or Perceived Impact – positive or negative – on the environment? ❍ Yes ● No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5.a. * Is the research performance site designated, or eligible to be designated, as a historic place? ❍ Yes ● No

5.b. If yes, please explain:

6.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ❍ Yes ● No

6.b. If yes, identify countries:

6.c. Optional Explanation:

7. Project Summary/Abstract	project_summary_abstract1025621133.pdf	Mime Type: application/pdf
8. Project Narrative	project_narrative1025621134.pdf	Mime Type: application/pdf
9. Bibliography & References Cited	bibliography_refCited1025620813.pdf	Mime Type: application/pdf
10. Facilities & Other Resources	facilities__1_1025621135.pdf	Mime Type: application/pdf
11. Equipment	Revised_equipment__2_1025621136.pdf	Mime Type: application/pdf

PROJECT SUMMARY/ABSTRACT

Heart failure affects 1 in 4 individuals during their lifetime, with non-ischemic cardiomyopathy being one of the least understood forms. Inherited variants account for ~30% of non-ischemic cardiomyopathy with truncating variants in the *TTN* gene (TTNtvs) being the most common sub-type. Titin is expressed in striated muscle cells and is essential for sarcomere formation and force generation. Since titin is also the largest known protein, I hypothesize that cellular control pathways can become overloaded in patients who have a TTNtv. This would lead to accumulation of damaged proteins, compromised sarcomere integrity, and diminished cardiac performance.

As an F31 Fellow, I will investigate how TTNtvs affect RNA and protein turnover, lipofuscin buildup, sarcomeric titin integration, and cardiac mechanics. The central hypothesis is that TTNtvs overload RNA surveillance and proteasomal systems, leading to defective protein turnover, accumulation of lipofuscin, and improper integration of truncated titin into sarcomeres. My experiments will leverage my lab's large biobank of human myocardium. I have identified 24 patients with TTNtvs and non-ischemic cardiomyopathy that are available for this project with many more samples from patients with non-genetic non-ischemic cardiomyopathies and organ donor controls.

Aim 1 focuses on how TTNtvs impact RNA degradation by analyzing nonsense-mediated and exosomal decay pathways in human samples. This will help determine whether TTNtvs upregulate the activity of RNA surveillance mechanisms to clear faulty transcripts.

Aim 2 will investigate the downstream effects of TTNtvs on proteasomal degradation and lipofuscin accumulation. Using immunoblotting and image segmentation techniques, I will measure K48-linked polyubiquitination of titin and quantify lipofuscin granules in affected myocardial tissue.

Aim 3 assesses the integration of truncated titin into sarcomeres and its impact on cardiac mechanics. Immunohistochemistry will illuminate truncated titin filaments in sarcomeric structures, and biophysical testing will measure maximal isometric and passive forces in triton-permeabilized fibers.

The outcomes will provide invaluable insights into how TTNtvs disrupt cellular protein quality control, promote disease progression, and alter myocardial mechanics. These findings could uncover biomarkers and therapeutic targets for TTNtv-associated non-ischemic cardiomyopathy, leading to potential treatments that enhance protein turnover and mitigate disease progression.

PROJECT NARRATIVE

This fellowship will provide me with advanced training in genetic mechanisms that lead to heart failure, the most common cause of hospitalization in the United States. My project will investigate why variants in the *TTN* gene are a frequent cause of the disease, and findings may uncover new therapeutic targets and improve treatment strategies.

BIBLIOGRAPHY AND REFERENCES CITED

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2. Bozkurt B, Ahmad T, Alexander K, et al. HF STATS 2024: Heart Failure Epidemiology and Outcomes Statistics An Updated 2024 Report from the Heart Failure Society of America. *J Card Fail.* 2025;31(1):66-116.
3. Chang AN, Potter JD. Sarcomeric protein mutations in dilated cardiomyopathy. *Heart Fail Rev.* 2005;10(3):225-235.
4. Lian H, Song S, Chen W, Shi A, Jiang H, Hu S. Genetic characterization of dilated cardiomyopathy patients undergoing heart transplantation in the Chinese population by whole-exome sequencing. *J Transl Med.* 2023;21(1):476.
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FACILITIES & OTHER RESOURCES

Laboratory: The Sponsor, Ken Campbell, PhD, is a Professor in the Division of Cardiovascular Medicine at the University of Kentucky. His lab space was remodeled in 2023 and now comprises 1800 square feet of contiguous space with specific areas allocated for wet experiments, sample processing and histology, and biophysical assays.

Office: Dr. Campbell has a new office (212 square feet) located on the same floor as his laboratory. His team have a dedicated conference room (348 square feet) as well as adequate desk-space for lab personnel. Dr Minton shares a large office (~320 square feet) with 3 other graduate students. Lab personnel share an all-in-one scanner/printer which is provided by the department.

Animal: Not applicable to this project.

Computing: Excellent computer resources are available for this project. Each member of Dr. Campbell's laboratory has a modern laptop supplied by the university. At least 5 additional computers are attached to experimental equipment. Three high-end Titan workstations (each with 256 GB of RAM and capable of running 128 threads simultaneously) are available as required. Austin is able to connect to these computers from off-campus which allows him to run analyses and access data from home or while traveling. The university has additional computing power which the team could access for free if it was useful.

All systems are password protected, backed up nightly to off-site storage systems, and protected by a firewall. Computer code, protocols, solution recipes, manuals, and templates are stored, shared, and backed up using GitHub. LabArchives is used as an electronic lab notebook.

Clinical: The University of Kentucky Chandler Hospital is a major academic medical center which provides quaternary level care for ~2 million people. 40,000 patients have been diagnosed with heart failure (ICD10 I50.9) The institution is currently performing ~1% of the world's cardiac transplants (~160 in the last 4 years) and implants another ~40 Ventricular Assist Devices per year. The Mikel D. and Annette C. Smith Echocardiography Lab was the first in Kentucky to receive national accreditation and performs ~100 clinical scans every day.

Dr. Campbell transitioned from the Department of Physiology to the Division of Cardiovascular Medicine in 2022 and became the division's inaugural Director of Translational Research. As part of that role, Dr. Campbell co-directs the Myocardial Recovery Alliance which focuses on heart failure and mechanical circulatory support.

Dr. Campbell leads 3 IRB protocols that cover procurement of biospecimens from patients who provide informed consent and unrestricted research-related access to clinical data from all patients who have received cardiovascular care at the institution (see Protection of Human Subjects).

Other

University of Kentucky Center for Clinical and Translational Sciences (CTSA) is the University of Kentucky's implementation of an NIH-funded CTSA center. The main purpose of this program is to foster and accelerate translational research. Dr. Campbell directs the Center's Biospecimens Core and leads an institution-wide biobanking program that has enrolled >60,000 patients since November of 2013. This center also provides seminars and workshops to discuss bioethics, handling clinical data, and other topics relevant to translational research, which Austin will take advantage of as part of his training plan.

Office of Research Integrity (ORI) is the University of Kentucky's central facility that supports 7 federally mandated review committees: 3 medical and 2 non-medical Institutional Review Boards (IRBs), the Institutional Animal Care and Use Committee (IACUC), and the Radioactive Drug Research Committee (RDRC). The university veterinarian provides guidance in animal care. The ORI maintains an extensive education and training program in all facets of basic and clinical research available for Austin as part of his continuous Responsible Conduct of Research training.

University of Kentucky Biostatistics Consulting Service is jointly supported by the University of Kentucky Colleges of Medicine and Public Health. This multidisciplinary unit provides a broad array of biostatistical and epidemiological consulting services to the entire University of Kentucky community. This service also assists with experimental design, data analysis, and power analysis for intramural and extramural grants.

University of Kentucky Environmental Health and Safety (EHS) is responsible for safety compliance in all operations, including research. EHS assists investigators with laboratory assessments to determine environmental, health, and safety needs and informs them of compliance requirements for their research and assigned space. Assessment results are used to direct investigators to appropriate research review and training resources, as needed (e.g., biological, chemical, or radiological safety). A mandatory chemical hygiene plan, personal protective gear, appropriate fume hoods, and eyewash/safety showers are core components of the annual laboratory inspection and certification process.

University of Kentucky Center for Muscle Biology (CMB) is a group of ~40 PIs and their respective laboratories conducting muscle-oriented research. The Center's mission is to integrate basic, clinical, and translational research at the University of Kentucky to catalyze research projects, strengthen grant applications, and serve as a hub for interdisciplinary research. The network allows for a community of openness between muscle researchers and resource-sharing amongst participating laboratories. The Center houses the Molecular Immunohistochemistry and Molecular Imaging Core and an automated data-processing program for immunohistochemistry (developed in-house). A member of Austin's advisory committee, Esther Dupont-Versteegden, PhD, serves as the director of the Center, which provides unique access to their resources.

University of Kentucky Medical Center Library consolidates the collections and services of all University of Kentucky health profession colleges, making them available to the entire University's community, along with patients and their families. As a resource library with the National Network of Libraries of Medicine (NNLM), Greater Midwest Region (GMR), and a designated outreach library for the Commonwealth of Kentucky, the Library supports outreach efforts designed to facilitate access to health information for users located across the Commonwealth.

University of Kentucky Light Microscopy Core is a multi-faceted facility that offers confocal (Aim 3a), super-resolution, and laser-capture microscopy, along with microscope slide scanners (Aim 2b). The Core contains a central laboratory for sample preparation and computer workstations for data analysis and image processing. Technical support offers training on instrumentation to allow independent use or complete processing and imaging by on-site staff. Austin has already completed trainings to independently book and use the confocal microscopes and slide scanners.

EQUIPMENT

The applicant has access to all major equipment needed for the completion of this project.

Tissue Processing and Biochemical Assays

Histology and biochemical assays will be performed in all Aims of this proposal using equipment in the Sponsor's lab, which is fully equipped for these techniques. Relevant equipment within the lab includes an ultra-pure deionized water supply, a top-ban balance, dissection microscopes, a modern cryostat, gel electrophoresis and Western blot setups, a ChemiDoc, ventilated fume hoods, refrigerated centrifuges, -20^{o}C freezers, a -80^{o}C freezer, cryogenic storage systems.

Slides in Aims 2.2 and 3.1 will be imaged using a Zeiss Axioscan Z7 and Nikon AXR Confocal microscope in the University of Kentucky's Light Microscopy Core.

Contractile Measurements

Contractile assays using permeabilized multicellular preparations will be performed as part of Aim 3 using 1 of 3 highly specialized setups available in the Sponsor's lab. Relevant equipment includes an inverted microscope with video attachment, a vibration isolation table, a force transducer, a length controller, and high speed (>1000 frames per second) video cameras.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Austin Middle Name Tyler Last Name*: Minton Suffix:

Position/Title*:	Graduate Student
Organization Name*:	University of Kentucky Research Foundation
Department:	Physiology
Division:	Medicine
Street1*:	780 Rose St
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City*:	Lexington
County:	
State*:	KY: Kentucky
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	40508-0000

Phone Number*: 859-323-8158 Fax Number: E-Mail*: austin.minton@uky.edu

Credential, e.g., agency login: AUSTIN.MINTON

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	biosketch_AustinMinton1025621137.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix: First Name*: Kenneth Middle Name S Last Name*: Campbell Suffix:

Position/Title*:	Professor/Director of Translational Research
Organization Name*:	University of Kentucky Research Foundation
Department:	Internal Medicine
Division:	Medicine
Street1*:	780 Rose Street
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Province:	
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Zip / Postal Code*:	40536-0298

Phone Number*: 8593238157 Fax Number: E-Mail*: KSCAMP3@uky.edu

Credential, e.g., agency login: KEN.CAMPBELL

Project Role*: Other (Specify) Other Project Role Category: Sponsor

Degree Type: Degree Year:

	File Name
Attach Biographical Sketch*:	biosketch_Campbell1025621138.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Austin Minton

eRA COMMONS USER NAME (credential, e.g., agency login): AUSTIN.MINTON

POSITION TITLE: Graduate Student

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Kentucky Wesleyan College, Owensboro, Kentucky	BS (Hons)	08/2018	04/2022	Chemistry
Kentucky Wesleyan College, Owensboro, Kentucky	BS (Hons)	08/2018	04/2022	Biology
University of Kentucky, Lexington, Kentucky	PHD	08/2022	05/2027	Physiology

A. Personal Statement

The mystery surrounding my sister's neuromuscular disorder first sparked my interest in translational science. Her geneticist identified an abnormality in chromosome 6 but could not determine how it contributed to her condition. This uncertainty—how genetic changes manifest in disease—ignited my passion for biomedical research. During my career, I hope to be able to help transform genealogical discoveries into clinical actions.

I quickly realized that accessing research opportunities was not going to be easy. I grew up in rural western Kentucky without access to resources that many of my PhD classmates take for granted. I was very fortunate to be able to attend Kentucky Wesleyan College as an undergraduate, but it is a small liberal arts institution with limited infrastructure for research. However, I persisted and secured an opportunity in the lab of Rachel Pritchard, PhD, where I studied soil-derived antibiotics as tools to combat the growing crisis of drug-resistant pathogens. I used preliminary data from this project to build a successful application for the Wesleyan Fellowship, which funded 2 years of research. I disseminated first-author works at various local, national, and international conferences.

After admission into a PhD program at the University of Kentucky, I joined the lab of my primary sponsor, Ken Campbell, PhD where I was given the opportunity to shift my focus towards the genetics of human heart failure. Non-ischemic cardiomyopathies have a strong genetic component, yet nearly half of cases are idiopathic, much like my sister's condition. Despite this, genetic screening remains vastly underutilized in clinical settings. Our lab has spent 17 years building a cardiac biobank that now contains more than 20,000 specimens matched to clinical data. I spearheaded our lab's initiative to obtain sequencing data for 350 patients, presenting findings at numerous conferences and laying the foundation for a multi-omic atlas of heart failure patients in the greater Bluegrass region.

My PhD project centers on titin-truncating variants (TTNtvs), which cause premature protein translation stoppage and are amongst the most prevalent genetic contributors to non-ischemic cardiomyopathy. However, how TTNtvs lead to pathophysiology remains unclear. My project aims to address this gap by investigating if and how TTNtvs lead to overloaded cellular turnover pathways, accelerated aggregation of cytosolic residuals, and truncated titin filaments in sarcomeres. By integrating genomic, (immuno)histological, and biomechanical analyses, I aim to uncover novel therapeutic targets that will advance current treatments towards proactive, genetics-informed interventions.

Under the mentorship of Dr. Campbell, I will use my previous experiences in drug discovery and scientific communication as a conduit to provide insight into the pathophysiological underpinnings of heart failure. My training will be a first-hand perspective of the interconnectedness between research and medicine. I believe that this, including my previous research experiences, will provide a solid foothold for my long-term goal of leading a research team in cardiovascular genetics.

a. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Multi-Omic and Biochemical Profiling of Heart Failure Specimens at the University of Kentucky. University of Kentucky Center for Clinical and Translational Research Spring Conference. 2025 (Podium Talk)
b. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Genomic and Biochemical Profiling of Heart Failure at the University of Kentucky. Biophysical Society Annual Meeting. 2025 (Poster)
c. **Minton AT**, Yackzan AT, Wellette-Hunsucker AG, Milburn GN, Gulbulak U, Campbell KS. Genomic Characterization of Patients with Advanced Heart Failure at the University of Kentucky. Madison Myofilament Meeting. 2024 (Poster)
d. Cortazar AS, **Minton AT**, Gulbulak U, Campbell KS. Whole Exome Sequencing of a Myocardial Repository at the University of Kentucky. National Institutes of Health STEP-UP Program Annual Conference. 2024 (Podium Talk)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2024 – **Member**, American Heart Association
2024 – **Member**, Biophysical Society
2023 – **Graduate Research Assistant**, Dr. Kenneth Campbell's Laboratory, Dept of Physiology, University of Kentucky College of Medicine
2020 – 2022 **Laboratory Assistant**, Div of Nat Sciences & Mathematics, Kentucky Wesleyan College
2020 – 2022 **Peer Tutor & Instructor**, Student Success Center, Kentucky Wesleyan College
2020 – 2022 **Member**, American Society for Microbiology
2019 – 2022 **Directed Researcher**, Div of Nat Sciences & Mathematics, Kentucky Wesleyan College

Honors

2023 **Featured in Fall 2023 Issue of *Pillars* as GOLD Alumnus**, institution's alumni magazine, Kentucky Wesleyan College
2022 **Invited Guest Speaker**, STEM Bridge Program, Kentucky Wesleyan College
2018 – 2022 **Presidential Scholarship**, partial tuition scholarship, Kentucky Wesleyan College
2020 – 2022 **Ellie Magnuson Memorial Endowed Fellowship Scholarship**, awarded to selected researchers majoring in chemistry, Kentucky Wesleyan College
2020 – 2022 **Wesleyan Fellowship**, awarded to selected researchers, Kentucky Wesleyan College
2020 – 2022 **Dean's List**, ≥3.5 semester grade point average, Kentucky Wesleyan College
2022 **President's Award**, awarded to a selected Greek Life member, Kentucky Wesleyan College
2022 **Program of the Year Award**, awarded to a selected organization leader who hosted a successful community-wide program, Kentucky Wesleyan College
2022 **Fraternity and Sorority Life Hall of Fame**, awarded to selected Greek Life members, Kentucky Wesleyan College
2022 **Student Government Association Senator of the Year**, Kentucky Wesleyan College
2022 **Order of Oak & Ivy Nominee**, institution's highest honor, Kentucky Wesleyan College
2022 **Chemistry Alumni Award**, awarded to a selected student majoring in chemistry, Kentucky Wesleyan College
2022 **Henry Milton Pyles Biology Award**, awarded to a selected student majoring in biology, Kentucky Wesleyan College
2021 **Interviewed on *Bench Talk: The Week in Science***, selected based on conference presentation award, Kentucky Academy of Science
2021 **Philip R. Edwards Microbiology Award**, awarded to a selected researcher in microbiology, Kentucky Wesleyan College
2021 **Oral Presentation Award Winner**, Kentucky Academy of Science, Eastern Kentucky University
2021 **Fraternity Man of the Year**, awarded to a selected fraternity member, Kentucky Wesleyan College
2021 **Dr. Ernest W. Abernathy Scholarship**, awarded to selected students majoring in chemistry or biology, Kentucky Wesleyan College
2020 **Oral Presentation Award Winner**, American Society for Microbiology, Vanderbilt University
2018 **Presidential Scholarship**, partial tuition scholarship, Kentucky Wesleyan College

C. Contributions to Science

1. **Genetic Variants in Heart Failure**

   Approximately 50% of patients with heart failure receive an idiopathic diagnosis. Moreover, animal models of many types of heart failure are nonrepresentative due to comorbidities such as hypertension, diabetes, and chronic lung diseases. There is a poor understanding of the link between heart failure and genetics, primarily due to the lack of genetic testing in this patient population. With Dr. Kenneth Campbell, I selected an experimental kit necessary to extract and purify nucleic acid eluants from specimens within the lab's myocardial repository. I used the extraction kit to derive a high-throughput protocol of extracting DNA and RNA from cryopreserved cardiac samples, which was utilized to extract DNA and RNA from 394 specimens. I coordinated with numerous genomics companies to determine the best sequencing platform, depth, coverage, and enrichment system to identify causal variants. 350 samples were sent for library preparation and whole exome/transcriptome sequencing. The collected data provided a genetic atlas of specimens within the myocardial repository, representative of heart transplant and ventricular assist device recipients in the greater Bluegrass region. Further analyses revealed trends in sequencing results and matched clinical data. Moreover, this dataset fostered research collaborations nationally and internationally.

   a. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Multi-Omic and Biochemical Profiling of Heart Failure Specimens at the University of Kentucky. University of Kentucky Center for Clinical and Translational Research Spring Conference. 2025 (Podium Talk)
   b. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Genomic and Biochemical Profiling of Heart Failure at the University of Kentucky. Biophysical Society Annual Meeting. 2025 (Poster)
   c. **Minton AT**, Yackzan AT, Wellette-Hunsucker AG, Milburn GN, Gulbulak U, Campbell KS. Genomic Characterization of Patients with Advanced Heart Failure at the University of Kentucky. Madison Myofilament Meeting. 2024 (Poster)
   d. Cortazar AS, **Minton AT**, Gulbulak U, Campbell KS. Whole Exome Sequencing of a Myocardial Repository at the University of Kentucky. National Institutes of Health STEP-UP Program Annual Meeting. 2024 (Podium Talk)

2. **Contribution of Variants in the Titin Gene to the Pathology of Dilated Cardiomyopathy**

   The Campbell Lab maintains and utilizes tissue from one of the world's largest human cardiac biobanks to perform cardiovascular research. Experimentation ranges from the single-myofibril to whole-organ level, providing insight applicable at the bench and the clinic. I collated clinical and whole exome sequencing data to identify patients who met dilated cardiomyopathy criteria and contained variants in the *TTN* gene. Using samples from these patients, I assisted in evaluating phosphorylation of proteins involved in myofilament calcium sensitivity (regulatory light chain, troponin I, and myosin-binding protein C) and relative abundances of contributors to intra/extracellular passive tension (collagen, alpha-tubulin, and titin). Findings differed from those previously collected by our lab, which included patients with truncating *TTN* variants (irrespective of dilated cardiomyopathy diagnosis). This hinted towards possible associations with the location of a genomic variant. To enable such comparisons, I mapped the exonic location of *TTN* variants based on the corresponding region of the sarcomere. Collected data has supported several conference presentations and serves as the basis of my dissertation research.

   a. **Minton AT**, Campbell KS. Effects of SGLT2i Treatment in Patients with Cardiac Titin Variants. University of Kentucky College of Medicine Department of Physiology Seminar Series: Trainee Talk. 2024 (Podium Talk)
   b. Wilkerson E, **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Campbell KS. Evaluating TTN Variants in Dilated Cardiomyopathy at the University of Kentucky. Kentucky Chapter of the American Physiological Society Annual Meeting. 2024 (Poster)

3. **Production of Novel Antibiotics from Soil Bacteria**

   Bacteria are becoming increasingly more resistant to commercially available antibiotics, leading to difficulty treating infections that were once subjective to such medications. Since antibiotics are commonly produced in bacteria inhabiting soil, this serves as a natural reservoir to identify and isolate novel antimicrobials. In coordination with Dr. Rachel Pritchard, I served as the lead investigator on a project that explored the ability to discover novel antibiotics from soil samples of various demographics. I revealed antibiotic production from thirteen bacterial isolates of four soil samples and assisted in optimizing an experimental technique to extract the antimicrobial compounds. Novelty of the bacteria was confirmed with 16S rRNA gene PCR, Sanger sequencing, and advanced biochemical testing. I cultivated stocks that were sent to the Tiny Earth Chemistry

Hub, a public database that preserves samples and records all experimental conditions, for use in further experimentation and possible application.

a. **Minton AT**, Pritchard R. Analysis of Purified Extracts from Antibiotic-Producing Bacterial Isolates. Kentucky Academy of Science Annual Meeting. 2021 (Podium Talk – Award Winner)
b. **Minton AT**, Pritchard R. Analysis of Bacterial Isolates Found in the Soil: Executing the Tiny Earth Project. Kentucky-Tennessee American Society for Microbiology Meeting. 2020 (Podium Talk – Award Winner)
c. **Minton AT**, Pritchard R. Analysis of Antibiotic-Producing Bacterial Isolates: Executing the Tiny Earth Project. Kentucky Wesleyan College Scholar's Day. 2021 (Poster)
d. **Minton AT**, Pritchard R. Analysis of Bacterial Isolates Found in the Soil: Executing the Tiny Earth Project. Tiny Earth Winter Symposium. 2020 (Podium Talk)

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	KENTUCKY WESLEYAN COLLEGE	
2016	Fundamentals of General Chemistry	P
2016	Fundamentals of General Chemistry Lab	P
2017	Fundamentals of Organic Chemistry	P
2017	Fundamentals of Organic Chemistry Lab	P
2017	Medical Terminology from Greek & Latin	P
2017	Writing I	P
2017	Music Appreciation	P
2018	Calculus AB	P
2018	English II	P
2018	General Biology I Lab	A
2018	General Biology I	C+
2018	General Chemistry Laboratory I	A
2018	General Chemistry I	B+
2018	Freshman Seminar	A
2018	Introduction to Religion	A
2019	General Biology II Lab	A
2019	General Biology II	C+
2019	General Chemistry Laboratory II	A-
2019	General Chemistry II	B+
2019	Introduction to Psychology	A
2019	Survey of Christian Traditions	A
2019	Microbiology I	B
2019	Organic Chemistry Laboratory I	A
2019	Organic Chemistry I	A-
2019	Analytical Chemistry	B-
2019	American Literature Survey	A
2020	Genetics	A-
2020	Directed Student Research	A
2020	Organic Chemistry Laboratory II	B+
2020	Organic Chemistry	A-
2020	Fitness and Wellness	A
2020	Directed Student Research	A
2020	Statistics in the Behavioral Sciences	A
2020	Natural Science Junior Seminar	A
2020	Principles of Sociology	A
2020	General Physics	P
2020	College Physics Laboratory	P

2021	Cellular/Molecular Biology	B+
2021	Directed Student Research	A
2021	Immunology	A-
2021	Inorganic Chemistry	A
2021	Biochemistry	A
2021	Introductory General Physics II	A
2021	Introductory General Physics II Laboratory	A
2021	Biology of the Mind	B
2021	Directed Student Research	A
2021	Senior Seminar	A
2021	Advanced Integrated Lab I	A
2021	Computer Literacy	P
2021	Introduction to Human Geography	A
2021	Survey of American History I	A
2021	Evolution	A
2022	Physiological Psychology	A
2022	Directed Student Research	A
2022	Investigations in Molecular Cell Biology	A
2022	Ecology	A-
2022	Instrumental Techniques of Biochemistry	B
2022	Advanced Integrated Lab II	A
	UNIVERSITY OF KENTUCKY	
2022	Biomolecules and Metabolism	B
2022	Molecular Biology and Genetics	B
2022	Seminar in Integrated Biomedical Sciences	S
2022	Research in Integrated Biomedical Sciences	A
2022	Critical Scientific Readings	A
2022	Practical Statistics	A
2023	Ethics in Scientific Research	A
2023	Cell Biology and Signaling	B
2023	Physiological Communication	A
2023	Seminar in Integrated Biomedical Sciences	S
2023	Research in Integrated Biomedical Sciences	A
2023	Genomics & Bioinformatics Tools	A
	---Joined the Campbell Muscle Lab---	
2023	Systems, Cellular & Molecular Physiology	A
2023	Graduate Seminar in Physiology	A
2023	Readings in Systems, Cellular and Molecular Physiology	A
2024	Fellowship Grant Writing Workshop	A
2024	Advanced Topics in Physiology	A
2024	Research in Physiology	A
2024	Graduate Seminar in Physiology	A
2024	Qualifying Exam Residency Credit	P

*Kentucky Wesleyan College Grading System: Pass (≥70%), Fail (<70%); A (100-93%), A- (93-90%), B+ (89-87%), B (86-83%), B- (82-80%), C+ (79-77%), C (76-73%), C- (72-70%), D+ (69-67%), D (66-63%), D- (62-60%), F (<60%)

**University of Kentucky Grading System: Satisfactory (S; ≥70%), Non-Satisfactory (NS: <70%); A (100-90%), B (89-80%), C (79-70%), D (69-60%), F (<60%)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kenneth S. Campbell

eRA COMMONS USER NAME (credential, e.g., agency login): ken.campbell

POSITION TITLE: Professor and Director of Translational Research for Cardiovascular Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Oxford, Oxford, UK	BA (Hons)	09/90 – 06/93	Physics
University of Birmingham, Birmingham, UK	PhD	09/93 – 04/98	Muscle physiology
University of Wisconsin-Madison, WI, USA	Postdoc	04/98 – 01/03	Muscle physiology

No variances from ordinary career progression

A. Personal Statement

My goal as the inaugural Director of Translational Research for Cardiovascular Medicine at the University of Kentucky is to accelerate research that has the potential to improve care for patients with cardiovascular disease. This includes (a) helping clinicians to develop research projects, (b) enrolling patients in trials, and (c) creating registries and biobanks. I try to lower the energy barriers that can prevent busy clinicians from performing meaningful research.

My own lab's research integrates biophysical, biochemical, and computational techniques to develop better therapies for heart failure. Much of our work, including that described in Austin Minton's proposal, uses samples of human myocardium that we have been collecting from organ donors and patients with advanced heart failure since 2008. Our cardiac biobank has procured a total of 20,000 samples and distributed ~5,000 samples to 40 groups around the world in the last 5 years.

Since joining the University of Kentucky in 2004, I have mentored ~60 trainees (high school to postdoc) and five junior faculty. I am a member of the Center for Clinical and Translational Science's clinician-scientist mentoring committee (TREE) and was recognized as the College of Medicine's Mentor of the Year as an Associate Professor. Roughly 20 undergraduates have performed research in our lab and applied to medical school. All have been accepted. Four of our six postdoc alumni are in faculty positions; three have tenure and two are R01-funded. Our lab currently comprises 3 PhD students (Austin is one), 1 MD/PhD student, 3 postdocs, 2 research coordinators, and 10 undergraduates. 16 of these 19 individuals are women. All trainees are making excellent progress.

I served as PI of T32 GM118292 after the original PI left our institution. I also completed the 12 hour "Entering Mentoring" curriculum developed by the National Research Mentoring Network (NRMN) in 2020. Following that, I led a session titled "Assessing Knowledge" for the College of Medicine's mentor training program for 3 years.

As the Director of Translational Research for Cardiovascular Medicine, I am responsible for training ~80 faculty, clinical fellows, and other trainees in the responsible conduct of research. This includes, but is not limited to, ensuring the safety of all individuals in the research environment. My lab also tries to set positive examples in rigorous and unbiased experimental design as well as the analysis, interpretation, and reporting of data. We document all data in LabArchives (an electronic lab management system) and version-control all computer code using GitHub. Last year, we published open-source software (GelBox) to improve rigor and reproducibility when analyzing gels and immunoblots.

Our team is fortunate to be supported by multiple NIH grants including:

1. R01 HL163977 (Corresponding MPI to 2026)
2. R01 HL146676 (MPI to 2028)
3. R01 HD 090642 (Co-I to 2026)
4. R01 HL 163585 (Co-I to 2026)

A. R01 HL173989 (MPI to 2028)

Manuscripts (from a total of ~130, h-index=42) that are representative of my work include:

1. **CAMPBELL, K. S.**, Yengo, C. M., Lee, L. C., Kotter, J., Sorrell, V. L., Guglin, M. & Wenk, J. F. (2019). Closing the therapeutic loop. *Arch Biochem Biophys.* 663, 129-131. PMC6377839.
2. Blair, C. A., Brundage, E. A., Thompson, K. L., Stromberg, A., Guglin, M., Biesiadecki, B. J. & **CAMPBELL, K. S.** (2020). Heart Failure in Humans Reduces Contractile Force in Myocardium From Both Ventricles. *JACC Basic Transl Sci.* 5, 786-798. PMC7452203.
3. **CAMPBELL, K. S.**, Chrisman, B. S. & Campbell, S. G. (2020). Multiscale Modeling of Cardiovascular Function Predicts That the End-Systolic Pressure Volume Relationship Can Be Targeted via Multiple Therapeutic Strategies. *Front Physiol.* 11, 1043. PMC7466769.
4. Sharifi, H., Mann, C. K., Wenk, J. F. & **CAMPBELL, K. S.** (2022). A multiscale model of the cardiovascular system that regulates arterial pressure via closed loop baroreflex control of chronotropism, cell-level contractility, and vascular tone. *Biomech Model Mechanobiol.* 21, 1903-1917. PMC10066042.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2025 - present	Associate Vice-Chair for Translational Research, Department of Internal Medicine, University of Kentucky
2023 - present	University of Kentucky, College of Medicine, Research Vision Committee
2023 - present	Editorial board, Circulation: Heart Failure
2023 - present	University of Kentucky, College of Medicine, Trainees in Research Advisory Committee
2022 - present	Director of Translational Research for Cardiovascular Medicine, University of Kentucky
2022	University of Kentucky Research Leadership Academy
2022 – present	Scientific Review Board, Sydney Heart Bank, Australia
2021	University of Kentucky College of Medicine Leadership Training
2020 - present	Grant review, multiple NIH panels
2020	Biophysical Society, Motility Sub-group, Co-leader
2020 - present	Course Director, Medical School Cardiology (100 hours at 3 campuses)
2020 - 2023	Director, COVID-19 Research Registry and Specimen Bank, University of Kentucky
2019 – 2021	Guest Editor, Archives of Biochemistry and Biophysics: special issue on muscle modeling
2019 - present	Editorial Board, Scientific Reports
2018 - 2019	Guest Editor, Biophysical Journal: special issue on cardiac modeling
2018 - present	Grant review, Wellcome Trust, United Kingdom
2018 - present	Professor (Tenured), Department of Physiology and Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY
2017 - present	Principal Investigator, Gill Cardiovascular Biorepository, University of Kentucky
2017 - 2023	Editorial Board, Life Sciences
2016 - 2022	Director of Graduate Studies, Department of Physiology, University of Kentucky
2015	Grant review, American Heart Association Established Investigator Award
2015 - 2018	Co-founder and Chief Technology Officer, MyoAnalytics, LLC
2015 - 2018	Associate Professor (Joint Appointment), Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY
2014	Symposium Speaker, Biophysical Society Annual Meeting

2014	Auckland Bioengineering Institute, New Zealand – 4 week visit supported by research grant from the Royal Society of New Zealand, Auckland, New Zealand
2014 - 2020	Grant review, NIH MTI, K99-R00 panel for NHLBI
2013	Grant review, NHLBI PPG
2013 - 2014	Grant review Chair, American Heart Association, Cardiac Biology and Regulation 1
2013 - present	Core Director, Biospecimens, Kentucky Center for Clinical and Translational Sciences
2012 - 2014	Grant review, NIH ZHL1 CSR-P (01)1 – Mentored Career Transition Scientist
2011	Co-Chair, Muscle Mechanics and Ultrastructure, Biophysical Society Annual Meeting
2011 - 2012	Grant review Co-Chair, American Heart Association, Cardiac Biology and Regulation 1
2011	Director, Modeling workshop for trainees in muscle biology, University of Kentucky, Lexington, KY
2010	Symposium Chair, 6th World Congress on Biomechanics, Singapore
2010 - present	Editorial Board, Frontiers in Cardiac Muscle Physiology
2009 - 2019	Executive Committee Member, Center for Muscle Biology, University of Kentucky
2009 - 2018	Associate Professor (Tenured), Department of Physiology, University of Kentucky
2008, 2010	Biophysical Society Annual Meeting Career Workshop Coordinator
2007	Symposium Chair, Experimental Biology, American Physiological Society Annual Meeting
2007 - 2009	Grant review, American Heart Association, Cardiac biology and regulation
2007, 2012, 2014	Grant review, National Science Foundation
2006 - 2012	Biophysical Society Early Careers Committee
2004 - 2009	Assistant Professor (Tenure-track), Department of Physiology, University of Kentucky
2004 - present	Member of the American Physiological Society
2003 - 2004	Assistant Scientist, Department of Physiology, University of Madison-Wisconsin
2001 - present	Member of the American Heart Association
1998 - present	Member of the Biophysical Society
1993 - 2010	Member of the Physiological Society (United Kingdom)

Honors

2006, 2010, 2014	Holsinger Award for Excellence in Teaching (University of Kentucky, Physiology)
2014	University of Kentucky CTSA Mentor Recognition Award
2012	Fellow of the American Heart Association
1993 - 1998	Wellcome Trust Prize Studentship (United Kingdom)

C. Contributions to Science

Contribution 1: Quantitative understanding of sarcomere-level function

Dr. Campbell has published ~30 manuscripts that quantify the mechanical properties of skeletal and cardiac muscles. Important insights from these publications include: (a) bound cross-bridges contribute to diastolic myocardial stiffness, (b) heterogeneity of half-sarcomere responses contributes to residual force enhancement, and (c) myocardial relaxation is independent of afterload but accelerated by end-systolic lengthening.

a) **CAMPBELL, K. S.**, Patel, J. R. & Moss, R. L. (2003). Cycling cross-bridges increase myocardial stiffness at submaximal levels of Ca^{2+} activation. *Biophys. J.* 84, 3807-3815. PMC1302962.
b) **CAMPBELL, K. S.** (2006). Tension recovery in permeabilized rat soleus muscle fibers after rapid shortening and restretch. *Biophys. J.* 90, 1288-1294. PMC1367280.
c) Campbell, S. G. & **CAMPBELL, K. S.** (2011). Mechanisms Of Residual Force Enhancement In Skeletal Muscle: Insights From Experiments And Mathematical Models. *Biophysical Reviews.* 3, 199-207. PMC3237401
d) Chung, C. S., Hoopes, C. W. & **CAMPBELL, K. S.** (2017). Myocardial relaxation is accelerated by fast stretch, not reduced afterload. *J Mol Cell Cardiol.* 103, 65-73. PMC5347980.

Contribution 2: Mathematical modeling of striated muscle

Dr. Campbell has published ~30 manuscripts that integrate mathematical modeling of skeletal and cardiac muscles with experimental data. The earliest manuscripts focused on the short-range mechanical properties of skeletal muscle and continue to influence the field of sensorimotor control. Three manuscripts from 2009 to 2011 showed that interactions between half-sarcomeres could explain residual force enhancement and apparent activation-dependent stiffening of muscle fibers. The latest work focuses on OFF/ON transitions in thick filament structure and their contribution to length-dependent activation in myocardium.

a) **CAMPBELL, K. S.** & Lakie, M. (1998). A cross-bridge mechanism can explain the thixotropic short-range elastic component of relaxed frog skeletal muscle. *J. Physiol.* 510, 941-962. PMC2231083.
b) **CAMPBELL, K. S.** (2009). Interactions between connected half-sarcomeres produce emergent mechanical behavior in a mathematical model of muscle. *PLoS Comput Biol.* 5, e1000560. PMC PMC2770126.
c) Campbell, S. G., Hatfield, P. C. & **CAMPBELL, K. S.** (2011). A mathematical model of muscle containing heterogeneous half-sarcomeres exhibits residual force enhancement. *PLoS Computational Biology.* 7, e1002156. PMC3182863.
d) **CAMPBELL, K. S.**, Janssen, P.M. & Campbell, S. G. (2018). Force-dependent recruitment from the myosin OFF state contributes to length-dependent activation. *Biophys. J.* 115, 543-553. PMC6084639.

Contribution 3: Open source software for scientific research

Dr. Campbell has a 16 year track record of creating scientific software and making it freely available to the research community. Major projects include: (a) SLControl, a package for acquiring and analyzing data relating to muscle mechanics, (b) GelBandFitter, a tool for analyzing closely-running bands on gels and immunoblots, (c) MyoSim, software for simulating the mechanical properties of half-sarcomeres, and (d) MyoVision, which automates image analysis for muscle cross-sections.

a) **CAMPBELL, K. S.** & Moss, R. L. (2003). SLControl: PC-based data acquisition and analysis for muscle mechanics. *AJP: Heart.* 285, H2857-2864. PMC not available. PMID 12907419.
b) Mitov, M. I., Greaser, M. L. & **CAMPBELL, K. S.** (2009). GelBandFitter--a computer program for analysis of closely spaced electrophoretic and immunoblotted bands. *Electrophoresis.* 30, 848-851. PMC2742644.
c) **CAMPBELL, K. S.** (2014). Dynamic coupling of regulated binding sites and cycling myosin heads in striated muscle. *J Gen. Physiol.* 143, 387-399. PMC 3933939.
d) Wen, Y., Murach, K. A., Vechetti, I. J., Jr., Fry, C. S., Vickery, C., Peterson, C. A., Mccarthy, J. J. & **CAMPBELL, K. S.** (2018). MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry. *J Appl Physiol (1985).* 124, 40-51. PMC6048460.

Contribution 4: Transmural variation in myocardium

Dr. Campbell's laboratory has demonstrated that rodent and human hearts exhibit transmural variation in contractile function and that disease changes the normal patterns. These results are important because they may explain changes in cardiac torsion and regional shortening that predict clinical outcomes.

a) Campbell, S. G., Haynes, P., Kelsey Snapp, W., Nava, K. E. & **CAMPBELL, K. S.** (2013). Altered ventricular torsion and transmural patterns of myocyte relaxation precede heart failure in aging F344 rats. *AJP Heart.* 305, H676-686. PMC3761331.
b) Chung, C. S. & **CAMPBELL, K. S.** (2013). Temperature and transmural region influence functional measurements in unloaded left ventricular cardiomyocytes. *Physiological Reports.* 1, e00158. PMC3871472.
c) Haynes, P., Nava, K. E., Lawson, B. A., Chung, C. S., Mitov, M. I., Campbell, S. G., Stromberg, A. J., Sadayappan, S., Bonnell, M. R., Hoopes, C. W. & **CAMPBELL, K. S.** (2014). Transmural heterogeneity of cellular level power output is reduced in human heart failure. *J Mol Cell Cardiol.* 72, 1-8. PMC4037376.
d) Zhang, X., Haynes, P., **CAMPBELL, K. S.**, & Wenk, J. (2015). Numerical evaluation of myofiber orientation and transmural contractile strength on left ventricular function. *J. Biomech. Eng.* 137:044502. PMCID not available. PMID25367232.

Contribution 5: Biobanking

Dr. Campbell's experience with biobanking started in 2008 when he initiated a collaboration with a cardiothoracic surgeon to collect samples of human myocardium. The project has now evolved into the Gill Cardiovascular Biorepository which Dr. Campbell leads as PI. The bank has acquired >20,000 myocardial samples from >650 organ donors and patients. The resource supports collaborations with ~40 groups in ~10 countries. Because of his experience, Dr. Campbell was chosen to lead an institution-wide biobanking program for the University of Kentucky CTSA-supported Center for Clinical and Translational Sciences. This program has enrolled ~60,000 patients to date and gives the institution permission to bank any sample that is procured as part of normal clinical care and that would otherwise be discarded. Starting in February 2020, Dr. Campbell worked with stakeholders from across the University of Kentucky to develop a COVID-related biobank to facilitate campus research during the pandemic. Dr. Campbell devotes 5% of his academic effort to these activities.

a) Blair, C. A., Haynes, P., Campbell, S. G., Chung, C., Mitov, M. I., Dennis, D., Bonnell, M. R., Hoopes, C. W., Guglin, M. & **CAMPBELL, K. S.** (2016). A protocol for collecting human cardiac tissue for research. *The VAD Journal.* 2, Article 12. PMC5199025.
b) Croker, J. A., Patel, R., **CAMPBELL, K. S.**, Barton-Baxter, M., Wallet, S., Firestein, G., Kimberly, R. P., & Elemento, O. (2021). Building biorepositories in the midst of a pandemic. *Journal of Clinical and Translational Science*. 10.1017/cts.2021.6. PMCID7785692.

Complete list of published work in NCBI My Bibliography

(~130 publications, h-index=42, i10-index is 85).

https://www.ncbi.nlm.nih.gov/myncbi/kenneth.campbell.1/bibliography/public/

PHS Fellowship Supplemental Form

Introduction

1. Introduction (for Resubmission applications)	

Fellowship Applicant Section

2. * Applicant's Background and Goals for Fellowship Training	background_and_goals_Revised1025621159.pdf

Research Training Plan Section

3. * Specific Aims	specific_aims_REVISED1025621149.pdf
4. * Research Strategy	research_strategy_Revised1025621160.pdf
5. * Respective Contributions	respective_contributions_REVISED1025621150.pdf
6. * Selection of Sponsor and Institution	selection_sponsor_institution_R1025621151.pdf
7. Progress Report Publication List (for Renewal applications)	
8. * Training in the Responsible Conduct of Research	training_in_RCR1025621139.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements	sponsor_statement_REVISED1025621152.pdf
10. Letters of Support from Collaborators, Contributors and Consultants	LOS_Ebbert1025621153.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training	institution_environment_training1025621154.pdf
12. Description of Candidate&s Contribution to Program Goals	

Other Research Training Plan Section

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? Yes ✔ No

13. Are vertebrate animals euthanized?

If "Yes" to euthanasia
Is method consistent with American Veterinary Medical Association (AVMA) guidelines?
If "No" to AVMA guidelines, describe method and provide scientific justification

14. Vertebrate Animals

PHS Fellowship Supplemental Form

Other Research Training Plan Information

15. Select Agent Research

16. Resource Sharing Plan

17. Other Plans

18. Authentication of Key Biological and/or Chemical Resources

Additional Information Section

19. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* Yes ✔ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

20. Alternate Phone Number: 270-775-3348

21. Degree Sought During Proposed Award:

Degree:	If "other", indicate degree type:	Expected Completion Date (MM/YYYY):
PHD: Doctor of Philosophy		05/2027

22. * Field of Training for Current Proposal: 175 Pathology, Human & Animal

23. * Current Or Prior Kirschstein-NRSA Support? Yes ✔ No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)

24. * Applications for Concurrent Support? Yes ✔ No

If yes, describe in an attached file:

25. * Citizenship

U.S. Citizen U.S. Citizen or Non-Citizen National? ✔ Yes No

Non-U.S. Citizen With a Permanent U.S. Resident Visa

With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here:

26. Change of Sponsoring Institution Name of Former Institution:*

PHS Fellowship Supplemental Form

Budget Section

All Fellowship Applicants:

27. * Tuition and Fees:

None Requested ✔ Funds Requested

Year 1	$3,204.00
Year 2	$3,364.00
Year 3	$3,465.00
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	$10,033.00

28. * Childcare Costs:

✔ None Requested Funds Requested

Year 1	
Year 2	
Year 3	
Year 4	
Year 5	
Year 6 (when applicable)	
Total Funds Requested:	

Senior Fellowship Applicants Only:

29. Present Institutional Base Salary: Amount | Academic Period | Number of Months

30. Stipends/Salary During First Year of Proposed Fellowship:

a. Federal Stipend Requested: Amount | Number of Months

b. Supplementation from other sources: Amount | Number of Months

Type (e.g., sabbatical leave, salary)

Source

Appendix

31. Appendix

APPLICANT'S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING

A. Doctoral Dissertation and Research Experience

Doctoral Dissertation

My PhD advisory committee consists of 2 muscle physiologists, an academic cardiologist, and my sponsor (Ken Campbell, PhD, a translational cardiovascular researcher). I presented an early version of this F31 proposal to them as part of my Qualify Exam and they approved my goal to quantify how titin-truncating variants impact cellular turnover pathways, accumulation of cytosolic waste, and cardiac mechanics.

My experiments will use myocardial samples donated by organ donors and patients with non-ischemic cardiomyopathy that I have helped to procure for the Gill Cardiovascular Biorepository led by Dr. Campbell. I will also work with the clinical data associated with each specimen. This will give me a unique background in translational and applied cardiovascular research that will strengthen my applications for postdoc positions in well-respected labs. My long-term goal is to lead my own research program and try to use research to help patients who develop cardiovascular disease due to inherited variants.

Prior Research Experience

Undergraduate Research Experience (2019 – 2022)

Growing up alongside my sister as she battled with an idiopathic neuromuscular disorder fueled my interest in translational research, particularly the interplay between genetics and pathology. My undergraduate institution was a small liberal arts college that lacked extensive research infrastructure, making it difficult to find clinically relevant research labs. I persisted and earned a researcher role in the lab of Rachel Pritchard, PhD, investigating antibiotic production from soil bacteria. 6 months after starting in Dr. Pritchard's lab, I took the lead on a new project and compiled preliminary data to support an application for an institutional award. My project was funded for 2 years during which I identified 13 bacterial isolates that produced broad spectrum antimicrobial compounds. I presented my findings at 6 conferences as 3 posters and 3 podium talks. My podium talks at the 2020 Kentucky-Tennessee American Society for Microbiology and 2021 Kentucky Academy of Science Annual Meetings received 1st-and 2nd-place presenter honors, respectively. Moreover, I served as 1 of 3 invited speakers at the 2022 STEM Bridge Program hosted by my undergraduate institution. These first experiences in research reinforced my interests and led to me applying to the more translational environment at my state's flagship university .

Graduate Research Experience (2023 – Present)

I was admitted into the Integrated Biomedical Sciences PhD Program at the University of Kentucky College of Medicine in August of 2022. The first year of this program involves taking undifferentiated core curriculum prior to joining a lab. Upon completion of these courses, I joined Dr. Campbell's lab, where I began working on the genetic characterization of our large cardiac biobank. The heart contains primarily post-mitotic cells, making it difficult to extract high quality nucleic acids from it. However, I optimized an extraction and purification protocol for tissue in our biobank, which I performed on over 350 human hearts.

Concurrently, I have become proficient and am still improving my skills in coding, particularly for batch data analysis. This has become especially useful for our multi-omics data since it exceeds 10 terabytes. I have used this knowledge to build scripts acceptable for a layperson to parse through the data efficiently (e.g., variants for one gene). This coding skillset has enabled me to identify 24 patients who have both non-ischemic heart failure and a titin-truncating variant. Moreover, I have built an image processing pipeline that executes precise and high-throughput segmentation of fluorescent scans, crucial for Aims 2 and 3 of this proposal. I have also customized this workflow for brightfield scans, making it the standard for analyzing histology in Dr. Campbell's lab.

Since joining Dr. Campbell's lab, I have presented my work at 8 conferences, including the 2024 Madison Myofilament and 2025 Biophysical Society Annual Meetings. In addition, I delivered a departmental podium talk focused on cardiac titin variants.

B. Training Goals and Objectives

My primary training goal during this fellowship is to develop expertise in a range of techniques that will help quantify links between genetics and heart failure pathophysiology. I will strengthen my scientific, collaborative, and communication skills while directly addressing the hypothesis outlined in this proposal. This experience will provide hands-on training in translational research while studying the bases of genetic characterization, RNA/protein turnover, accumulation of cellular waste, and muscle mechanics in human heart failure. I will gain technical expertise in genomic analysis, biochemistry, and biophysics while engaging with scientists across related disciplines. The resulting research outputs will demonstrate meaningful progress and productivity, supporting future applications for postdoctoral positions and extramural awards. Ultimately, this fellowship will serve as a springboard towards achieving my short- and long-term goals (listed below).

Annual Research Product Goals

- Manuscripts/Publications
    - 2 First-Author
    - 2 Co-Author
- Conference Presentations (*1 abstract selected for podium talk)
    - 2 Regional
    - 1 National/International

Short-Term Research and Academic Objectives

Research

1. Develop skills in genomics, biochemical assays, and muscle mechanics to a degree of producing publication-quality data (Aims 1-3).
2. Enhance my writing abilities by producing high quality manuscripts, grant proposals, and conference abstracts, along with participating in the process of peer review.
3. Broaden my network with translational and clinical researchers with attendance and presentation at local and national/international conferences.
4. Supplement my rigor and reproducibility of data collection and analysis by developing and subsequently publishing MATLAB scripts for data management, statistical testing, and figure generation.

Academic

1. Expand my clinical knowledgebase of cardiology and heart failure by auditing the medical school's cardiology course (directed by my sponsor, Dr. Campbell), bi-weekly shadowing of cardiologist Vedant Gupta, MD (advisory committee member), and attending cardiology fellow meetings.
2. Further my understanding of ethical and responsible research by partaking in responsible conduct of research trainings and courses.

Long-Term Research and Academic Objectives

Research

1. Network with NIH-funded labs in search of post-doctoral positions by attending and presenting my work at conferences.
2. Strengthen my public-speaking skills by giving oral presentations at seminars and conferences.

Academic

1. Mentor high school and undergraduate students to develop skills in leadership and scientific communication.
2. Establish myself as a productive and innovative researcher by meeting the benchmarks outlined in *Annual Research Product Goals* and successfully defending my dissertation research.

C. Activities Planned Under This Award

My lab's research focuses on heart failure and spans 4 broad areas: (1) computer modeling of contraction, (2) biochemistry, (3) muscle mechanics, and (4) biobanking. The lab has built a large repository of human myocardium (currently >20,000 samples from 650 patients) and specializes in clinically supported translational research. This expertise and unique access to human samples allow experimental results to extend from sarcomere- to organ-level function. The activities planned under this award include using our experimental techniques and sharpening my scientific repertoire (Table 1).

Table 1. Planned Activities and Percent Effort

Activity	Effort (%)
F31 Research Aims (experimentation, data analysis)	70
Scientific Writing (abstracts, manuscripts)	10
Conferences/Networking	5
Responsible Conduct of Research (trainings)	5
Coursework (department curriculum, communication skills workshops)	4
Leadership (mentor undergraduate/high-school students, collaborative projects)	3
Seminars (seminar series', forums, specialized meetings)	3
Total	**100**

F31 Research Aims

My prime focus will be to complete the 3 Specific Aims described in the Research Strategy and described in Table 2 below.

Table 2. Skills Learned in Specific Aims

Aim		Focus	Resultant Skill(s) Learned
1		RNA turnover pathways	Western blotting to measure protein abundance
2	.1	K48-linked polyubiquitination of titin	Electrophoresis of ultra-large proteins
	.2	Lipofuscin granule accumulation	Cryosectioning, immunostaining, and imaging tissue (slide scanner and confocal microscope); Analyzing fluorescent images
3	.1	Truncated titin in sarcomeres	
	.2	Contractile dysfunction	Tissue permeabilization and multicellular muscle mechanics

Scientific Writing

I will complete "Duke Graduate School Scientific Writing Resource", an online course that will strengthen my conveyance of science into words. Also, I will write and submit at least 2 first-author manuscripts from data collected for this proposal and other research questions explored with the techniques honed during this fellowship.

Conferences/Networking

I will attend at least 2 regional and 1 national/international conference(s) per year to disseminate my work. Examples of such events include the biannual Myofilament Meeting, American Heart Association Basic Cardiovascular Sciences Scientific Sessions, and the European Muscle Conference.

Responsible Conduct for Research

I have and will continue to attend bimonthly, in-person seminars provided by the Bioethics department on a variety of topics related to responsible conduct of research. Also, I will take the “Fundamentals of Bioethics” course offered by the Department of Pharmacology during this award period. This will provide >35 contact hours of RCR training, well above the NIH requirement of 8 hours.

Coursework

I will audit the medical school’s cardiology course (directed by my sponsor, Dr. Campbell) to enhance my understanding of the clinical aspects of our research. Also, I will continue to strengthen my ability to speak about science in communication skills workshops and professionalism courses.

Leadership

I meet, and will continue to meet, one-on-one with Dr. Campbell in-person for 30 minutes every Tuesday . Additionally, we will continue hosting weekly meetings with lab members and collaborators to discuss current projects, grants, and manuscripts in preparation. I will have an advisory committee meeting every 6 months to present updates on my progress toward dissertation defense and receive feedback.

I will mentor at least 1 undergraduate or high-school student each semester throughout this fellowship. I have been mentoring Elizabeth Wilkerson, a junior undergraduate at the University of Kentucky, and Angela Cortazar, a senior at Bryan Station High School, for 1 year. Elizabeth has presented our work as posters at the 2024 Kentucky Chapter of the American Physiological Society Annual Meeting and the 2025 University of Kentucky Center for Clinical and Translational Research Spring Conference. Angela has presented our work as a poster at the 2024 Kentucky Chapter of the American Physiological Society Annual Meeting and a podium talk at the 2024 National Institutes of Health STEP-UP Program Annual Meeting.

This will strengthen my understanding and execution of the experiments within each Aim, along with building mentorship skills.

Seminars

I will continue to attend weekly departmental/cardiovascular seminars and present my work at such gatherings yearly. Also, I will attend weekly cardiology fellow meetings and continue bi-weekly shadowing of cardiologist Vedant Gupta, MD (advisory committee member).

F31 Timeline

Table 3. Activity Timeline During F31 Proposal

Activity	Year 1		Year 2		Year 3	
Courses						
Aim 1: RNA Turnover						
Aim 2: Protein Turnover & Waste Accumulation						
Aim 3: Sarcomere Ultrastructure & Mechanics						
Manuscript Preparation						
Conferences						
Mentoring Students						
Training in RCR						

Proposal Aims

Scientific Dissemination

Professional Development

Research Ethics

Pre-Award Period

During the time of this submission and potential award, I will become proficient at performing muscle mechanics with rat skeletal muscle. Then, I will transition to human cardiac tissue. Concurrently, I will collate summary results from my analysis of our whole exome and transcriptome sequencing results, and continue with allelic phasing, burden analysis, and expression clustering. This will serve as the basis for one of my first-author manuscripts. Also, I will continue to provide around-the-clock support for tissue collections for our myocardial repository.

SPECIFIC AIMS

Truncating variants in the *TTN* gene (TTNtvs) are strongly associated with non-ischemic cardiomyopathy (NICM), a common presentation of heart failure. Titin, the largest known protein in humans, plays an essential role in sarcomere assembly and force generation. In patients with a TTNtv, truncated titin may disrupt cellular turnover mechanisms, leading to accumulation of cytosolic waste (lipofuscin), and improper titin integration into sarcomeres. However, the mechanisms by which TTNtvs contribute to NICM remain poorly understood. This project aims to investigate how TTNtvs disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration, leading to cardiac dysfunction in affected individuals.

Innate turnover pathways exist at the DNA, RNA, and protein levels to limit penetrance of genomic mutations. Two key players in RNA quality control are Up-Frameshift Protein 1 (UPF1) and Exosome Complex 10 (EXOSC10), which are involved in nonsense-mediated and exosomal decay pathways, respectively. At the protein level, damaged proteins are tagged with K48-linked polyubiquitin chains for proteasomal recognition and degradation. Titin's atypical size may overwhelm these RNA and protein checkpoints, resulting in accumulation of ubiquitinated titin and lipofuscin.

Working with Dr. Campbell et al., I have developed experiments to test the **global hypothesis that TTNtvs contribute to NICM pathogenesis by overloading RNA surveillance and proteasomal degradation systems, leading to defective protein turnover, lipofuscin accumulation, and incorporation of truncated titin into sarcomeres.** To test this hypothesis, procured human myocardium from 3 groups will be used: (1) organ donors (control), (2) patients with NICM, and (3) patients with NICM and a TTNtv. Presence of a NICM phenotype and TTNtvs will be confirmed using matched clinical data and whole exome sequencing results, respectively.

Aim 1: Test the hypothesis that samples with a TTNtv have higher UPF1 and EXOSC10 abundance. Homogenized left ventricular tissue will be analyzed using Western blotting to quantify UPF1 and EXOSC10 abundance. 2,2,2-trichloroethanol will be used for total protein normalization, and bands will be quantified using peer-reviewed software developed by our lab.

Aim 2: Test the hypothesis that samples with a TTNtv have higher K48-linked polyubiquitinated titin and lipofuscinogenesis. (*2.1*) Homogenized left ventricular tissue will be analyzed using agarose gel electrophoresis to resolve titin. After transfer to a PVDF membrane, K48-linked polyubiquitinated titin will be quantified with immunoblotting. Oriole fluorescent staining will be used for total protein normalization, and bands will be quantified peer-reviewed software developed by our lab. (*2.2*) Left ventricular tissue will be cryosectioned (10-μm thickness) and immunostained for alpha actinin. A laser within lipofuscin's autofluorescent excitation spectrum (~650 nm) will be used to excite lipofuscin during imaging with fluorescent microscopy. Total alpha actinin will be used to calculate the relative proportion of lipofuscin. Images will be analyzed using custom analysis software that I have trained to write.

Aim 3: Test the hypothesis that samples with TTNtv incorporate truncated titin filaments into sarcomeres and exhibit lower intracellular passive and maximal isometric forces. (*3.1*) Left ventricular tissue will be cryosectioned (10-μm thickness) and immunhistochemically stained for the M-9 epitope of titin, which is near the sarcomeric M-line, and for alpha actinin. Cryosections will be imaged with super-resolution microscopy. Total alpha actinin will be used to calculate the relative proportion of titin. Images will be analyzed using custom analysis software that I have trained to write. (*3.2*) Triton-permeabilized left ventricular tissue will be anchored between a force transducer and length controller to evaluate passive tension and maximal isometric force. Myofilament destabilizing solutions KCl and KI will be used to parse out extracellular and intracellular contribution to passive tension.

Overall Impact

Heart failure remains a leading cause of hospitalization, with a significant gap in understanding the molecular mechanisms underlying NICM, particularly those involving TTNtv. This proposal directly assesses human myocardial function to uncover how TTNtvs disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration. By bridging molecular, cellular, and mechanical analyses, this research could identify new biomarkers and therapeutic targets, offering potential advances in personalized treatments for TTNtv-associated heart failure. I have developed these experiments to extend our understanding of heart failure's multimodal disease onset and have worked with Dr. Campbell to formulate a training plan that will help me develop into a distinguished principal investigator at the forefront of clinical advancement.

RESEARCH STRATEGY

Significance

Heart failure is the leading cause of hospitalization in the US and will affect 1 in 4 people during their lifetime[1,2]. The syndrome has different forms with non-ischemic cardiomyopathy (NICM) being the least understood. The most likely genetic cause of NICM is a truncation variant in the *TTN* gene (TTNtv), which encodes titin[3-9].

Titin, the largest known protein in humans, spans half sarcomeres and is essential for sarcomere assembly, passive tension, and force generation[7,9,10]. Myocyte quality control pathways must attempt to adapt to the atypical size of titin to rid the cell of its faulty transcripts and filaments. Nonsense-mediated and exosomal decay are two major surveillance mechanisms responsible for eliminating faulty transcripts, while the ubiquitin-proteasome system works similarly at the protein-level. The nonsense-mediated decay pathway, mediated by up-frameshift protein 1 (UPF1), recognizes and degrades truncated mRNA species before they are translated[11,12]. Similarly, the exosomal decay pathway, regulated in part by exosome complex 10 (EXOSC10), processes and degrades aberrant transcripts[13,14]. The ubiquitin-proteasome system uses ubiquitin to tag damaged proteins, typically via a K48-polyubiquitin linkage, for proteasomal degradation[15-17].

In NICM hearts, these pathways may be overloaded due to the burden of TTNtvs, which could lead to accumulation of cellular debris (lipofuscin) and/or incorporation of truncated titin into sarcomeres. The status of these mechanisms in TTNtv-associated NICM and their influence on disease progression is unknown.

This project will investigate how TTNtvs disrupt RNA and protein turnover, lipofuscin accumulation, sarcomeric titin integration, and myocardial mechanics (Fig. 1). By utilizing human tissue with matched clinical and multi-omics data, this study bridges molecular, cellular, and mechanical changes with direct translational implications.

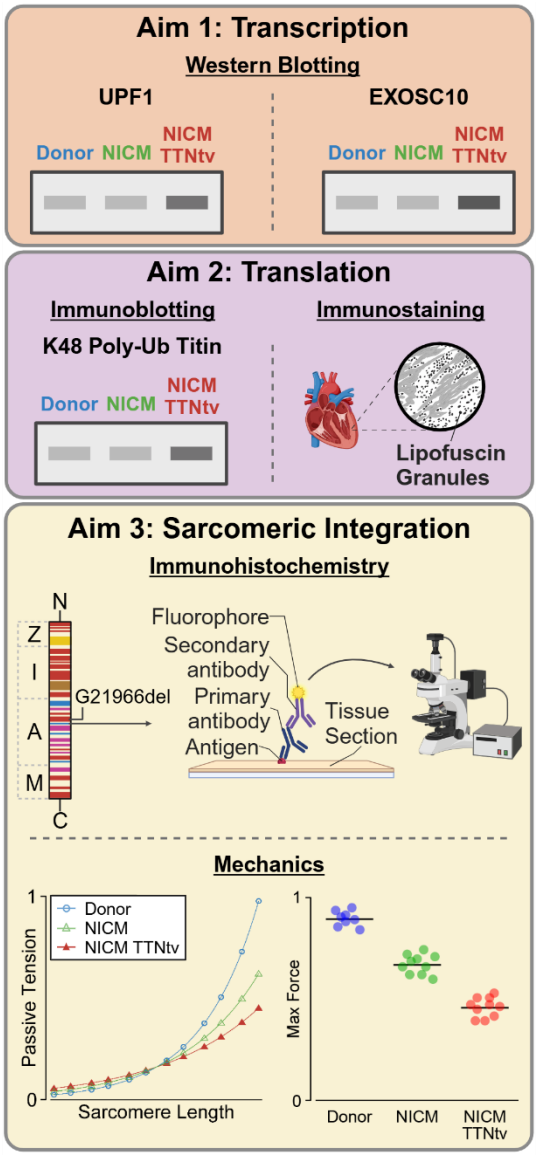


Figure 1. Project Overview

Innovation

This study integrates human myocardial tissue, multi-omics, and clinical data to examine disease mechanisms in TTNtvs. Unlike studies relying on animal models or in vitro systems, this approach provides a direct assessment of human myocardial function.

This research focuses on TTNtv-related inefficiencies in RNA and protein turnover, bridging nonsense-mediated and exosomal decay, proteasomal processing, and lipofuscinogenesis. While previous studies have emphasized titin's structural role, this project expands to explore protein quality control and degradation pathways.

By examining how truncated titin integrates into the sarcomere and affects intracellular tension and force generation, this work offers new insights into the mechanical consequences of TTNtvs. A newly developed image segmentation pipeline ensures accurate quantification of lipofuscin deposition, overcoming challenges posed by autofluorescence.

Potential Path to Clinical Application

Better understanding the pathogenic mechanisms of TTNtvs has direct clinical implications. If truncated titin disrupts sarcomere integrity and reduces contractile function, therapies targeting protein quality control pathways—such as autophagy enhancement or proteasomal regulation—could be explored to mitigate pathogenic effects. Additionally, if TTNtv-associated mechanical dysfunction follows specific patterns, this knowledge could improve risk stratification, refine patient-specific interventions, and guide treatment decisions.

These techniques may distinguish high-risk TTNtv carriers for NICM onset and progression, supporting personalized treatments and advancing precision medicine for TTNtv-associated cardiomyopathies.

Preliminary Studies

The University of Kentucky performs ~1% of the world's heart transplants, and my lab has banked these myocardial specimens for over a decade, amassing over 20,000 samples from 650 patients. I coordinated the whole exome sequencing of 348 patients and analyzed the results to identify 24 patients who had NICM and a TTNtv (Table 1). I also have transcriptomic and (by May 2025) proteomic data for these individuals. I will use samples from age and sex-matched organ donors who did not have NICM or a TTNtv as physiological controls, along with patients with NICM (Table 1). To our knowledge, this is one of the largest datasets of TTNtv-related omics data available world-wide.

Table 1. Patient Characteristics

	Donor (n=24)	NICM (n=24)	NICM TTNtv (n=24)	*p-value*
Age, mean ± SD	39.7 ± 12.7	45.5 ± 15.9	45.6 ± 15.9	0.31
Male, %	66.7	66.7	66.7	
White, %	91.7	70.8	87.5	0.36
Diabetic, %	16.7	16.7	16.7	
BMI, mean ± SD	29.2 ± 6.4	28.3 ± 7.8	29.9 ± 6.0	0.70

Categorical and continuous variables were analyzed with chi-squared tests and one-way ANOVA.

Approach

Aim 1. Test the hypothesis that samples with a TTNtv have higher UPF1 and EXOSC10 abundance.

Rationale

Our transcriptomics data confirm prior studies showing that pathways regulating nonsense-mediated and exosomal decay are upregulated in patients who have TTNtvs[7,8,10,18]. Since nonsense-mediated and exosomal decay are central to clearing faulty transcripts, higher UPF1 and EXOSC10 activity in TTNtv samples is likely to manage the increased presence of aberrant RNAs.

UPF1 identifies and facilitates degradation of mRNAs with premature stop codons[8,10]. By comparison, EXOSC10 interacts with cofactors for transcript processing and serves as part of degradation machinery[14].

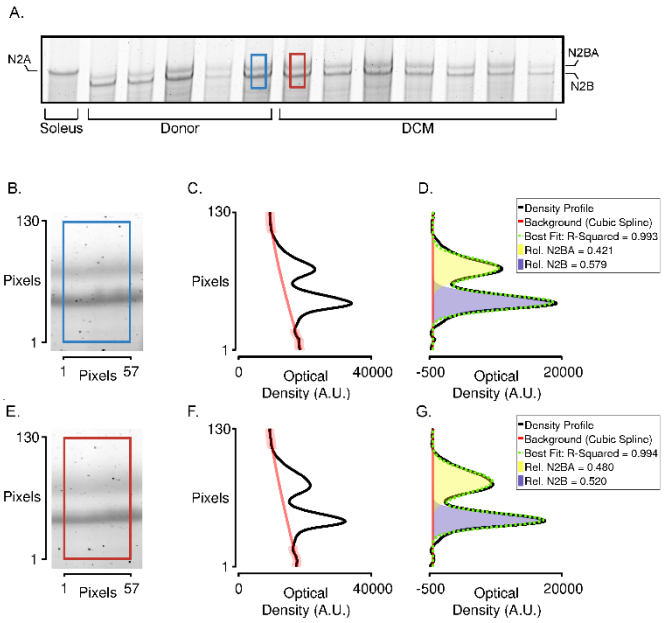


Figure 2. GelBox corrects for background variation and partially resolved bands to semi-automatically quantify gels.

Experimental Design

Left ventricular tissue from organ donors and NICM patients (with and without a TTNtvs) will be analyzed for UPF1 and EXOSC10 abundance via Western blot (n=24/group; Table 1).

Western Blotting

Samples will be resolved on 10% SDS-PAGE gels, transferred to PVDF membranes, and probed for UPF1 and EXOSC10. Total protein will be normalized using 2,2,2-trichloroethanol, and bands will be quantified via GelBox (Fig. 2)[19].

Table 2. Expected Results of Aim 1

Protein	Abundance compared to other experimental groups
UPF1	↑
EXOSC10	↑

Challenge and Potential Solution

Human cardiac tissue is difficult to procure and process consistently. This study circumvents this limitation by utilizing out biobank, which cryopreserves samples within 50 minutes post-explant, a method shown to preserve protein integrity and contractile function[20].

Outcomes

TTNtvs are expected to elevate UPF1 and EXOSC10 abundance, indicating increased activity of RNA degradation pathways in affected myocardium (Table 2).

Aim 2. Test the hypothesis that samples with a TTNtv have higher K48-linked polyubiquitinated titin and lipofuscinogenesis.

Rationale

Damaged proteins are targeted for degradation via K48-linked polyubiquitination, yet proteasomal efficiency declines with age, leading to lipofuscin accumulation[15-17]. These processes are accelerated in disease, compounding protein quality control deficits[15,21].

Titin's large size and abundance may overwhelm proteasomal degradation, promoting lipofuscin formation.

Experimental Design

Using the same myocardial samples as in Aim 1, K48-linked polyubiquitination of titin and lipofuscin granule accumulation will be measured via immunoblot/stain, respectively.

Aim 2.1 K48-Linked Polyubiquitination of Titin

As shown in Figure 2, Samples will be resolved on agarose-stabilized 1% gels, transferred to PVDF membranes, and probed for K48-linked polyubiquitin. Total protein will be normalized using Oriole fluorescent staining and quantified via GelBox[19].

Aim 2.2 Lipofuscin Granule Accumulation

10-µm cryosections will be immunohistochemically stained for alpha actinin and imaged with lasers to exploit lipofuscin's innate autofluorescence (~650 nm) and the attached fluorophore (~555 nm). I have written code to segment fluorescent images and normalize lipofuscin to total cardiomyocyte area (Fig. 3).

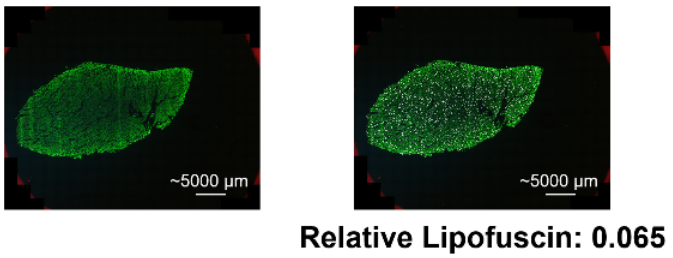


Figure 3. Customized image segmentation pipeline calculates relative lipofuscin from immunofluorescent images.

Challenge and Potential Solution

The size difference between titin (~3,000 kDa) and ubiquitin (~8.5 kDa) make it difficult to distinguish clear signals from noise in immunoblots. Moreover, variability in lipofuscin autofluorescence affects consistency of quantification. This investigation bypasses these hurdles by using highly sensitive and adaptive analysis tools to quantify gels and segment images (Figs. 2 & 3)[19].

Outcomes

TTNtvs are expected to increase titin polyubiquitination and lipofuscin accumulation, highlighting proteasomal inefficiency in NICM (Table 3).

Table 3. Expected Results of Aim 2

Target	Abundance compared to other experimental groups
Aim 2.1: Immunoblotting	
K48 Poly-Ub Titin	↑
Aim 2.2: Immunohistochemistry	
Lipofuscin	↑

Aim 3. Test the hypothesis that samples with a TTNtv incorporate truncated titin filaments into sarcomeres and exhibit lower intracellular passive and maximal isometric forces.

Rationale

Truncated titin is meant to be removed from the cell via quality control mechanisms, but prior studies have shown that TTNtv reduce sarcomere stability and alter kinase/phosphatase activity[9,11-13,15-17,22]. Cellular turnover pathways are modulated by kinase/phosphatase activity, suggesting that faulty titin from a TTNtv may overload turnover pathways, leading to sarcomeric integration of these filaments.

Experimental Design

I will use specimens from the same samples studies in Aims 1 and 2 to test how TTNtv impact muscle mechanics. I will also quantify if truncated titin filaments are incorporated into sarcomeres using immunohistochemistry.

Data will be collected from 3 cryosections or permeabilized cardiac fibers per patient (n=72). Power calculations (G*Power, Cohen's f = 0.17) indicate that this design can detect small-to-medium effect sizes. Statistical analyses will use linear mixed models to account for repeated measures, with a significance threshold of 5% ($p<0.05$).

Aim 3.1 Incorporation into Sarcomeres

10-µm cryosections will be immunohistochemically stained for a titin epitope near the sarcomeric M-line and alpha-actinin (Fig. 4). A custom image segmentation pipeline will quantify the relative proportion of titin by normalizing to total alpha-actinin content.

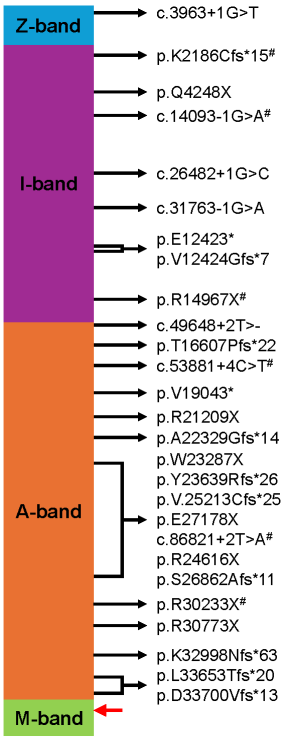


Figure 4. Sarcomeric locations of TTNtv. Red arrow indicates titin epitope. '#': frequency is >1 patient.

Aim 3.2 Biophysical Deviation

~100 mg of tissue per patient will be permeabilized to make ~200-µm x 600-µm cardiac fibers. These fibers will be anchored between a force transducer and length controller to generate force traces upon execution of stretch protocols[23]. Experiments will mimic physiological temperature (37°C) and sarcomere length (2.0 µm).

Fibers will be stretched before and after incubation with potassium chloride/iodide, which depolymerizes myofilaments, to separate intra/extracellular contributions to passive tension.

Fibers will be placed in calcium solutions of varying concentrations (pCa), and force traces will be fit to the Hill Equation, $F = F_0 + F_{max}([Ca^{2+}]^{nH}/([Ca^{2+}]^{nH} + [Ca_{50}^{2+}]^{nH}))$, to determine maximum isometric force (F_{max}; Fig 5).

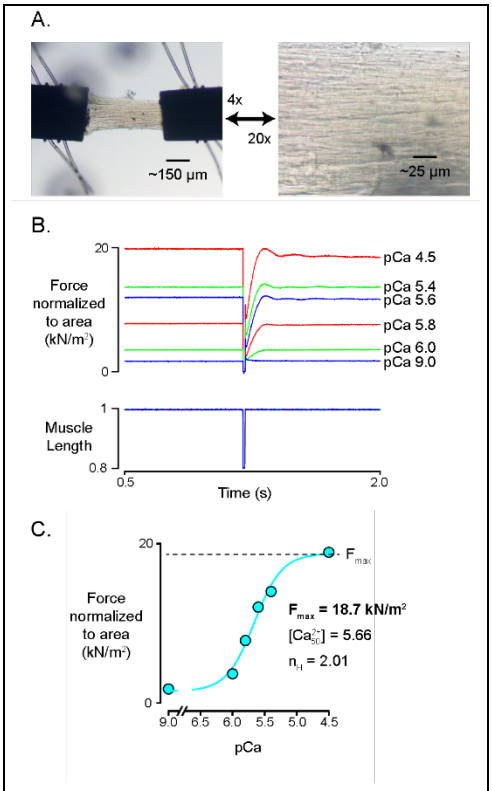


Figure 5. Muscle mechanics experiment I performed with tissue from a NICM patient with a TTNtv.

Challenge and Potential Solution

Detecting truncated titin may be difficult due to low expression and/or masking by full-length titin. This project utilizes a published, epitope-specific titin antibody to ensure adequate detection sensitivity (Myomedix TTN-M9).

Force measurements can be influenced by tissue heterogeneity, fiber quality, and/or uncontrolled sarcomere lengths. To maintain fiber quality and reproducible sarcomere length measurements, these experiments utilize a calibrated camera and exclude fibers with significant pre-experimental damage. Moreover, the design of triplicate measures per patient enhances statistical power and minimizes variability.

Outcomes

TTNtvs are expected to induce sarcomeric integration of truncated titin, leading to reduction of intracellular passive and maximal isometric forces (Table 4).

Conclusions

This study investigates the contribution of TTNtvs to NICM by integrating molecular, histological, and biomechanical analyses. By characterizing the burden of TTNtvs from transcript to protein and its impact on sarcomere function, I aim to clarify whether truncated titin filaments are incorporated into sarcomeres and how they affect myocardial mechanics.

Elucidating the status of myocyte quality control mechanisms in TTNtv-bearing NICM hearts will not only advance our understanding of NICM pathogenesis but also identify potential biomarkers and therapeutic targets for enhancing RNA and protein turnover.

Table 4. Expected Results of Aim 3

Metric	**Abundance compared to other experimental groups**
Aim 3.1: Immunohistochemistry	
Stain Intensity	↓
Aim 3.2: Muscle Mechanics	
Intracellular Passive Force	↓
Maximal Isometric Force	↓↓

RESPECTIVE CONTRIBUTIONS

The applicant's section of this proposal was prepared entirely by Austin Minton. The Sponsor, Dr. Kenneth Campbell provided edits and suggestions for revisions for clarity and format.

The global hypothesis that TTNtvs contribute to NICM pathogenesis by overloading cellular turnover pathways, leading to increased lipofuscin accumulation, incorporation of truncated titin into sarcomeres, and reduced isometric active and passive forces, was developed by the applicant, Austin Minton. The applicant developed the aims and experiments proposed in this project with some input and refinement by his PhD advisory committee.

Development of Training Plan

Austin aims to pursue a career in translational medicine and basic science, which has led him to conduct research in the laboratory of Dr. Campbell. Dr. Campbell's background in cardiac muscle biophysics, biochemistry, and computational modeling will help him develop the skills needed to succeed as an independent scientist. The applicant has started off strong since joining the lab by leading the effort of multi-omic characterization of over 300 human hearts, learning MATLAB code to build segmentation script for analyzing all histological images (fluorescent and brightfield), building skills in muscle mechanics using rat skeletal and human cardiac tissue, and presenting corresponding data at 8 conferences. The training plan developed in this proposal aims to provide Austin with the training and mentorship necessary to maintain a high level of productivity and pursue a career in research as a principal investigator.

Review and Editing of Training Plan

Austin developed the training plan and other submitted documents with edits from Dr. Campbell. Austin presented this proposal to his PhD advisory committee after passing his qualifying exam and becoming a PhD candidate. Members of his advisory committee provided written feedback on the proposal, which Austin used to make revisions and improvements. Additional edits to the training plan were discussed during weekly 1-on-1 meeting with Dr. Campbell, allowing a personalized training plan to form.

Respective Roles in Accomplishing the Proposed Research

Austin Minton will be the principal investigator for this project, conducting all experiments and analyzing subsequent results. Drs. Ebbert and Kampourakis will provide support when their expertise will be helpful. Dr. Campbell will continue to provide mentorship on various aspects of each assay and support with experimental design, data analysis, and interpretation.

SELECTION OF SPONSOR AND INSTITUTION

Institution

I chose the University of Kentucky because it is my state's flagship university . It is 1 of only 22 institutions in the U.S. to have an NIH Clinical and Translational Science Award, NIH Alzheimer's Disease Center, and NIH National Cancer Institute. The College of Medicine contains 7 basic science and 18 clinical departments, along with numerous biomedical centers in areas such as cardiovascular, diabetes and obesity, drug addiction, and infectious diseases. I found interest in the College of Medicine's Integrated Biomedical Sciences Program, which provides 1 year of undifferentiated curriculum to serve as an entry point for 6 doctoral programs. Admission to this Program allowed me to commit 32 weeks of laboratory rotations in the Departments of (1) Neuroscience, (2) Microbiology, Immunology and Molecular Genetics, and (3) Physiology.

Of the 3 Departments I rotated in, the Department of Physiology stood out as ideal for my doctoral training. My interest sparked due to the cohesivity of investigators in distinct research areas, which allows trainees to participate in multi-disciplinary science ranging from molecular to broad-scale levels. The Department has 36 full-time primary and 26 associated faculty. Collectively, these investigators received $15.2 million in extramural funding in 2024, ranking 7th-highest on the Blue Ridge Rankings for Departments of Physiology.

The Department of Physiology is committed to fostering an environment that promotes individual development and networking skills for trainees, including instruction to provide a knowledgebase of cellular, molecular, and organ-system physiology. As a graduate student in the Department, I attend weekly research seminars with 12 seminars hosting external speakers each semester. Along with providing an opportunity to learn about current scientific advancements, the Department holds an informal lunch with each seminar presenter to promote collaboration between trainees and guest speakers. Additionally, the Department offers a broad range of research equipment for trainee utilization, preventing limitations based on instrument availability.

Sponsor

I selected Kenneth Campbell, PhD, as my mentor and sponsor due to his distinguished reputation in translational research pertaining to heart failure. Dr. Campbell is the Director of Translational Research in the Division of Cardiovascular Medicine and the Director of the Biospecimens Core in the Kentucky Center for Clinical and Translational Science. He has a strong publishing history (>130 publications, h-index of 42 including manuscripts in Nature, JCI, and PNAS) and a successful record of mentoring trainees into academia and industry. According to the Blue Ridge Institute for Medical Research, Dr. Campbell was in the 8th percentile of most-funded Physiology investigators in 2023.

Dr. Campbell's lab embraces the complexity of heart failure by incorporating experimentation involving muscle mechanics, biochemistry, histology, and computational modeling. The lab also maintains a myocardial repository containing an excess of 20,000 samples from 650 human hearts. Utilizing worldwide connections in cardiovascular physiology, Dr. Campbell shares samples with labs from over 30 institutions, 1, of which, resulted in a Nature publication describing the first single-molecule-level structural depiction of the cardiac thick filament (*Cryo-EM structure of the human cardiac myosin filament*). A high level of productivity is sustained in Dr. Campbell's lab, as he grounds the team's projects in synchrony with medical professionals at the Albert B. Chandler Hospital, the flagship component of UK HealthCare.

I spent 18 of my 32 weeks of laboratory rotations in the lab of Dr. Campbell. During this time, he offered the opportunity to lead a project that involved identifying genetic variants within his myocardial repository. I eagerly assumed this role, which built upon my previous research experiences in genetics and has led to numerous poster/oral presentations at regional and national/international conferences. In this endeavor, Dr. Campbell unveiled my everlasting desire to use bench science as a conduit to improve medicine and overall treatment of patients in heart failure.

Collectively, Dr. Campbell's expertise, mentorship, and research-orient align directly with my research interests and will be crucial in my aspiration to become a renowned principal investigator in translational cardiac research.

TRAINING IN RESPONSIBLE CONDUCT OF RESEARCH

Completed Training

The University of Kentucky Graduate School utilizes unique opportunities to train individuals in Responsible Conduct of Research (RCR), occurring, both, in the first year Integrated Biomedical Sciences Program curriculum and subsequent years of dissertation research. During my first year, I completed the 'Ethics in Scientific Research' course, which met 1 hour each week for 15 weeks and was directed by Isabel Mellon, PhD. The course held lectures and interactive activities led by numerous biomedical researchers and clinicians, outlined below:

Weeks 1 - 3: Mentoring / Laboratory Supervision

Weeks 4 & 5: Plagiarism / Authorship / Publication

Week 6: Ethics and Regulations of Animals in Research

Weeks 7 - 10: Data Management / Scientific Misconduct / Commercializing Scientific Discovery

Weeks 11 - 15: Case Studies / Human Subject Research

In subsequent years, I completed additional trainings/courses, outlined below:

Collaborative Institutional Training Initiative Courses: Biomedical Investigators and Key Personnel, Health Insurance Portability and Accountability Act (HIPPA) and Human Research

Department of Laboratory Animal Research: Euthanasia of Research Animals, Pain Management of Laboratory Animals, Common Compliance Issues

Planned and Ongoing Training

I will enroll in the 'Fundamentals of Bioethics' course offered by the University of Kentucky Department of Pharmacology (PHS711). Also, I will take advantage of bimonthly University of Kentucky Bioethics Program seminars, annual RCR trainings, and guidance from my sponsor and advisory committee members.

Format: PHS711, the University of Kentucky Bioethics Program seminars, and RCR trainings are in-person. My sponsor, Dr. Campbell, trains me in RCR practices during our weekly one-on-one meetings.

Subject Matter: Curriculum of PHS711 covers many topics, outlined below:

Weeks 1 & 2: Mentor-Mentee Relationships / Responsible Authorship

Weeks 3 & 4: Ethical Data Interpretation / Ethical Use of Vertebrate Animals

Weeks 5 & 6: Ethical Research Administration / Conflict of Interest

Weeks 7 & 8: Ethical Human Resource Management / Diversity and Inclusivity

Weeks 9 & 10: Data Management / HIPPA and Privacy of Human Subjects

Weeks 11 & 12: Ethics of Using Human Subjects / Intellectual Property

Weeks 13 & 14: Research Misconduct / Ethics of Legal intervention

Recent topics of the University of Kentucky Bioethics Program seminars include laboratory breaches, bioethics of non-human primate use, and HIPPA compliance. The University of Kentucky RCR trainings contain seven modules and an annual refresher course, spanning a breadth of related topics. My sponsor and I frequently discuss rigor and reproducible research, impartial data reporting, and protection of human subjects.

Faculty Participation: PHS711 is team-taught by Joseph Chappell, PhD, and Oleg Tsodikov, PhD. The University of Kentucky Bioethics Program seminars has rotating guest speakers from various academic backgrounds and is hosted by Sara Rosenthal, PhD, Director of the Bioethics Program. Advisory committee members John McCarthy, PhD, and Esther Dupont-Versteegden, PhD, will be available to discuss proper experimental design and responsible analysis/reporting of biochemical data. Additional topics of patient privacy and ethics when working with human samples and clinical data will be discussed with advisory committee members Yuan Wen, MD/PhD, and Vedant Gupta, MD.

Duration and Frequency of Instruction: PHS711 lasts one semester, 2 hours weekly. The University of Kentucky Bioethics Program seminars are held bimonthly for 1 hour. I meet, at least, weekly and every 6 months with my sponsor and advisory committee, respectively.

In total, this training plan has greater than 35 contact hours, exceeding the 8 hours required by NIH during this F31 award period.

SPONSOR'S STATEMENT

A. **Research Support Available**

We are fortunate to be funded by multiple MPI grants focusing on quantitative analysis of contractile function in heart failure.

Funding source	ID	Title	PI(s)	Dates	Fiscal year total costs to Campbell
NHLBI	R01 HL146676	Computer modeling of myosin binding protein-C and its effects on cardiac contraction	Stelzer / Campbell (MPIs)	04/2019 – 03/2028	$256,265
NHLBI	R01 HL163977	Data-driven optimization of therapy for heart failure	Campbell / Wenk / Lee (MPIs)	05/2022 – 04/2026	$227,727
NHLBI	R01 HL173989	Biological basis of genetic cMyBP-C myopathies	Stelzer / Campbell (MPIs)	072024 – 06/2028	$224,256
NSF	2406028	Machine-learning enhanced computational models of cardiac pathophysiology	Wenk / Lee / Campbell (MPIs)	09/2024 – 08/2028	$28,077

We also have additional subcontracts where I serve as Co-Investigator (3 R01s and an AHA Transformational Project) as well as 3 pending R01s as MPI. These resources provide complete support for Austin's training.

B. **Sponsor's Previous Fellows/Trainees**

I have trained 2 prior PhD students, 5 prior postdocs, and current mentor 3 PhD students (Austin is one), 1 MD/PhD student, and 3 postdocs. All prior trainees remain in science with 4 having tenure or tenure-track positions. I have also mentored 5 junior faculty members as they apply for independent funding and >50 high school and undergraduate students. Some examples are listed below.:

Trainee Name	Training Period (Trainee stage)	Title of Project	Current Position of past trainee
Haynes, P.	2009 - 2014 (PhD Student)	Transmural variation in human myocardial contraction	Went to Postdoc at University of Washington. Currently a Translational Research Scientist at Bristol Myers Squibb
Campbell, SG (No relation)	2010 - 2012 (Post-doc)	Single myocyte mechanics	Went to tenure-track position at Yale. Now full Professor
Chung, C	2012 - 2015 (Post-doc)	Myocardial relaxation	Went to tenure-track position at Wayne State University. Currently an Associate Professor with tenure
Blair, C.	2013 - 2017 (PhD Student)	Interventricular differences in human myocardial contraction	Went to Postdoc at Stanford & UCSB. Currently tenure-track Assistant Professor at the University of Kentucky
Kosta, S.	2020 – 2022 (Post-doc)	Dual filament regulation of myocardial power output	Transitioned to a position as a Statistical Python Developer at IDDI (International Drug Development Institute) in Belgium

C. **Training Plan, Environment, Research Facilities**

Training plan to develop the applicant's research capabilities

The training plan for Austin Minton has four components. Each has a defined goal, an implementation plan, and objective metrics to quantify success. Austin's PhD advisory committee (1 academic cardiologist and 3 muscle physiologists) will assess his progress every 6 months and provide both Austin and myself with written recommendations. We have specifically chosen a committee with both clinical and basic science expertise to ensure Austin's training will prepare him for his intended career as a clinically-relevant cardiovascular researcher.

A) Experiments with permeabilized multicellular preparations

Current status: Austin is learning to measure the contractile properties of permeabilized skeletal and cardiac muscle but is not yet an expert in the technique.

Goals: Austin can perform publication-quality experiments measuring the contractile properties of chemically permeabilized human myocardium, trouble-shoot apparatus, analyze data, and conduct experiments independently.

Plan: I (Dr. Campbell) will spend 2 hours per week training Austin to (a) operate and troubleshoot the muscle mechanics apparatus, (b) attach permeabilized multicellular cardiac preparations to the equipment, (c) use SLControl software to measure contractile properties of myocardium and (d) automate the analysis of large datasets.

Metrics: Independent ability to perform contractile assays (yes/no), analyze experimental data (yes/no), interpret results (yes/no)

B) Biochemical analysis of human myocardium

Current status: Austin has experience with histological analysis of human myocardium but will learn additional biochemical assays to develop his skills and produce robust data.

Goals: Austin will perform (1) cast and run 10% SDS-PAGE gels to quantify UPF1 and EXOSC10, (2) cast and run specialized 1% SDS-agarose gels for quantifying K48 poly-ubiquitinated titin, (3) use immunostaining to measure relative lipofuscin in tissue, (4) use M-line titin antibodies to quantify truncated titin, and (5) use custom software to analyze gels and histology/IHC data.

Plan: Austin will optimize existing laboratory protocols to carry out experiments to quantify a) UPF1, EXOSC10, K48 poly-ubiquitinated titin, and truncated titin abundance, and b) lipofuscin.

Metrics: Independent ability to produce high-quality biochemical data (yes/no), analyze experimental data (yes/no), interpret results (yes/no)

C) Scientific Writing

Current status: Austin drafts abstracts, protocols, and manuscripts independently with edits from Dr. Campbell but can, like most scientists, further refine and enhance the clarity of his writing.

Goals: Austin will continue to write and submit high-quality manuscripts and grant proposals in addition to assisting in the editing of collaborators' manuscripts.

Plan: Austin will complete the online course in scientific writing developed by the Graduate School at Duke University. Dr. Campbell will spend 2 hours per week helping Austin to improve his manuscripts and grants.

Metrics: Applications submitted for predoctoral fellowships (F31 and AHA) (yes/no). The number of manuscripts submitted for which Austin has made significant writing contributions (goal of 2 first author papers from this project plus 2 middle author).

D) Networking and Career Development

Current status: As an aspiring principal investigator, Austin interacts at least weekly with both basic scientists and academic cardiologists.

Goals: Austin develops a reputation as an outstanding PhD student and builds relationships that will be useful as he progresses in his career and establishes himself as a principal investigator.

Plan: Austin will present a first author poster and, if the opportunity arises, platform talks at the Biophysical Society (spring) and AHA BCVS (summer) meetings. He will also give poster or oral presentations at the annual Cardiovascular Research Day hosted by the Gill Cardiovascular Institute at the University of Kentucky.

Metrics: Number of talks (goal of 2) and posters (goal of 8) from this project.

E) Additional Seminars and course-work:

Course/Event	When	Why
MD 826: Medical School Cardiology (audit)	Fall semester	Provides an additional clinical perspective
PGY 603: Foundations of Experimental Design and Analysis	Fall semester	Improve understanding the principles and pitfalls of experimental design, biostatistics, and data analysis
PHS 711: Responsible Conduct of Research	Spring semester	Further learn the fundamental principles of ethical and responsible conduct of reporting of his research
Physiology seminar series	Tuesday mornings	Improve knowledge and learn latest techniques within the department
Cardiovascular seminar series	Friday mornings	Improve knowledge and learn latest clinical advancements

F) Training in responsible conduct of research and rigor and reproducibility:

Austin will take PHS711, 'Responsible Conduct of Research' which satisfies NIH's requirements and complete additional university-mandated training that includes online activities and in-person discussions focused on data archiving, rigor and responsibility, and ethical conduct of research. Lastly, he will complete the CITI-based RCR refresher training yearly.

G) Strategy for insufficient progression

Given Austin's success to date and his academic trajectory, we expect he will achieve all the goals outlined above. I meet with Austin weekly during our scheduled one-on-one meetings to discuss his progress, goals, and aspirations. If he fails to meet some of the defined metrics for an unforeseen reason, Austin will work with his PhD advisory committee (Esther Dupont-Versteegden, PhD, John McCarthy, PhD, Vedant Gupta, MD, Yuan Wen, MD/PhD) to refine the training plan and explore additional opportunities.

Relationship of training plan to applicant's career goals.

After completing his PhD, Austin plans to continue translational research in cardiovascular disease. This project will help him develop essential research skills and increase his scientific depth. He has not ruled out switching from a traditional academic path to industry in the medium term (5 to 10 years) but his immediate goals are to complete a meaningful PhD followed by a rigorous postdoc and a potential transition to PI status/assistant professor position.

Training Environment/Facilities

Personnel: Currently, the lab has 4 graduate students and 3 post-doctoral scholars. Our research is supported by 2 research coordinators, and a project manager. We have exemplary logistical support for human subjects research and grants management from the Division of Cardiovascular Medicine.

Laboratory: The Campbell laboratory has a total area of ~1500 sq. ft. of contiguous space with 2 additional rooms for specialized microscopy. Besides the Zeiss Axioscan Z7 and Nikon AXR Confocal microscope, all equipment required for Aims 1, 2, and 3 of this project are already available in the Campbell Laboratory. Aims 1 and 2.1 use gel electrophoresis and Western blot setups. Aims 2.2 and 3.1 use ventilated fume hoods. Aim 3.2 uses a specialized muscle mechanics setup with an inverted microscope with video attachment, a vibration isolation table, a force transducer, and a length controller. Austin has been trained on and has access to the Zeiss Axioscan Z7 and Nikon AXR Confocal microscope in the University's Light Microscopy Core, used in Aims 2.2 and 3.1 respectively.

Additional equipment available in the laboratory includes a Bio-Rad ChemiDoc, a SpectraMax i3x Multi-mode Microplate Reader, a ThermoFisher cryostat, a Thermofisher Nanodrop, dissection microscopes (x6), various centrifuges, a pH meter, refrigerators (x2), -20°C freezers (x2), a -80°C freezer, liquid nitrogen tanks, 4 LocatorPlus cryogenic storage systems, top-pan balances, and an ultra-pure deionized water supply.

Office: Our graduate students share a large office (400 square feet). Austin has an assigned desk with space for 3 computer monitors. My own office (202 square feet) is located adjacent to Austin's office and is fully equipped. All-in-one scanners/high-speed printers are supplied as a Departmental resource.

Clinical: The University of Kentucky currently performs ~1% of the world's cardiac transplants (~160 in the last four years) and implants another ~40 Ventricular Assist Devices per year. Dr. Campbell is the PI of the Gill Cardiovascular Biorepository and leads an IRB protocol that allows researchers to procure specimens that would otherwise be discarded from any patient undergoing any cardiovascular procedure. Myocardial samples are acquired directly from the Operating Room by Dr. Campbell's team and transferred to the basic science laboratories (~5-minute walk) for further study. More than 20,000 samples (each ~500 mg) have been acquired from ~650 patients and organ donors since 2008. Most are snap-frozen and stored long-term in the vapor phase of liquid nitrogen, but Dr. Campbell's team also performs experiments using living trabeculae and freshly isolated myocytes. Cardiac slices are currently under development.

D. **Number of Fellows/Trainees to be Supervised During the Fellowship**

I currently mentor 4 graduate students and 3 post-doctoral scholars. Two of the students will graduate in the summer of 2025. One of our postdocs will transfer to medical residency. I meet in-person weekly one-on-one with each trainee for 30 minutes. There is an additional 90-minute lab meeting once per week. We all work in person and share a heavily used coffee machine so I interact informally with each trainee most days.

Trainee Name	Training Period (Trainee stage)	Title of Project	Source of Support for Trainees
Wellette-Hunsucker, A.	2021 -present (PhD student)	Biochemical changes of sarcomeric proteins in dilated cardiomyopathy	F31 HL170558 (to 2027)
Milburn G.	2021- present (PhD student)	Effects of mechanical unloading on eccentric growth signaling	AHA 24PRE1181511 (to 2025)
Minton, A.	2023 – present (PhD student)	Genomics of human dilated cardiomyopathy	HL163977 (to 2026)
Roth, C.	2024 – present (PhD student)	Cardiac slices	Supported by HL HL173989 (to 2028)
Squarci, C.	2023 – present (Postdoc)	Single myofibril mechanics in human heart failure	Supported by HL HL173989 (to 2028)
Daneshgar, N.	2024 – present (Post-doc)	DNA damage and cardiac pathology	Supported by HL HL146676 (to 2026)
Pakbaz, M.	2025 – present (Postdoc)	Cardiac dysfunction in HfrEF	Research endowment

E. **Applicant's Qualifications and Potential for a Research Career**

Austin joined our lab ~24 months ago to complete his PhD after rotating through the lab for 16 weeks. In his first year, he leveraged his undergraduate research experience to isolate DNA and RNA from 350 hearts in our bioank. We then invested ~$250,000 to generate genomic, transcriptomic, and proteomic data for these samples yielding ~30 TB of raw data and one of the largest multiomic cardiac datasets worldwide.

Austin has taken point on this project and has become our 'omics expert. I have taken great pleasure in watching him evolve as a scientist and become proficient at coding and analysis of extremely large datasets. He is creative, thoughtful, and ingenious. His performance during his qualifying exam was exemplary and notable for the scientific independence that he demonstrated. I see no limit to what he can achieve going forward and am totally committed to his future in research. With 4 MPI R01s in-hand and 28 grant applications (fellowships to multi-PI trials) submitted in 2024, our lab is as well positioned as any at our university to support his development.

Austin's performance would be remarkable for any student but is particularly noteworthy for an individual who grew up with very limited resources. He was raised in rural western Kentucky in a home that lacked a consistent

and safe water supply. The electricity failed (or was cut-off for non-payment) twice per week and Austin's main source of nutrition as a teenager was the wild game he could shoot for his family. It is astounding to me (but also a source of great hope) that a student raised with these challenges is now working at the cutting edge of omics-based cardiovascular research. He has my full and unflinching support.

Sanders-Brown Center on Aging

MARK T. W. EBBERT, PH.D.
ASSOCIATE PROFESSOR
SANDERS-BROWN CENTER ON AGING
DEPARTMENT OF INTERNAL MEDICINE
P: 859.218.0125
MARK.EBBERT@UKY.EDU

March 31, 2025

Dear Austin:
I am pleased to confirm my support for you and your F31 pre-doctoral fellowship proposal titled: "*Contribution of Titin-Truncating Variants to Human Non-Ischemic Cardiomyopathy*". I have worked as a translational bioinformatician and omics expert for two decades, providing invaluable expertise in the links between genomic variants and human pathology.

Your proposal sounded very interesting during our previous meetings about your lab's sequencing data. Your aims span potential aberrations at each level of the central dogma, which has been a crucial focus of my professional journey. The novelty of your hypothesis—titin-truncating variants overloading cellular turnover mechanisms—makes any findings from this proposal extremely beneficial to the fields of science and medicine. Moreover, the utilization of human tissue and large sample sizes significantly strengthens the translation of your proposal.

Again, I am delighted to assist you with this project and are always welcome to meet with me whenever needed. More specifically, I have helped you with handling and analyzing your DNA and RNA sequencing datasets, along with building the sample size of patients with titin-truncating variants to 24 by introducing deep-learning tools like SpliceAI. I would be more than willing to help you develop skills to use additional variant prediction tools and discuss interpretation of multi-omic findings.

If you have any questions or need additional information, please contact me at 859.218.0125 or mark.ebbert@uky.edu.

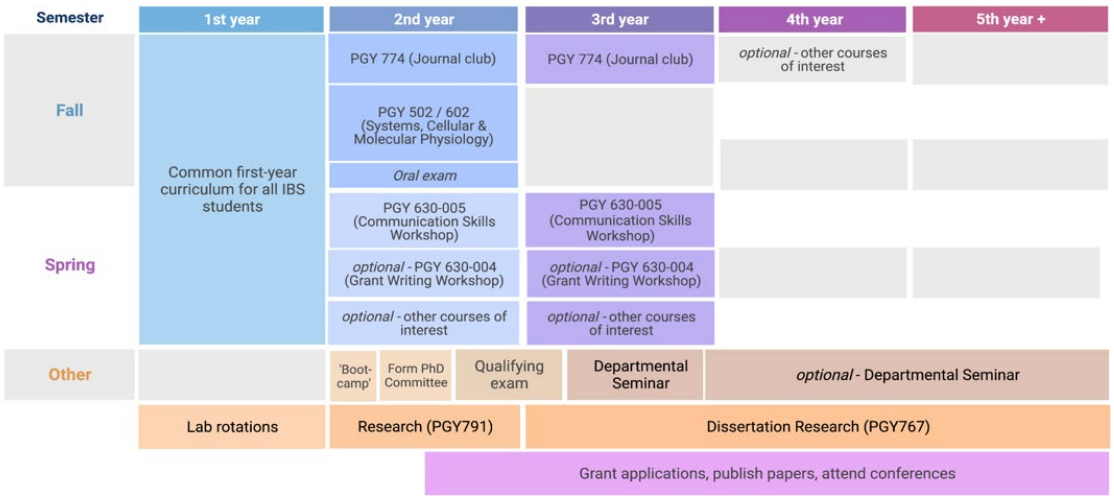
Sincerely,

Mark T. W. Ebbert, Ph.D.
Associate Professor
Sanders-Brown Center on Aging
Biomedical Informatics Division of Internal Medicine Department
Associate Director, Statistical 'Omics' Research Collaborative
University of Kentucky

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING

The University of Kentucky is the state's flagship public university with ~32,000 students (8,773 graduate students), 16 colleges, 112 undergraduate, 85 masters, and 55 doctoral programs. University research funding was over $527 million for fiscal year 2024, with $225 million of that supporting research in the College of Medicine. The Department of Physiology is one of 6 basic science departments in the College of Medicine and has 36 full-time primarily appointed faculty, 26 joint faculty, and 49 current PhD students, and numerous postdocs and staff. Extramural support for the department totaled ~$15 million in 2023, ranking 7th nationally in NIH funding. The Physiology faculty teach ~2,400 undergraduate, professional, and graduate students in ~50 courses each year.

Educational Information



First year: Most PhD students in the College of Medicine are admitted through a common Integrated Biomedical Sciences Program which enrolls 20-25 students per year. These trainees spend their first year completing a core curriculum that includes biomedical ethics, team-taught classes in cellular and molecular biomedicine, elective mini-courses and four separate 8-week research rotations. Students select a mentor from 200 full-time participating faculty within 6 basic science departments. Those choosing a mentor in the Department of Physiology join the Physiology training program at the end of their second semester in.

Second year: Incoming Physiology students take PGY502 (didactic) and PGY602 (discussion based) classes during fall semester which provide 8 hours per week of advanced training in systems, cell, and molecular level physiology. As part of the PGY602 course, students complete a combined written and oral comprehensive examination process consisting of 8 hours of written examinations and a 2-hour oral exam administered by eight Physiology faculty instructors. Students must perform at B-grade or better on the comprehensive exams to continue in the Physiology program.

Additional required coursework in the 2nd year includes PGY774, a one credit, discussion-based class that focuses on reading scientific literature, learning to review manuscripts, and the nuances of experimental design. Spring semester students also complete a Communication Skills Workshop, a weekly meeting that teaches effective presentation and writing styles and in which students present oral presentations related to their research project and receive faculty and student feedback.

Typically, in spring of their 2nd year, PhD students form their advisory committee which consists of:

1) The Dissertation Director (who is generally the primary advisor)
2) Two or more additional faculty members from the Department of Physiology
3) At least one member from a supporting area outside the department

Faculty members in Physiology have diverse research interests, spanning neuroscience, aging, cardiovascular physiology, muscle biology, and respiratory physiology. Thus, nearly all student committees are multidisciplinary. As part of biannual advisory committee meetings, student progress is evaluated by each committee member using standardized metrics. The departmental Graduate Affairs Committee monitors student progress annually.

Third year: All students must complete their formal qualifying exam before the end of their 3rd year fall semester. The procedures for this exam are as follows:

1) Students develop and present two separate 500-word abstracts summarizing research proposals which are distinct from the primary advisor's submitted grants.

2) The advisory committee selects one of these projects which the student then expands into a F31-style NIH grant proposal over a 4–6-week period.
3) The advisory committee examines the student, testing their ability to formulate novel hypotheses and logical experimental designs as well as their knowledge of physiology and their chosen specialized field.

Required coursework includes one semester of PGY774 and one semester of Communication Skills Workshop.

Subsequent years: Students who pass their qualifying exam enroll in PGY767 (2 research credit hours) until they are ready to defend their thesis. The final exam consists of a public one-hour seminar followed by a closed-door meeting with the advisory committee and an outside examiner appointed by the University of Kentucky Graduate School. Students must demonstrate a detailed knowledge of their field of study and defend the conclusions that they present in their written thesis. The quality and extent of the work must be such that the advisory committee regards it as suitable for publication in a reputable scientific journal.

Summary data: The Department of Physiology typically accepts 6 to 8 PhD students per year from the Integrated Biomedical Sciences Program and/or the College of Medicine MD/PhD program. On average, 6 students graduate from the program each year with a mean time to completion of 4.9 years. Recent graduates have moved on to post-docs at nationally ranked institutions, prominent industry positions, and leadership roles at several research Foundations.

Additional training opportunities

In addition to research-specific seminars and journal clubs, all Physiology trainees attend weekly departmental seminars throughout their entire training and present in this forum during in their 2nd and 3rd years.

All students in the program are also encouraged to complete:

1) Physiology bootcamp – a summer-long program taken at the end of the first year which focuses on biomedical statistics and experimental design, data and image acquisition, presentation of results, funding strategies, and career development skills.
2) Teaching skills workshop – an informal program led by the department's Director of Teaching which helps students to gain experience lecturing to undergraduates and leading discussion groups, laboratory sessions, and lab demonstrations.

All students also attend a wide range of regional, national, and international-level scientific meetings. Typical events include: Kentucky Chapter of the American Physiological Society, Experimental Biology, Biophysical Society, Society for Neuroscience, and American Heart Association Scientific Sessions.

Status of Applicant

Austin Minton is in the 3rd year of his PhD studies at the University of Kentucky. He transitioned into the Physiology program during the summer of 2023 after successfully completing his first year in the Integrated Biomedical Sciences Program. He is in good academic standing and has passed his qualifying exam.

Individual providing this information

Lance A. Johnson, PhD
Director of Graduate Studies, Department of Physiology
Associate Professor, Sanders Brown Center on Aging
University of Kentucky

PHS Assignment Request Form

Funding Opportunity Number: PA-23-272

Funding Opportunity Title: Ruth L. Kirschstein National Research Service Award (NRSA) Individual Predoctoral Fellowship (Parent F31)

Awarding Component Assignment Request *(optional)*

If you have a suggestion for an awarding component (e.g., NIH Institute/Center) assignment, use the link below to identify the appropriate short abbreviation (e.g. "NCI" for National Cancer Institute") and enter it below in the boxes for "Suggested Awarding Components". All suggestions will be considered; however, not all assignment suggestions can be honored.

Awarding Components: https://grants.nih.gov/grants/phs_assignment_information.htm#AwardingComponents

Suggested Awarding Component:

Study Section Assignment Request *(optional)*

If you have a suggestion for a study section assignment, use the link below to identify a study section(s). Enter the short abbreviation for that study section in the boxes for "Suggested Study Sections." Remove all hyphens, parentheses, and spaces. All suggestions will be considered; however, not all assignment suggestions can be honored.

For example, enter "CAMP" if you wish to suggest assignment to the NIH Cancer Molecular Pathobiology study section, or "ZRG1HDMR" if you wish to suggest assignment to the NIH Healthcare Delivery and Methodologies SBIR/STTR panel for informatics.

Study Sections: https://grants.nih.gov/grants/phs_assignment_information.htm#StudySection

Assign to Study Section:
Each entry is limited to 20 characters

PHS Assignment Request Form

Rationale for assignment suggestions ***(optional)***

Entry is limited to 1000 characters

List individuals who should not review your application and why ***(optional)***

Entry is limited to 1000 characters

Identify scientific areas of expertise needed to review your applications ***(optional)***

Note: Do not provide names of individuals

Expertise:
Each entry is limited to 40 characters

PHS Human Subjects and Clinical Trials Information

Use of Human Specimens and/or Data

* Does any of the proposed research involve human specimens and/or data? ●Yes ❍No

Provide an explanation of why the application does not involve human subjects research

Are Human Subjects Involved ●Yes ❍No

Is the Project Exempt from Federal regulations? ❍Yes ●No

Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

Other Requested Information

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Gill Cardiovascular Biorepository	No

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

Section 1 - Basic Information (Study 1)

1.1. * Study Title (each study title must be unique)

Gill Cardiovascular Biorepository

1.2. * Is this study exempt from Federal Regulations? ❍Yes ●No

1.3. Exemption Number ❏ 1 ❏ 2 ❏ 3 ❏ 4 ❏ 5 ❏ 6 ❏ 7 ❏ 8

1.4. * Clinical Trial Questionnaire

1.4.a. Does the study involve human participants?	●Yes	❍No
1.4.b. Are the participants prospectively assigned to an intervention?	❍Yes	●No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍Yes	●No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍Yes	●No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable NCT00753974

Section 2 - Study Population Characteristics

2.1. Conditions or Focus of Study

- Cardiovascular disease

2.2. Eligibility Criteria

All patients undergoing cardiovascular surgery / procedure at the University of Kentucky who are at least 18 years og age.

2.3. Age Limits	Min Age: 18 Years Max Age: N/A (No limit)
2.3.a Inclusion of Individuals Across the Lifespan	inclusion_across_lifespan1025620984.pdf
2.4. Inclusion of Women and Minorities	inclusion_of_women_and_minorities1025620985.pdf
2.5. Recruitment and Retention Plan	recruitment_and_retention1025620986.pdf
2.6. Recruitment Status	Recruiting
2.7. Study Timeline	study_timeline1025620987.pdf
2.8. Enrollment of First Participant	06/01/2008 Actual

Inclusion Enrollment Reports

Entry#	Enrollment Location Type	Enrollment Location
IER 1	Domestic	University of Kentucky Chandler Medical Center

Section 3 - Protection and Monitoring Plans

3.1. Protection of Human Subjects

protection_of_human_subjects1025620988.pdf

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site? ❍ Yes ● No ❍ N/A

If yes, describe the single IRB plan

3.3. Data and Safety Monitoring Plan

data_safety_and_monitoring_board1025620989.pdf

3.4. Will a Data and Safety Monitoring Board be appointed for this study? ❍ Yes ● No

3.5. Overall Structure of the Study Team

overall_structure1025620990.pdf

Section 4 - Protocol Synopsis

4.1. Study Design
4.1.a Detailed Description
4.1.b. Primary Purpose
4.1.c. Interventions

Type	Name	Description

4.1.d. Study Phase
Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ❍ No

4.1.e. Intervention Model
4.1.f. Masking ❍ Yes ❍ No

❏ Participant ❏ Care Provider ❏ Investigator ❏ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description

4.3. Statistical Design and Power
4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/Investigational Device Exemption (IDE) status
4.6 Is this an applicable clinical trial under FDAAA? ❍ Yes ❍ No

4.7. Dissemination Plan

Section 5 - Other Clinical Trial-related Attachments

5.1. Other Clinical Trial-related Attachments

Inclusion Enrollment Report 1

1. * Inclusion Enrollment Report Title: Inclusion enrollment for Gill Cardiovascular Biorepository

2. * Using an Existing Dataset or Resource: ●Yes ❍No

3. * Enrollment Location Type: ●Domestic ❍Foreign

4. Enrollment Country(ies): USA: UNITED STATES

5. Enrollment Location(s): University of Kentucky Chandler Medical Center

6. Comments: Actual enrollment shows cumulative data since the inception of the biorepository in 2008. Planned enrollment is calculated as the actual enrollment prorated to an expected number of 75 patients per year.

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	2	1	0	0	3
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	15	39	1	3	58
White	76	211	6	17	310
More than One Race	2	2	0	0	4
Total	95	253	7	20	375

Cumulative (Actual)

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			Total
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	1	0	0	0	0	0	0	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	23	57	0	0	1	0	3	9	0	93
White	115	324	0	1	5	0	15	37	0	497
More than One Race	0	2	0	1	1	0	0	0	0	4
Unknown or Not Reported	0	0	0	0	0	0	0	7	43	50
Total	139	383	0	2	7	0	18	53	43	645

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN

All participants will be over the age of 18. There is no upper age limit.

Children will not be included in this study because the University of Kentucky does not have a pediatric heart transplant or assist device program. Patients over the age of 18 who are receiving transplants and/or assist devices are the main source of tissue in this study. In addition, pediatric patients would be a very small cohort with a variety of diseases (mostly congenital) which would make it difficult to draw meaningful conclusions.

The protocol will be expanded to include children if/when the University of Kentucky develops pediatric cardiac transplant and/or assist device programs.

INCLUSION OF WOMEN AND MINORITIES

This study analyzes myocardium procured from patients at the University of Kentucky who are undergoing cardiac surgery. The experiments in this proposal will use tissue that is procured directly from the operating room and frozen in liquid nitrogen before being shipped to the experimental sites.

Two enrollment reports are provided. The first describes the patients who have already donated samples. The second documents planned enrollment during the project.

Distribution of Subjects by Sex/Gender and Racial/Ethnic Group

Subjects will not be selected for this study based on their sex/gender or racial/ethnic group. The research team attempts to recruit every patient who is listed for cardiac transplant and/or is scheduled to receive a Ventricular Assist Device. The distribution of subjects is representative of the patients who have advanced heart failure in Kentucky.

Proposed Outreach

There are no plans to develop an outreach program to recruit specific groups. Recruiting patients to the study solely to collect tissue would expose them to unnecessary risk.

Proposed Exclusion

No sex/gender or racial/ethnic group will be excluded from the study.

RECRUITMENT AND RETENTION

Recruitment

Members of the research team seek informed consent from every patient at the University of Kentucky who is listed for cardiac transplant and/or is scheduled to receive a Ventricular Assist Device. Almost all patients (>99%) enroll in the study.

Retention

Patients do not need to be retained in the study after the surgery. They complete their involvement by donating tissue samples that were excised as part of normal care and which would otherwise have been discarded.

STUDY TIMELINE

The Gill Cardiovascular Biorepository already contains >15,000 myocardial specimens collected from >550 patients and organ donors. This timeline shows additional patients who will be recruited during the course of this project.

Years after notice award	1	2	3	4	5
Cumulative number of patients recruited	75	75	75	75	75

PROTECTION OF HUMAN SUBJECTS

Background

Cardiovascular disease accounts for 1 in 3 deaths in the United States and costs the country >$300 billion per year (2016 American Heart Association Statistical Update). Major disease sub-groups include myocardial ischemia, vascular disease, and heart failure with many patients presenting with more than one condition. For example, myocardial ischemia often leads to heart failure which in turn can worsen vascular disease.

Treatment options for the millions of Americans who have cardiovascular disease are currently limited by inadequate understanding of cardiovascular biology and pathophysiology. Animal models of cardiovascular disease can be used in many research projects but biospecimens from patients are required for translational studies. Blood samples can be obtained with minimal risk from most cardiovascular patients. Myocardial and vascular specimens are often removed as part of standard care during clinical procedures.

This protocol allows researchers to collect some of these biospecimens for research and to use these tissues and blood samples in studies that could ultimately help to improve treatment options for patients who have cardiovascular disease.

Objectives

Use biospecimens procured from patients and organ donors to improve understanding of molecular, cellular, and tissue-level processes produced by cardiovascular disease and therapeutic interventions.

RISKS TO HUMAN SUBJECTS

Study Design

This is a prospective evaluation of patients undergoing cardiovascular procedures at the University of Kentucky Medical Center.

Study Population: Inclusion criteria

All patients undergoing cardiovascular surgery/procedure at the University of Kentucky who are at least 18 years of age.

Subject Recruitment Methods and Privacy

Subjects will be recruited from the Gill Heart & Vascular Institute, the University of Kentucky Emergency Department, or other clinics and services at UK HealthCare.

Whenever possible, subjects will be informed of the opportunity to participate in this study after they have been informed of their need for a cardiovascular procedure. A member of the research team will then provide a detailed description of the research and obtain informed consent. The procedures for retrospective consents (which should be used only for emergency procedures) are described below.

Research Procedures

This protocol covers the procurement of tissue samples and blood samples from patients undergoing any cardiovascular surgery and/or clinical procedure.

Any tissue sample that is procured as part of a normal cardiovascular procedures and which would otherwise be discarded can be collected under this protocol. Examples of these tissue samples include:

- Ventricular myocardium
- Atrial myocardium
- Epicardial fat
- Aortic segment
- Peri-aortic fat
- Blood vessels (arterial or venous)
- Skeletal muscles including pectoralis, intercostal, and diaphragm

Peripheral blood samples may also be collected from patients. These are the only biospecimens that will be procured specifically for this research and that are not collected as part of normal cardiac care. These samples will consist of 5 to 10 cc of peripheral blood and may be collected:

- Prior to procedure
- Immediately after procedure
- At different time intervals after the procedure during the hospital stay. These samples will not exceed the frequency and volume limits detailed in the IRB regulations
- At the first outpatient follow-up after procedure
- Annually at an office-visit
- Upon readmission if hospitalized

When possible, these collections will be coordinated with clinical laboratory tests to minimize the need for additional needle sticks. However, collection of blood from a peripheral vein may be required.

Sample utilization

This research aims to advance understanding of cardiovascular disease and to support the development of improved therapies.

Many of the biospecimens will be used in experiments assessing cellular-level function and changes in mRNA and protein content associated with cardiovascular disease. However, the investigators also request permission to use the biospecimens in assays that are not specifically described in this application. This does not present additional risks to the patients (because the samples have already been collected) and it will allow the investigators to test hypotheses and to explore research directions that could not be envisaged at the beginning of the study.

Being able to use the banked samples for many types of experiments is important because there are only a few opportunities per year to collect some of the specimen types (for example, discarded cardiac tissue from patients who are receiving a heart transplant but have not been supported by a mechanical assist device or from patients who have an open repair of their abdominal aortic aneurysm.) If the researchers had to reapply to the IRB each time they needed to add a new assay, and then collect new samples (which could take several years), the rate of research would become extremely slow. This would seriously hinder the investigators' goal to support the development of improved therapies for patients with life-threatening cardiovascular disease.

Experimental collaborations

Researchers at the University of Kentucky can perform many of the experiments that are likely to be useful. However, there are some experiments that require specialized equipment or unusual skills that are not available on our campus. To facilitate the research, the investigators therefore request permission to share biospecimens with collaborators at other institutions who can help to advance the research goals.

Before samples are shared, researchers at the University of Kentucky will:

1) De-identify the biospecimens (that is, provide them to the collaborator in packaging that is labeled with a randomized code that the collaborator cannot link to an identified person.)

2) On request, provide de-identified summary data about the patient or organ donor that each sample came from (for example, diagnosis, age in years, dimensions of cardiac chambers, diabetic status, medications at time of specimen collection). Under no circumstances will PHI data be disclosed.

The ability to share specimens with collaborators will accelerate the rate of research and does not expose the patients and organ donors to additional risks. Since the collaborators will not be able to associate the specimens with identified people, it is likely that most IRBs will decide that the collaboration does not meet the criteria for human subjects research and does not need additional review.

Payment

Patients will not receive any financial inducements for their participation in this study.

Costs to Subjects

All costs associated with this study are considered standard of care. There are no research related costs. The patients' insurance company, Medicare or Medicaid will be responsible for the costs of all care and treatment that they receive during this study that they would normally receive for their condition. These are costs that are considered medically reasonable and necessary and will be part of the care the patient would receive if they did not take part in this study.

Subject Complaints

If patients have complaints in relationship to their participation in this research study, they may contact anyone on the research team or the Office of Research Integrity.

Research Involving Non-English Speaking Subjects or Subjects from a Foreign Culture

Not applicable

HIV/AIDS Research

Not applicable

ADEQUACY OF PROTECTION AGAINST RISKS

Informed Consent Process

Subjects will be interviewed by a member of the research team and have the nature and purpose of the study described as well as any potential risks involved with participation. All attempts will be made to give the patient ample time to consider research participation prior to procedure. If the subject agrees to participate, he/she will be required to provide written informed consent.

The researchers may ask patients or their legal surrogate for retrospective consent in cases where the biospecimens could only be obtained during an emergency clinical procedure (for example, percutaneous coronary intervention for an acute myocardial infarction, or surgical repair of a ruptured abdominal aneurysm). Retrospective consent is the only practical option in these emergencies because it would not be possible to obtain standard informed consent using the normal procedures. Biospecimens collected under these conditions would be saved until the patient or their legal surrogate was able to participate in the informed consent process. If they declined or were not able to consent, specimens and research data would be destroyed.

Resources

Clinical research staff are available 24 hours/7 days/week to address any research concerns. All research activities will be conducted by cardiovascular personnel at the University of Kentucky Medical Center.

Potential Risks

The main risk associated with this study is likely to be that associated with data collection and patient confidentiality.

Tissue collection will not present patients with additional risk because all of the samples that are used for research were already going to be removed as part of normal clinical care. These tissues would have been discarded if they had not been saved for research.

There are some minor risks associated with collecting peripheral blood. These include bleeding, soreness, bruising, and infection. In rare instances, fainting may occur.

There may also be some risks that are not known.

Safety Precautions

Provisions to guard against the potential risks and discomforts discussed above are as follows:

Every precaution to prevent a direct study injury will be taken by medical personnel and the investigator. Patients will be followed by physicians, nurses, pharmacists and other staff during the duration of the patient's hospital stay. All usual care for a patient with severe sepsis will be provided by hospital staff. Emergency medical equipment, medications, and supplies will be at the physician's disposal should the patient have an acute untoward reaction. Patients will be medically evaluated by physicians, nurses, pharmacists and other staff during their clinic visits for potential adverse events.

Significant efforts will be made to maintain the confidentiality of study records. PHI will be stored in locked compartments and/or in secure REDCap databases. All data collected during the study will be associated with a randomly generated hashcode. Only personnel listed on this IRB protocol will be able to link this code to an individual patient.

As described above, the main risk associated with the study is likely to be associated with data collection and patient confidentiality.

Available Alternative Treatments

There are no alternative treatments available to patients who choose not to participate in this study. These patients will receive the same care as they would have if they had given research consent.

Research Materials, Records and Privacy

Cardiovascular specimens will be obtained and processed as described above. This information will be used for research purposes only. The collection of these samples will be documented in the patients' medical record. Patients will be assigned a unique patient number that can only by linked to PHI by study personnel. This master list will be stored in a locked compartment and/or a secure REDCap database.

Confidentiality

Investigational records from this study will be maintained in a confidential manner; subjects' names will not be associated with any published results. All samples will be used for the purpose of research. Samples will be kept for up to 1 year after the complete study ends. After the database is locked, all study records are kept on site for a minimum of 2 years, then possibly sent to Kentucky Underground Storage, Inc., 3830 High Bridge Rd., Wilmore, KY, 40390 until regulatory storage requirements are met.

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO PARTICIPANTS

Benefits vs. Risk

Patients are unlikely to derive a direct benefit from participating in this study. It is unlikely that any of the research results could have an immediate impact on a patient's short-term clinical care. Most of the research will be conducted in non-CLIA-approved laboratories which increases the ethical complexity. For these reasons, the investigators have chosen not to return individualized results to the patients.

Subjects may gain an indirect benefit from participating in the study. They will know that their involvement supports translational science that aims to improve therapies for patients with different forms of cardiovascular disease.

IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

The biospecimens and clinical data that are procured and collated under this protocol are used in research that seeks to improve understanding of cardiovascular disease. The goal of the research is to support the development of better therapies for patients. Although individual participants may not benefit directly from the study, they will be helping doctors and scientists to better understand and/or treat others.

DATA SAFETY AND MONITORING PLAN

Not applicable

OVERALL STRUCTURE OF STUDY TEAM

Not applicable