Smith Family Foundation: Odyssey Award
A program of the Richard and Susan Smith Family Foundation
2024 Award Recipients

- **Franziska Bleichert, Ph.D.**
  Assistant Professor of Molecular Biophysics and Biochemistry
  Yale University

  "In situ Structural Biology of Eukaryotic DNA Replication"

  Key Words: DNA replication, Replisome, Genome integrity, Cryogenic electron microscopy

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The replication of chromosomal DNA is essential for cell division and proliferation. It is accomplished by a complex and dynamic molecular machinery, called replisomes, that are assembled anew every cell cycle. Eukaryotic replisomes contain 40+ protein components and synthesize complementary DNA strands while moving away from replication origins bidirectionally, ensuring that all DNA is efficiently and accurately replicated. While structures of core replisome components or subcomplexes have been determined, the architecture of eukaryotic holo-replisomes remains unclear, preventing the formulation of precise structural models for the coordination of leading and lagging strand DNA synthesis and for replication-coupled events essential for preserving genome stability. Here, we aim to delineate the structures of DNA replication assemblies directly in cells at high resolution using an approach that integrates single-particle cryogenic electron microscopy with cryogenic focused ion beam milling of metazoan cells. Our work will define the 3D structural organization of eukaryotic replication forks, multi-megadalton replisomes, and other replication complexes in action in their native environment in human cells (Aim 1) and in fly embryos (Aim 2). In the long-term, these efforts will help explain disease mechanisms linked to DNA replication stress and genome instability, a first step towards devising novel strategies for treatment.

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- **Florian Douam, Ph.D.**
  Assistant Professor of Microbiology
  Boston University

  "Characterizing A Novel Humanized Mouse Platform To Model Arbovirus Transmission"

  Key Words: Arbovirus, Mouse models of viral infection, Humanized mouse models, Viral transmission,
  Arthropod-borne diseases, Arthropod-borne viruses, Human immune responses to viruses, Cutaneous responses to viruses

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Arthropod-borne viruses (ABV), such as Zika virus (ZIKV), cause significant public health concerns. ABV are transmitted to humans through the bites of infected arthropods (e.g., mosquitoes). Upon bite, cutaneous responses define the course and clinical outcome of an ABV infection. However, these processes remain poorly understood due to the lack of animal models able to recapitulate human cutaneous responses to ABV transmission, impeding the development of countermeasures against ABV. Mice engrafted with a human immune system (HIS) poorly recapitulate ABV transmission due to limited reconstitution in human myeloid lineages and structural differences between the mouse and human skin. Here, we will develop a mouse model (SKIN mice) co-engrafted with a HIS enriched in myeloid lineages and autogenic human skin organoids (HSO). We hypothesize that SKIN mice can recapitulate arthropod-to-human ABV transmission and that myeloid engraftment of HSO is critical in this process. We will test if SKIN mice mount physiologically relevant human cutaneous responses when HSO are reconstituted with myeloid lineages (Aim1). Then, we will determine if HSO myeloid engraftment is critical for effective mosquito-mediated ZIKV transmission (Aim2). Our project will showcase how SKIN mice can revolutionize our understanding of ABV transmission and aid in developing countermeasures against these viruses.

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- **Jessica Fetterman, Ph.D.**
  Assistant Professor, Medicine
  Boston University School of Medicine

  "Development Of Novel Approaches For Editing Mitochondrial Genetic Variants In Patient-Derived Stem Cells"

  Key Words: Mitochondrial Genetics, Mitochondria Disease, Mitochondria

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Mitochondrial diseases range from severe pediatric syndromes to aging-related diseases. No therapies are approved for the treatment or cure of mitochondrial diseases. Our understanding of the genetics and mechanisms of mitochondrial diseases is hindered by our limited ability to genetically manipulate the mitochondrial DNA. CRISPR-Cas9 has been successfully targeted to the mitochondrial matrix but a key barrier in the field is the inability to get CRISPR guide RNAs, required for CRISPR-Cas genome engineering, into the mitochondrial matrix. Mitochondria have no known import mechanism for nucleotide sequences. The goal of our proposed project is to develop, optimize, and validate two innovative approaches for transporting guide RNAs into the mitochondrial matrix by exploiting the mitochondrial protein import pathway. We will engineer viral RNA-binding proteins with a mitochondrial matrix targeting sequence. We will add modular RNA hairpin domains to guide RNAs recognized by the RNA-binding proteins with the guide RNA serving as a scaffold RNA. Additionally, we will covalently link the guide RNAs directly to the Cas9 deaminase with a mitochondrial matrix targeting sequence. The ability to use CRISPR-Cas gene editing tools in mitochondria will transform our understanding of mitochondrial genetics and biology in human health and disease.

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- **Esther Rheinbay, Ph.D.**
  Assistant Professor of Medicine
  Massachusetts General Hospital

  "Impact Of Somatic Y Chromosome Loss In Immune Cells On Tumor Response"

  Key Words: Sex bias in cancer, sex chromosomes, Y chromosome, Tumor microenvironment, Aging

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Mosaic loss of the male Y chromosome (mLOY) in hematopoietic cells (including lymphoid and myeloid immune cells) affects ~ 40% of men at age 701, and occurs in a few to 100% of cells. mLOY has been associated with increased mortality, including from solid and hematologic malignancy, Alzheimer's disease and cardio-vascular disease. The exact reasons are largely unknown and could directly involve several ubiquitously expressed genes as well as several immune genes encoded on the Y chromosome, suggesting that LOY might directly impact immune cell function. It is largely unknown whether and how LOY alters the properties of tumor-infiltrating immune cells and their response of immune cells to solid tumors, whose incidence also increases in the later decades of life. I plan to address this question through two specific aims: (1) computational analysis of the composition and regulatory pathways of tumor-associated LOY and wildtype Y immune cells obtained from single-cell transcriptomics of solid tumor microenvironments. And (2) testing the efficiency of LOY compared to wildtype Y immune cells to respond to a solid mouse tumor model, with and without immune checkpoint therapy. Contingent on the hypothesis being correct, future research directions also include evaluating hematopoietic LOY as a prognostic and/or predictive biomarker that is easily accessible from a blood draw; and identification of therapeutics that specifically target LOY cells.

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- **Shruti Sharma, Ph.D.**
  Assistant Professor
  Tufts University School of Medicine

  "Immune pathways safeguard endothelial integrity and mitigate atherosclerotic severity in aging: a novel target."

  Key Words: STING, cGAS, Innate Immunity, Cell-intrinsic, Endothelium, Endothelial Cells, Macrophages, Vascular Disease, Arterial Health, Arterial integrity, Atherosclerosis.

Vascular dysfunction is considered a key driver of age-associated decline. Despite its importance in aging, molecular components that regulate vascular function, including mechanisms that modulate a key aspect, endothelial integrity, remain unknown. These gaps in the field contribute to the paucity in therapies targeting this highly significant arm of aging.

In this novel proposal, we explore an unexpected role for a critical innate immune node, the cGAS/STING pathway, in promoting vascular health with aging. The proposed studies will confirm and extend our discovery that STING promotes endothelial integrity and healing. We will assess the explicit contribution of STING-signaling to molecular and cellular mechanisms in the endothelium to the initiation of atherosclerosis in pre-clinical mouse models and in vitro approaches of endothelial cell function (Specific Aim1). By leveraging a one-of-a-kind cohort of aging patients with peripheral artery disease, we will correlate STING loss-of-function polymorphisms with atherosclerosis severity in patients, uncovering new translational avenues (Specific Aim2).

The long-term objectives of this project are to define the specific molecular pathways downstream of STING that promote endothelial repair and contribute to the preservation of an aging vascular system. The studies in humans will be integrated into a translational platform to identify patients prone to vascular diseases and thus candidates for specific therapeutics.