Perelman SCHOOL OF MEDICINE
UNIVERSITY of PENNSYLVANIA

Spring 2025 PMI Symposium

From Molecules to Movement: Cytoskeletal Insights into Muscle Function and Neuronal Health

Thursday, April 17th

Gaulton Auditorium and Lobby
Biomedical Research Building (BRB II/III)
421 Curie Blvd, 1st Floor

Andrew Somlyo Honorary Lectures

Lukas Kapitein, PhD
Utrecht University
Navigating microtubule diversity in neurons

Charlotte Sumner, MD
Johns Hopkins University
The role of the mechanosensitive TRPV4 channel in motor neuron disease

Joseph and Jean Sanger Honorary Lecture in Muscle Biology

Rachelle Crosbie, PhD
University of California, Los Angeles
Targeting Cell-Matrix Interactions for the Muscular Dystrophies

New Perspectives Lecture

Ewa Bomba-Warczak, PhD
University of Pennsylvania
Long-lived mitochondrial cristae proteins in mammalian brains and hearts

Grad Student and Postdoc Lectures

Emily Scarborough, PhD (Prosser Lab)
Microtubule dynamics control directional growth in the heart

Carris Borland (Holzbaur Lab)
Investigating the novel role of KIF1A trafficking in autophagy

Libby Nunn (Titchenell/Baur Labs)
Skeletal muscle and adipose tissue-specific roles of *ActRIIA/B in regulating body composition and metabolic homeostasis during obesity*

Elana Baltrusaitis (Dominguez Lab)
Structural-functional characterization of the MIRO1-TRAK1 complex

Mengqi Xu, PhD (Ostap Lab)
Myosin-I Synergizes with Arp2/3 Complex to Enhance Pushing Forces of Branched Actin Networks

Ronan Lordan, PhD (Fitzgerald Lab)
Endurance exercise training partially rescues aged metabolic phenotypes and improves healthspan in mice lacking a functional circadian clock

Organized by Kaya Matson, Linda Pang & Erika Holzbaur

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Image Credit: Julia Riley

Pennsylvania Muscle Institute
Spring 2025 Symposium & Retreat

From Molecules to Movement:
Cytoskeletal Insights into Muscle Function and Neuronal Health

Thursday, April 17, 2025

Location: BRB Gaulton Auditorium & Lobby,
Biomedical Research Building (BRB II/III),
421 Curie Blvd., 1st Floor, Philadelphia, PA 19104

Sponsored by the Physiological Society of Philadelphia and the Pennsylvania Muscle Institute
Organized by Kaya Matson, Linda Pang, and Erika Holzbaur
www.med.upenn.edu/pmi

8:15 – 9:00am **Registration Check-in, Poster Setup, Breakfast, Coffee**
Location: BRB Gaulton Auditorium & Lobby*
**Table seating available in BRB 14th Floor Lounge*

9:00 – 9:15am ***Welcome***
Erika Holzbaur, PhD
Director, Pennsylvania Muscle Institute
University of Pennsylvania

E. Michael Ostap, PhD
Senior Vice Dean and Chief Scientific Officer, Perelman School of Medicine at the University of Pennsylvania

Session 1 - Tracks and Traffic: Microtubules in Polarity, Transport, and Cellular Function

9:15 – 9:20am ***Introduction: Andrew P. Somlyo Honorary Lecture***
Jayne Aiken, PhD

9:20 – 10:00am ***Andrew P. Somlyo Honorary Lecture***
Lukas Kapitein, PhD
Utrecht University
Navigating microtubule diversity in neurons

10:00 – 10:15am **Emily Scarborough, PhD (Prosser Lab)**
University of Pennsylvania
Microtubule dynamics control directional growth in the heart

Poster Session

10:15 – 11:00am **Odd Numbered Posters and Coffee Break**

Session 2 – Clinical Connections: Mechanisms of Disease

11:00 – 11:05am ***Introduction: Andrew P. Somlyo Honorary Lecture***
Nick Marotta

11:05 – 11:45am ***Andrew P. Somlyo Honorary Lecture***
Charlotte Sumner, MD
Johns Hopkins University
The role of the mechanosensitive TRPV4 channel in motor neuron disease

11:45am – 12:00pm **Carris Borland (Holzbaur Lab)**
University of Pennsylvania
Investigating the novel role of KIF1A trafficking in autophagy

12:00 – 12:15pm **Libby Nunn (Titchenell/Baur Labs)**
University of Pennsylvania
Skeletal muscle and adipose tissue-specific roles of ActRIIA/B in regulating body composition and metabolic homeostasis during obesity

Lunch

12:15 – 1:15pm **Lunch**
Location: Table seating in BRB 14th Floor Lounge and Room 1412
Boxed Lunches Available in BRB Lobby

1:15 – 1:30pm **Networking and Posters**
Location: BRB Lobby

Session 3 - New Perspectives

1:30 – 1:35am ***Introduction: New Perspectives Session***
Nick Palmer

1:35 – 2:00pm **Ewa Bomba-Warczak, PhD**
University of Pennsylvania
Long-lived mitochondrial cristae proteins in mammalian brains and hearts

2:00 – 2:15pm **Elana Baltrusaitis (Dominguez Lab)**
University of Pennsylvania
Structural-functional characterization of the MIRO1-TRAK1 complex

2:15 – 2:30pm **Mengqi Xu, PhD (Ostap Lab)**
University of Pennsylvania
Myosin-I Synergizes with Arp2/3 Complex to Enhance Pushing Forces of Branched Actin Networks

Poster Session

2:30 – 3:15pm **Even Numbered Posters and Coffee Break**

Session 4 – Muscle Physiology in Aging and Disease

3:15 – 3:30pm **Ronan Lordan, PhD (Fitzgerald Lab)**
University of Pennsylvania
Endurance exercise training partially rescues aged metabolic phenotypes and improves healthspan in mice lacking a functional circadian clock

3:30 – 3:35am ***Introduction: Jean and Joseph Sanger Honorary Lecture***
Kaya Matson, PhD

3:35 – 4:15pm ***Jean and Joseph Sanger Lecture in Muscle Biology***
Rachelle Crosbie, PhD
University of California, Los Angeles
Targeting Cell-Matrix Interactions for the Muscular Dystrophies

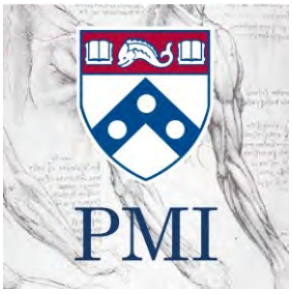
4:15 – 4:25pm **Concluding Remarks, Awards, and Future Plans**
Erika Holzbaur, PhD
Director, Pennsylvania Muscle Institute
University of Pennsylvania

Reception

4:30 – 5:45pm **Reception, Posters**
Location: BRB Lobby

5:45pm **Farewell, Posters take-down**
Location: BRB Lobby

Scan to vote for best trainee talks



Pennsylvania Muscle Institute: Cytoskeletal Research @Penn

Perelman School of Medicine
University of Pennsylvania

The Pennsylvania Muscle Institute (PMI) is an internationally renowned center for research on muscle, motility, and the cellular cytoskeleton. The PMI was founded to explore mechanisms of muscle function and muscle disease. Progress on these important research topics has led us to broaden our focus to include the exploration of related questions investigating cytoskeletal dynamics and the role of these dynamics in cellular functions including cell motility, intracellular transport, and organelle trafficking. Our institute is dedicated to innovative, collaborative, and cross-disciplinary research, and the application of our basic science discoveries to the treatment of human disease.

Our mission is to:

- Discover mechanisms driving muscle function and motile biological systems through innovative and cross-disciplinary research
- Develop state-of-the art technologies for the study of muscle and motile systems
- Apply our discoveries to the development of new therapies for disorders involving skeletal and cardiac muscle as well as neurodevelopmental and neurodegenerative diseases
- Provide education and training in muscle biology, motility, and cytoskeletal dynamics to scientists, physicians, and students at all stages of discovery
- Maintain an outstanding, interactive, and collegial environment that facilitates interactions that will lead to new discoveries and that supports the progress of all interested scientists

Research is conducted by more than 60 laboratories using biophysics, biochemistry, cell biology, genetics, physiology and ultrastructure to understand cell motility, cell migration, cytoskeletal dynamics, intracellular transport, molecular motors, cell division, muscle contraction, muscle development, muscle pathologies, neurodevelopmental and neurodegenerative diseases, and therapies targeted to treatment of these disorders. We are leaders in technological and methodological development, with advanced expertise in light microscopy, super-resolution microscopy, structural biology, nanotechnology, biochemical kinetics, live cell imaging, image processing, and viral gene targeting. Collaborative grants, seminars, symposia, and journal clubs are initiated and supported by the PMI. Additionally, the PMI sponsors vigorous graduate and post-doctoral training activities, including a NIAMS-supported training program in “Muscle Biology and Muscle Disease” and active journal clubs focused on muscle biology, motility and the cytoskeleton, and the cell biology of neuroscience.

For questions or inquiries about PMI membership, please contact:

Erika Holzbaur, Ph.D.
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Professor of Physiology
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Pennsylvania Muscle Institute (PMI)
Perelman School of Medicine at the University of Pennsylvania
700A Clinical Research Building
415 Curie Blvd. Philadelphia, PA 19104
www.med.upenn.edu/pmi

About: Faculty Speakers

Andrew P. Somlyo Honorary Lectures

Lukas C. Kapitein, PhD
Professor of Molecular and Cellular Biophysics,
Utrecht University
Navigating microtubule diversity in neurons

Dr. Lukas Kapitein is full professor of Molecular and Cellular Biophysics and director of the Biology Imaging Center at the Department of Biology at Utrecht University. His research focuses on the architecture and dynamics of cellular structures, particularly in neurons. His work addresses a fundamental question in neurobiology: how do neurons develop and sustain their complex morphology?

Lukas studied Experimental Physics at the VU University in Amsterdam, where he earned his Master's degree in 2002. He remained at VU to pursue his PhD in Biophysics, which he completed in 2007 with a thesis on the motility of mitotic kinesins. Bridging the gap between physics and biology, he then undertook postdoctoral research in Neurobiology at the Erasmus Medical Center, supported by a ALW-VENI grant from the Netherlands Organisation for Scientific Research (NWO) and an Erasmus MC Fellowship. There, he started to investigate cytoskeletal organization and polarized cargo transport in neurons.

In 2011, Kapitein joined Utrecht University as an assistant professor and became a full professor in 2018. His approach to cellular biology integrates principles of physics with cutting-edge technologies such as advanced light microscopy—including super-resolution techniques—cellular engineering, and optogenetics. His lab develops and applies active control strategies to study and manipulate intracellular transport, cellular architecture, and cell function in real-time. Over the years, his research has been recognized with several major grants and fellowships. These include VENI, VIDI and VICI grants from NWO, as well as a Starting Grant (2013) and a Consolidator Grant (2018) from the European Research Council (ERC). He also codirects the Gravitation project IMAGINE! (2022–2032), aimed at pioneering innovative microscopy and cellular guidance in native environments. With a career at the intersection of physics, biology, and advanced imaging, Kapitein continues to explore the fundamental principles of life at the cellular level, thereby contributing to our understanding of neuronal form and function.

Charlotte J. Sumner, MD
Professor of Neurology, Neuroscience, and Genetic Medicine,
Johns Hopkins University
The role of the mechanosensitive TRPV4 channel in motor neuron disease

Dr. Charlotte Sumner is a Professor of Neurology, Neuroscience and Genetic Medicine at Johns Hopkins University School of Medicine. She is a Daniel Nathans Scientific Innovator and the Vice Chair for Clinical Research in the Department of Neurology. She is also President of the Peripheral Nerve Society. She received her B.A. from Princeton University and M.D. from the University of Pennsylvania. She completed internal medicine internship and neurology residency at the University of California San Francisco and further clinical and scientific fellowship training at Johns Hopkins and the

National Institute of Neurological Disorders and Stroke. Dr. Sumner cares for patients with genetically-mediated neuromuscular diseases and co-directs the Johns Hopkins Muscular Dystrophy Association Care Center, the Spinal Muscular Atrophy (SMA), and the Charcot-Marie-Tooth (CMT) clinics, which deliver multidisciplinary clinical care, engage in international natural history studies, and provide cutting edge therapeutics. Dr. Sumner's laboratory research focuses on the genetic and cellular pathogenesis of motor neuron and peripheral nerve disorders with particular attention to identification of disease genes, characterization of molecular and cellular disease mechanisms, and preclinical development of therapeutics. Her work contributed to the scientific foundations leading to three new approved gene directed therapeutics in SMA. Her work has been recognized by receipt of a NINDS R35 Research Program Award and elected membership in the American Society of Clinical Investigators (ASCI), the Interurban Clinical Club, and the American Association of Physicians (AAP). She serves as an advisor to multiple SMA, CMT, and peripheral neuropathy nonprofit foundations, government, and private companies. Dr. Sumner's teaching and mentorship have been recognized with awards from medical students, residents, and research mentees including the NINDS Landis Award for Outstanding Mentorship.

Jean and Joseph Sanger Honorary Lecture in Muscle Biology

Rachelle H. Crosbie, PhD
Professor and Chair, Integrative Biology and Physiology,
University of California, Los Angeles
Targeting Cell-Matrix Interactions for the Muscular Dystrophies

Dr. Rachelle Crosbie is Professor and Chair of the Department of Integrative Biology and Physiology at UCLA. The Crosbie group focuses on investigating cell-matrix interaction in the context of muscular dystrophy and cardiomyopathy. The lab has developed methods for on-slide decellularization of biological tissues to generate acellular scaffolds that can be used for interrogating cell-matrix interactions using live cell imaging and correlating these findings to the mechanical and biochemical characteristics of the matrix. This platform enables observation and quantification of cell adhesion, proliferation, differentiation, gene expression, matrix remodeling, and motility on biologically relevant matrices. Using acellular scaffolds from dystrophin-deficient muscle as a model system with mechanically distinct regions of the matrix, the lab demonstrated that stiff fibrotic scars inhibit all cell function and are resistant to remodeling. The Crosbie team has focused on the function and therapeutic potential of sarcospan, a myoprotective gene that mediates cell-matrix interactions. Sarcospan is a transmembrane scaffolding protein that regulates cell surface expression of mechanosensors, including integrins. Loss of sarcospan diminishes expression of the laminin-binding complexes and renders skeletal and cardiac muscles susceptible to injury. Overexpression of sarcospan in dystrophin-deficient *mdx* mouse model of Duchenne muscular dystrophy and the gamma-sarcoglycan deficient limb-girdle muscular dystrophy (LGMDR5) murine model restores myofiber binding to the matrix, preventing muscle damage and ameliorating muscle degeneration and cardiomyopathy. Dr. Crosbie is Director of the NIH NIAMS T32 "Muscle Cell Biology, Pathogenesis, and Therapeutics" Training Program that supports pre- and postdoctoral fellows at UCLA. She is a recipient of the UCLA Chancellor's Distinguished Teaching Award for innovations in the classroom.

New Perspectives

Ewa Bomba-Warczak, PhD
Assistant Professor of Physiology,
University of Pennsylvania
Long-lived mitochondrial cristae proteins in mammalian brains and hearts

Dr. Ewa Bomba-Warczak received her PhD in Neuroscience from the University of Wisconsin – Madison in 2017 in the laboratory of Dr. Edwin Chapman, and HHMI Investigator, followed by postdoctoral studies under the mentorship of Dr. Jeffrey Savas at Northwestern University. She received her B.S. in Biological Sciences with honors from University of Illinois at Chicago. She is a recipient of a Jerzy Rose Award for most outstanding graduate thesis in Neuroscience at the University of Wisconsin-Madison, NIH F32 Fellowship and NIH MOSAIC K99 Pathway to Independence Fellowship to Promote Diversity. She has been named a Leading Edge Fellow (class of 2023) and recently a Keystone Fellow (class of 2025). She is also a co-founder of MITOchats, an online seminar series featuring graduate students and postdoctoral fellows working in the field of mitochondria.

Dr. Bomba-Warczak's research investigates how cells with exceptionally long lifespans – neurons and oocytes - establish and maintain their mitochondrial networks throughout their life, and how the stability of mitochondrial components contributes to cell's health and age-dependent degeneration. In her laboratory she combines in vitro cell and in vivo mouse models with mass spectrometry-based proteomics, biochemistry, genetics, and fluorescent imaging to define the mechanisms governing the lifelong mitochondrial homeostasis in health and disease.

About: Honorary Lectures

Andrew P. Somlyo Honorary Lectures

Andrew P. Somlyo, MD (1930 – 2004)

Professor of Physiology and Pathology and founding Director of the Pennsylvania Muscle Institute, Dr. Somlyo was a luminary in the field of smooth muscle physiology. His research (in collaboration with Dr. Avril Somlyo) played a key role in showing that actin-myosin interactions are responsible for force generation in smooth muscle. With colleagues at the University of Pennsylvania, Dr. Somlyo developed electron probe microanalysis to determine local ion concentrations in tissues at nanometer resolution. Additionally, his pioneering work in signaling revealed the mechanisms that regulate contraction of smooth muscle independently of the membrane potential – a process he termed pharmacomechanical coupling. Dr. Somlyo had a passion for science that is evident in the remarkable imprint that he left on the field of muscle physiology and on his students and colleagues. He was also a noted collector of Asian art. Dr. Somlyo left Penn Medicine in 1988 to chair the Department of Molecular Physiology and Biological Physics at the University of Virginia School of Medicine.

Jean and Joseph Sanger Lecture in Muscle Biology

Jean M. Sanger, PhD

Professor, Department of Cell and Developmental Biology
SUNY Upstate Medical University

Joseph W. Sanger, PhD

Professor, Department of Cell and Developmental Biology
SUNY Upstate Medical University

Drs. Jean and Joseph Sanger are pioneers in the development and use of fluorescently labeled proteins to examine the architecture and dynamics of a range of biological processes in developing and mature cells. As former members of the Department of Cell and Developmental Biology, they were founding members of the Pennsylvania Muscle Institute. The Sangers were among the first cell biologists to take advantage of probes to follow the assembly and changing localizations of cytoskeletal components in living cells. Their research led to impactful new discoveries about cell division, actin based bacterial infections, and assembly and maintenance of myofibrils in muscle cells. Importantly, the Sangers were the first scientists to visualize and quantify the kinetics of sarcomeric proteins entering newly developing and mature myofibrils. In real time, they followed key components of the contractile machinery during myofibrillogenesis. The revolutionary models they formulated for how Z-bands, thick and thin filaments, and other sarcomeric components are templated during development are still the standards in the field. More recently, the Sangers were the first to determine the role of the ubiquitin–proteasome system in the progression of nascent myofibrils to maturity, and possible mechanisms for the off-target effects on hearts by chemotherapeutics. In addition to their scientific achievements, the Sangers have been leaders and role models in the Cell Biology community as educators, mentors to trainees and faculty, editors, reviewers, conference organizers, and administrators. Dr. Joseph Sanger served as interim chair of PSOM's Department of Cell and Developmental Biology. The Sangers left Penn in 2006 for SUNY Upstate Medical University, where Dr. Jean Sanger became Professor and Dr. Joseph Sanger became Professor and Chair of Cell and Developmental Biology. They were and are parts of the soul of the Pennsylvania Muscle Institute.

ABSTRACTS

*[Poster presenter(s) in **bold**]*

1.

Unconventional Gliding of Kinetochore-Associated Ndc80 Protein Along Microtubules Under Dragging Force

Vladimir Demidov, **Fedor Balabin**, Ivan Gonchar, Fazly Ataullakhanov, and Ekaterina Grishchuk

During cell division, kinetochore-localized microtubule-associated proteins maintain mobile bonds with the wall of spindle microtubules under forces that vary in both magnitude and direction. A key component of this "mobile glue" is the Ndc80 protein complex, which undergoes Brownian diffusion along microtubules in vitro. However, the mechanism by which Ndc80 glides along the microtubule under a dragging force remains unclear. We utilized a laser trapping system to oscillate a microtubule "dumbbell" near a "pedestal" bead coated with human Ndc80 protein. With the application of an ultrafast force-clamp regime, we detected single-molecule gliding events under constant forces ranging from 2 to 15 pN. Our results reveal that Ndc80 translocates at different velocities depending on the direction of movement. Specifically, when Ndc80 is pulled toward the microtubule plus-end, its velocity is lower than predicted by its force-free diffusion coefficient, a phenomenon we refer to as the mobile catch-bond translocation mechanism. When multiple Ndc80 molecules glide together in the constant velocity regime, the translocation pattern consists of periods of near-constant velocity, interrupted by brief "slipping" events. This motility pattern deviates from simple diffusion-based predictions and appears to emerge from the mobile catch-bond translocation mechanism. Our preliminary data suggest that when multiple Ndc80 molecules glide together, they can maintain a stable velocity despite increasing force by dynamically adjusting their friction with the microtubule.

2.

Structural-functional characterization of the MIRO1-TRAK1 complex

Elana Baltrusaitis, Erika Ravitch

Mitochondrial Rho GTPase (MIRO) features N- and C-terminal GTPase domains (nGTPase and cGTPase) flanking two pairs of EF-hands, and functions as a master scaffold on the outer mitochondrial membrane. It regulates mitochondrial motility by recruiting trafficking kinesin-binding protein (TRAK), which in turn recruits kinesin-1 and dynein-dynactin. The MIRO-TRAK interaction remains incompletely understood. Here, we describe the cryo-electron microscopy structure of $TRAK1_{569-623}$ bound to MIRO1. TRAK1 binds in a cleft between the nGTPase and first EF-hand pair, inserting side chains into hydrophobic pockets of both domains. MIRO1-TRAK1 forms a dimer, mediated by interactions through the second EF-hand pair, cGTPase, and TRAK1. Another MIRO1-binding site, mapped to $TRAK1_{425-428}$, was found to target a pocket between the last EF-hand pair and cGTPase. Both binding sites were validated by mutagenesis and binding assays, showing no clear dependence on cofactor conditions (Ca^{2+}, EGTA, GDP, GTP). In cells, both sites contribute to TRAK1's mitochondrial localization.

3.

Decoding the Mitophagic Stress Response: a stress-dependent pathway modulating mitochondrial quality control in neurons

Bishal Basak[1,2], Erika Holzbaur[1,2]

[1] *Department of Physiology, Perelman School of Medicine, University of Pennsylvania, PA 19104, USA*
[2] *Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA*

	Pink1/Parkin-mediated mitophagy is critical for maintaining mitochondrial homeostasis and preserving neuronal health, with dysregulation of this process linked to Parkinson's disease (PD). Here, we describe a neuron-specific stress response pathway termed the Mitophagic Stress Response (MitoSR), that complements Pink1/Parkin to enhance mitochondrial clearance during oxidative damage. MitoSR facilitates mitochondrial turn-over by selectively degrading the negative regulators of autophagy, which are Myotubularin-related phosphatase 5 (MTMR5), MTMR2, and Rubicon. These proteins are ubiquitinated and targeted to the proteasome, alleviating their inhibitory actions on autophagic machinery. Degradation of the MTMR5-MTMR2 complex results in increase in autophagosome numbers. Critically, our study uncovers the unexplored role of Rubicon in neurons. We show that Rubicon represses lysosomal acidification and function, impairing autophagosome-lysosome fusion in the soma. Rubicon localizes to lysosomes in a Rab7-dependent manner, and its degradation upon MitoSR activation restores lysosomal maturation, thus promoting efficient removal of damaged mitochondria. By integrating enhanced autophagosome biogenesis with improved lysosomal function, activation of MitoSR thus ensures efficient mitochondrial quality control in neurons. These findings place Rubicon as a central regulator in neuronal mitophagy, and point to MitoSR as a potential therapeutic way to restore mitochondrial health in PD.
4.	**Cell contractility effects on fibrillar collagen in developing and mature hearts** **Susanna Belt**, Karanvir Saini, Dennis Discher *Introduction:* Tissue fibrosis causes almost half the deaths in the developed world, and cardiac fibrosis alone leads to heart failure in >6-million U.S. adults. Collagen-rich extracellular matrix (ECM) accumulates in fibrosis and both stiffens hearts and compromises beating. Current treatment efforts for progressive cardiac fibrosis primarily focus on ECM rather than the cell forces that conceivably regulate ECM levels. Cardiac myosin-II is one possible target based on recent studies, but the Rho kinases (ROCK) expressed in varying amounts by many cell-types including fibroblasts seem to also promote expression of ECM genes possibly via acto-myosin contractility. A new ROCK inhibitor (ROCKi) was FDA-approved recently for chronic Graft-versus-host disease (cGVHD) that affects many organs in recipients of stem-cell graft transplants. To visualize any effects of such contractility inhibitors on heart function and ECM collagen levels, we studied beating hearts from chick embryos. To track real-time kinetics of fibrillar collagen during perturbations with various myosin-II inhibitors, we used second harmonic imaging (SHG). *Materials and Methods:* For studying cellular contractility-based ECM effects and heart functions, we selected isolated embryonic chick hearts that beat autonomously (without any electrical stimulation) in contrast to mature hearts and comprises of different cell-types namely cardiomyocytes, fibroblasts as revealed by single-cell RNA sequencing. Acto-myosin based cellular contractility was perturbed by several small-molecule inhibitors namely ROCK-2 (Belumosudil) and Blebbistatin (a pan myosin-II inhibitor) whereas a pan matrix metalloproteinase inhibitor (MMPi) and collagenases were used to perturb ECM protein levels. The heart function, defined by beating strain magnitude, was accessed by means of wide-field time-lapse imaging during above mentioned perturbations in cellular-contractility and ECM protein levels (Fig.1) whereas second harmonic imaging (SHG) was used to assess the real-time kinetics of fibrillar collagen levels in the tissues. *Results and Discussion:* Exposure of the heart tissues to ROCKi resulted in heart beating strain suppression similar to that of Blebbistatin treated hearts (Fig.2). Given that ROCK-2 is primarily expressed by fibroblastic cells in the heart and cardiomyocytes express extremely low levels, the slower rate of beating strain inhibition during ROCKi treatments (~30 mins) compared to blebbistatin treated hearts (<15 mins) likely results from cell-type specific distribution of ROCK-2 within the heart tissue while blebbistatin targets several myosin types abundant in most cell-types present in the heart. Considering the propagation of contractile wave among sparse immature cardiomyocytes within the heart relies on robust cell-ECM interactions, ROCK-2 inhibition likely promotes ECM catabolism and

	thus, disables contractile wave propagation in the heart. Such effects of altered ECM turnover on beating strain are evident during the presence of pan-MMPi when tissue function recovery is observed following drug-washouts (Fig.2). For various mature mice tissues and beating embryonic chick hearts, we find collagen-sensitive second harmonic generation (SHG) image intensity scales non-linearly versus tissue stiffness, aligning well with the results from cellularized gels of collagen. Chick hearts beating at ~5% strain maintain collagen levels until their contractile strain is suppressed by myosin-II inhibition and endogenous matrix metalloproteinases then degrade collagens within ~30-60 minutes based on SHG (Fig.3). *Conclusions:* Our results have potential to deepen the understanding of cell-based contractile forces on ECM in development, adaptation, and disease. The modulation of cellular forces during pathologies via targeting ROCK-pathway or other means might help target fibroblastic cells over other cell-types. Given that fibroblasts are primarily responsible for synthesis and degradation of a tissue ECM, such targeting of fibroblasts might help restore "normal" ECM and function of fibrotic hearts and other tissues.
5.	**Investigating the role of KIF1A in neuronal autophagy** **Carris Borland**, Jayne Aiken, Jacob Popolow, Erika Holzbaur KIF1A Associated Neurological Disorder (KAND) is a group of neurodevelopmental and neurodegenerative diseases caused by mutations in the *KIF1A* gene. KIF1A is a neuron-specific molecular motor that anterogradely transports cargo along the axon and is essential for cell viability, synaptic health, and development. Because intracellular trafficking is vital and is intricately connected to many neuronal pathways, there is a need to further understand the mechanisms by which KIF1A trafficking affects different pathways. Furthermore, to create treatments for KAND patients, we need to understand the molecular basis for the disease and how aberrant trafficking by KIF1A leads to disease manifestation. Autophagy, a well-studied, well- conserved and essential pathway for neuronal homeostasis is frequently disrupted in neurodegenerative diseases and is a promising target for therapeutic approaches to various diseases. There is evidence that KIF1A-mediated trafficking regulates autophagy. First, a study in *Caenorhabditis elegans (C. elegans)* showed that ATG9 vesicle transport is dependent on KIF1A. ATG9 is a transmembrane scramblase necessary for autophagosome biogenesis. In addition, KIF1A has been shown to traffic lysosomes, which are degradative organelles needed for autophagosome maturation. These previous results suggest that KIF1A trafficking may affect autophagosome biogenesis and/or maturation. However, the mechanism by which KIF1A trafficking affects autophagy in human neurons is unknown. We investigated the role of KIF1A in autophagy biogenesis and maturation using human IPSC-derived neurons using biochemical and imaging approaches, comparing wild-type (WT) neurons to C92* homozygous mutant neurons, which lack KIF1A expression. We found that ATG9 localization is dependent on KIF1A, as ATG9 vesicles accumulated at the TGN and were depleted from axon in C92* neurons. We also observed significantly less LC3-labeled autophagosomes distributed along C92* axons compared to WT, suggesting that autophagy biogenesis is impaired. We also found that KIF1A co-transports with LAMP-1 labeled lysosomes in human neurons, and that loss of KIF1A in the C92* mutant drastically reduces lysosomal density along the axon. Furthermore, we saw less acidified autophagosomes in C92* axons, suggesting that autophagy maturation is impaired. Together, these results suggest that KIF1A-mediated transport is critical to maintain neuronal autophagy and that targeting autophagy defects could be a promising therapeutic strategy for alleviating symptoms of KAND.
6.	**Investigating the molecular mechanisms of thin-filament length regulation at the pointed end** **Shayna Brotzman**, Malgorzata Boczkowska, and Roberto Dominguez

	The length of thin filaments, primarily composed of filamentous actin (F-actin), in striated muscle is tightly regulated to ensure optimal overlap with thick filaments, a key determinant of contraction strength and efficiency. Dysregulation of TFL is a major cause of cardiac and skeletal muscle diseases, most notably dilated cardiomyopathy (DCM) and nemaline myopathy (NM). In the sarcomere, the basic contractile unit of striated muscle, the pointed end of thin filaments serves as the primary site for actin subunit exchange. Despite this dynamic exchange, thin filaments display a uniform length across the sarcomere, suggesting that regulatory mechanisms mediated by actin-binding proteins (ABPs) maintain this uniformity. However, these mechanisms remain poorly understood. Two ABPs, tropomodulin (Tmod) and tropomyosin (Tpm), play critical roles in regulating thin filament length. Recent studies in cell and animal models suggest that a Tmod homolog, leiomodin (Lmod), plays a role in thin filament pointed end elongation. However, Lmod-mediated elongation has not been demonstrated in vitro, and the underlying molecular mechanism remains unknown. Additionally, it is unclear how Tmod and Lmod, despite sharing similar domain architectures, perform distinct functions. To address these gaps, I have solved preliminary cryo-electron microscopy (cryo-EM) structures of Lmod-Tpm and Tmod-Tpm complexes bound to the pointed end of F-actin. These structures support a preliminary mechanism for Lmod-mediated elongation and reveal structural differences that likely underlie the functional divergence between Lmod and Tmod. These findings will provide novel insights into thin filament length regulation and thin filament pointed end structure.
7.	**Microtubule depolymerization at kinetochores restricts anaphase spindle elongation** **Geng-Yuan Chen**[1], Changfeng Deng[2], David M. Chenoweth[2], Michael A. Lampson[1] *[1]Department of Biology, University of Pennsylvania, Philadelphia, PA, 19104* *[2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, 19104* Anaphase chromosome segregation depends on forces exerted by spindle microtubules. Current models propose two mechanisms of force generation: kinetochore microtubules (kMTs) depolymerize to pull chromosomes toward the spindle poles (anaphase A), while antiparallel microtubule sliding in the central spindle further separates the chromosomes by elongating the spindle (anaphase B). Experimental evidence in cells supports the sliding mechanism, but contributions of the depolymerization mechanism remain unclear. Here we show that kMT depolymerization limits spindle elongation rather than moving chromosomes apart. We developed a chemical optogenetic approach to recruit a microtubule depolymerase to kinetochores at anaphase onset, thereby increasing the rate of kMT depolymerization without perturbing earlier stages of mitosis. We find that increased depolymerization slows the velocity at which spindle poles move apart without changing kinetochore separation velocities. Our findings support a model in which kinetochores selectively couple to central spindle microtubules parallel to their kMTs, so that antiparallel sliding drives chromosome segregation while kMT depolymerization pulls poles inward.
8.	**Novel gene therapy designs for Duchenne Muscular Dystrophy ameliorate disease progression in mouse models** **Dongwook C. Choe**, Coral Kasden, Gargi Ghosh, Tanvi Singh, and Hansell Stedman *Introduction*: Duchenne Muscular Dystrophy (DMD) is a genetic muscle wasting disease caused by deletions in the dystrophin gene. DMD is a target for gene therapy; however, full-length dystrophin cDNA greatly exceeds the packaging size of standard gene therapy delivery platforms such as adeno-associated virus. Therefore, miniaturized versions of dystrophin and its evolutionary cousin utrophin have been closely studied for potential therapeutic effect. Here, we describe a series of experiments used to discriminate between multiple DMD gene therapy candidates that we generated using novel design approaches rooted in evolutionary and structural biology.

	Methods: We injected a DMD mouse model (mdx) with nine different DMD gene therapy candidates at a high and/or low dose seven days after birth. Low dose mice were sacrificed at 4 weeks for histology. High dose mice were subjected to multiple assays over the course of nine weeks including treadmill running, force grip assessment, and AngII/PE challenge. *Results*: Some gene therapy treatment groups demonstrated, via immunofluorescence, a lower proportion of centrally-nucleated myofibers relative to the untreated DMD control group. While mdx mice showed a statistically-significant decline in grip force over the course of the force grip assay, two treatment groups did not. Similar trends towards the wild-type baseline may be present for treatment groups undergoing AngII/PE injection and CK/NTFT measurement after challenge via treadmill. *Conclusions*: Our initial results indicate that some of our gene therapy candidates may protect against genetic DMD. Additional experiments are necessary to provide further information and improve statistical power.
9.	**TRPML1 Positions Lysosomes to Regulate the Plasticity of Peripheral Astrocytic Processes** **Maeve Coughlan**[1], Madison Fuller[1], Serena Chen[1], & Sandra Maday[1] *[1]Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania* Late endosomes/lysosomes play crucial roles in the maintenance of neuronal synapses. Astrocytes, like neurons, have complex morphologies and form thousands of synaptic contacts; suggesting lysosome traBicking must be highly coordinated to serve local functions. Yet, little has been described regarding the traBicking or functions of lysosomes within astrocytic branches. Recent literature implicates the lysosomal cation channel TRPML1 in regulating lysosome motility in neurons and other cell types. Loss of function mutations in TRPML1 manifest as the lysosomal storage disorder mucolipidosis type IV, which has prominent neuronal and glial pathology. How TRPML1 regulates lysosome traBicking in astrocytes is unknown. To address this question, we co-cultured cortical astrocytes and neurons, allowing astrocytes to form stellated, branched morphologies. By live-cell imaging, we find that TRPML1 activation arrests lysosome motility within astrocytic branches. Conversely, inhibition or loss of TRPML1 increased astrocytic lysosome motility. Combined pharmacological and genetic tools revealed this arrest is mediated by tethering onto the actin cytoskeleton via myosin-Va. Lysosome tethering occurs rapidly after TRPML1 activation and a subset of arrested lysosomes fuse with the plasma membrane, preceding the extension of peripheral astrocytic processes (PAPs). PAPs are dynamic, actin-enriched protrusions that can envelope synapses. PAPs are enriched for the actin-membrane linkers ezrin-radixin-moesin (ERM). Changes in ERM phosphorylation status are thought to contribute to the structural plasticity of PAPs. However, mechanisms underlying PAP plasticity are still largely unknown. We find that TRPML1 activation promotes a rapid dephosphorylation of ERM proteins. Conversely, phosphorylated-ERM accumulates in TRPML1-KD astrocytes. Thus, we propose TRPML1 positions lysosomes to regulate the structural plasticity of PAPs through both actin and membrane remodeling.
10.	**Comparative Analysis of iPSC-Derived and Primary Skeletal Myocytes in a Neurovascular Triculture Model** **Jay Dave**[1,2], Wenli Yang[4,5], D. Kacy Cullen[1,2,3], Suradip Das[1,2] *[1]Department of Neurosurgery, Center for Brain Injury & Repair, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.* *[2]Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Afairs Medical Center, Philadelphia, PA 19104, USA.*

[3]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA.
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Skeletal myocytes derived from induced pluripotent stem cells (iMyo) or isolated from a muscle tissue sample (Primary skeletal myocytes, pMyo) are promising sources for skeletal muscle regeneration and disease modelling. However, the extent of their similarities and diferences remains unclear. In this study, we established a triculture system by co-culturing iMyo and pMyo with induced pluripotent stem cell (iPSC)-derived motor neurons and primary human umbilical vein endothelial cells (HUVECs) to better replicate the native muscle microenvironment. To investigate the impact of neurovascular signalling on myocyte maturation, we performed gene expression analyses. While both iMyo and pMyo cultures exhibited comparable myofiber morphology, they demonstrated distinct expression patterns of key structural and neuromuscular genes when co-cultured with motor neurons and endothelial cells. MYH3 was expressed in iMyo whereas absent in the pMyo cultures. We also observed that endothelial cells enhanced expression of MYH4 in iMyo whereas motor neurons promoted this expression in pMyo. The observed diferences in MYH3 and MYH4 expression provide insights into myocyte maturation, as MYH3 is exclusively expressed during embryonic development, whereas MYH4 is associated with later stages of myogenesis and a more mature muscle phenotype. Diferential gene expression analysis indicated that iMyo retains a relatively immature phenotype, whereas pMyo exhibits a gene expression profile more characteristic of mature adult myocytes. Understanding these diferences can provide insights into stage and eficiency of myocyte development and regeneration under various conditions, including aging and disease, and encourage further investigation into the impact of myocyte origin in tissue engineering.

11.

Impaired Myofibril Relaxation Across Human HFpEF Subphenotypes

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Heart failure with preserved ejection fraction (HFpEF) is commonly accompanied by significant diastolic dysfunction, yet its subcellular origins remain incompletely understood. The syndrome is further complicated by the impact of obesity and other comorbidities, which has resulted in the classification of different subphenotypes categorized by patients with comparably lower obesity and the highest levels of hypertension and hypertrophy (H/H), and those with comparably lower hypertension and hypertrophy, but with more severe obesity and likelihood of diabetes (O/D). Individuals with high levels of both features comprise a third subphenotype (Mixed). We previously reported that isolated cardiomyocytes (CMs) of H/H patients had elevated tension at lower calcium concentrations (diastolic) but preserved tension at higher concentrations (systolic), while CMs from O/D patients had preserved diastolic tension but significantly depressed systolic tension. CMs from Mixed patients exhibited both phenotypes, with elevated diastolic tension and depressed systolic tension. Our identification of higher tension at diastolic calcium suggests the presence of mechanisms which may impair relaxation at the myofilament level. To assess this directly, force production and relaxation kinetics were measured in myofibrils isolated from human right ventricular biopsies using a custom-built apparatus. Maximal calcium-activated tension was significantly reduced in O/D and Mixed myofibrils compared to non-failing (NF) controls but was unchanged in H/H, complementing our findings from CMs. Upon the rapid removal of calcium, the initial, linear phase of myofibrillar relaxation was prolonged in all HFpEF groups, indicating sustained actomyosin interactions with delayed inactivation. Thus, myofibrillar dysfunction is likely a common contributor to diastolic abnormalities within HFpEF, while systolic

dysfunction may be uniquely linked to obesity. We also performed X-ray diffraction experiments on the same biopsies to determine whether anomalous structural relationships between myofilament proteins could contribute to the myofibrillar functional differences. We found that myofilament lattice spacing ($d_{1,0}$) was increased and the intensity ratio of the equatorial reflections ($I_{1,1}/I_{1,0}$) was decreased in O/D, but not H/H or Mixed, myocardium compared to NF, indicating increased lattice spacing and decreased actomyosin proximity, respectively, which together could contribute to the reduced maximal force in O/D CMs and myofibrils. These data provide further justification for the therapeutic targeting of myofilament-based mechanisms, particularly those regulating relaxation, in the treatment of HFpEF, but also suggest that the severity of obesity should be considered due to its potential influence on myofilament activation.

12.

Characterization of stiffness-dependent phosphorylation events downstream of TCR engagement

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One of the key characteristics of cells is their ability to respond to external stimuli. This includes chemical stimuli and physical cues such as substrate stiffness, pressure, and shear force. The response to physical cues is termed mechanosensing. In the immune system, T lymphocytes use mechanosensing to move through body tissues and identify tumor cells and virally infected cells as targets for destruction. Our lab showed previously that substrate stiffness modulates T cell activation, as measured by upregulation of cell surface markers, enhanced proliferation, and production of the growth factor IL-2. However, the relevant molecular pathways are unknown. To ask which steps of the TCR signaling cascade respond to mechanical cues, we prepared acrylamide hydrogels ranging in stiffness from 4-50kPa, coated with antibodies to the T cell receptor component CD3 and the costimulatory ligand CD28. CD4+ T cells were then stimulated on these surfaces, and protein phosphorylation was measured by Western blotting and immunofluorescence microscopy. We find that the overall level of tyrosine phosphorylation increases with substrate stiffness, as does phosphorylation of ERK, a known regulator of IL-2 production. Current studies are aimed at assessing stiffness-dependent phosphorylation of specific molecules in the TCR signaling cascade, and asking how this response relates to T cell spreading and morphological features of the immunological synapse. Our long-term goal is to identify molecules that are required for stiffness-dependent increases in T cell proliferation and cytokine production and define the role of cytoskeletal forces in this response.

13.

Structural Characterization of TRPML1 Modulation and Lysosomal Tubulation

Aria Garrett, Elaine Mihelc, Ruth Pumroy, José Jesús-Peréz, Bridget McVeigh, Vera Moiseenkova-Bell

Lysosomes are essential cellular organelles that orchestrate metabolic regulation and degradative processes. Lysosomal storage diseases and neurological disorders with altered endo-lysosomal flux often feature aberrant activity of the transient receptor potential mucolipin 1 (TRPML1) ion channel. TRPML1 plays a critical role in lysosomal signaling, impacting cellular processes such as lysosomal morphology, degradation, movement, turnover, and cellular autophagy. TRPML1 is a target for therapeutic development, yet there remain structurally uncharacterized modulators of the channel. TRPML1 activity is also linked to the poorly understood phenomenon of lysosomal tubulation. To address this, I aim to solve the single particle cryo-electron microscopy structures of TRPML1 in complex with structurally undescribed modulators. Additionally, I plan to utilize cryo-focused ion beam scanning electron

	microscopy and tomography to investigate *in situ* lysosomal tubulation. Advancing our understanding of TRPML1 activity and its role in lysosomal morphology will not only enhance our knowledge of basic lysosomal biology but also offer potential avenues for therapeutic development. Targeting TRPML1 activity is currently being explored as a strategy for treating various diseases, including Parkinson's and Alzheimer's disease.
14.	**Differential Modulation of β-Cardiac Myosin Conformations by Cardiomyopathy-Associated Mutations in the Converter Domain** **Jinghua Ge**[1], Sebastian Duno-Miranda[2], Arun Kumar Somavarapu[3], Skylar M.L. Bodt[1], Ruchi Gautam Sharma[3], Samantha E. Previs[2], Angela Ploysangngam[2], Roger Craig[3], Raul Padron[3], David M. Warshaw[2] and Christopher M. Yengo[1] *[1]Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA, USA* *[2]Department of Molecular Physiology and Biophysics, Cardiovascular Research Institute, University of Vermont, Burlington, VT, USA* *[3]Department of Radiology, University of Massachusetts Chan Medical School, Worcester, MA, USA* Cardiomyopathy-associated mutations in the converter domain of β-cardiac myosin (M2β) can significantly alter myosin function, impacting heart muscle contraction and leading to heart failure. We demonstrated using the M2β 15 heptad (15HP) long tailed construct that, compared to wild-type 15HP, the hypertrophic cardiomyopathy (HCM) mutation R723G 15HP enhances actin-activated ATPase activity and in vitro motility while the dilated cardiomyopathy (DCM) mutation F764L 15HP depresses both actin-activated ATPase activity and in vitro motility. The HCM R723G 15HP destabilizes both the super-relaxed state (SRX) and interacting heads motif (IHM), while the DCM F764L 15HP stabilizes the SRX state without affecting IHM formation. To expand on these findings, we utilized the M2β 2 heptad (2HP) construct, which is double-headed and short-tailed that cannot form the full IHM. The HCM R723G 2HP mutant demonstrated no significant difference from wild type in terms of SRX, ATPase activity and motility. In contrast, the DCM F764L 2HP mutation exhibited an increase in the SRX state (salt insensitive) with only minor changes in actin-activated ATPase activity. F764L 2HP also demonstrated slower in vitro sliding velocity compared to both wild-type and R723G 2HP. Our previously reported results with R723G and F764L M2β subfragment 1 (S1) demonstrated no significant changes in actin-activated ATPase activity compared to WT S1. These results suggest that F764L may stabilize an alternative auto-inhibited conformation that does not fully form the IHM. Experiments are in progress using negative stain electron microscopy, which may provide mechanistic insights into novel autoinhibited structural states. Overall, these findings support the hypothesis that cardiomyopathy mutations differentially modulate SRX and IHM states, potentially through distinct auto-inhibitory mechanisms.
15.	**Model of membrane delivery and flow during cytokinesis** **Shuhan Geng**, Dimitrios Vavylonis During cytokinesis the cell surface area increases by up to 20%, requiring plasma membrane delivery to the division plane to prevent rupture through global stretching. As tension gradients develop, the plasma membrane must also undergo two-dimensional fluid-like deformations while preserving mechanical integrity. To understand membrane mechanics and hydrodynamics during cytokinesis, we focused on fission yeast where the rate of contractile ring constriction and spatial distribution and rates of exocytosis and endocytosis have been experimentally measured. We model the cell membrane as a 2D fluid with forces by cell wall and osmotic pressure pinning its shape along a curved shape. The contractile ring/septum growth provides a moving boundary condition of speed varying with time as measured in prior experiments. Conservation of mass and balance of forces determine time evolution local membrane

	density and flow. We find that membrane strain is higher at early stages of cytokinesis, which may relate to recent observations of Ca spikes primarily at these stages of division. Delivery of vesicles, especially the "rim" of the division plane through the TRAPP-II and exocyst complex pathways, leads to divergent flows with an outward flow pattern away from the division plane, as was recently observed with low mobility peripheral membrane proteins, and inward flows closer to the contractile ring. We discuss the importance of the spatiotemporal pattern of lipid trafficking in relationship to the unknown drag forces exerted by transmembrane proteins anchored to the cell wall, which determine the rate of membrane tension propagation and maximum strain. Overall, these theoretical results provide insights into how cells maintain membrane integrity for successful division
16.	**Peri-mitochondrial actin filaments inhibit Parkin assembly via disruption of ER- mitochondrial contact** **Amrapali Ghosh**[1*], Tak Shun Fung[2*], Maite R Zavala[1], Zuzana Nichtova[1], Dhaval Kumar Shukal[1], Marco Tigano[1], Gyorgy Csordas[1], Henry N Higgs[3] and Rajarshi Chakrabarti[1#] *[1]Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia PA, USA;* *[2]Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA* *[3]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth College, Hanover NH, USA* Mitochondrial damage represents a dramatic change in cellular homeostasis, necessitating metabolic adaptation as well as clearance of the damaged organelle (i.e. mitophagy) to sustain cell survival and health. One rapid response to mitochondrial damage is peri-mitochondrial actin polymerization within 2 mins, which we term ADA (acute damaged-induced actin) (PMID: 31413070). This rapid response is temporally distinct from a second round of actin assembly that occurs >1 hour after mitochondrial damage (PMID: 29398621). ADA requires two parallel signaling pathways initiated by increased cytoplasmic calcium and AMPK activation, and culminating in activation of two actin assembly factors, the Arp complex and FMNL formins, respectively (PMID: 35290799). Recently we have shown that ADA is vital for a metabolic shift from oxidative phosphorylation to glycolysis upon mitochondrial dysfunction (PMID: 36102863). In the current study we investigated the effect of ADA on Pink1/Parkin mediated mitochondrial quality control. We show that inhibition of proteins involved in the ADA pathway, including Arp2/3 complex, FMNL formins, WAVE complex significantly accelerates Parkin recruitment onto depolarized mitochondria in HeLa and U2OS cells. Conversely, treatments that prolong ADA significantly delay Parkin accumulation onto depolarized mitochondria in wild-type cells but not in Arp2/3 complex depleted cells. We next addressed the mechanism by which ADA resists Parkin recruitment onto depolarized mitochondria. Through confocal and TEM microscopy we found that the ADA acutely disrupts close contacts between ER and mitochondria. Interestingly, over- expression of ER-Mitochondrial tethers overrides the effect of ADA, allowing rapid recruitment of Parkin after mitochondrial depolarization. Finally, Arp2/3 inhibition or depletion results in higher LC3 accumulation on damaged mitochondria. In a more chronic mitochondrial damage induced by mtDNA depletion ADA-like filaments persist for days and disrupt close contacts between ER and mitochondria in an Arp2/3 dependent manner. Arp2/3 inhibition using CK666 in these cells results in Parkin and LC3 recruitment speeding up mitophagy. Taken together, we propose that, beyond its metabolic role, ADA acts as a protective mechanism, allowing damaged mitochondria the chance to recover before mitophagy pathways are engaged for organelle clearance.
17.	**Interfering with ER Calcium Stores Inhibits Clustering of IRE-1 in Response to ER Stress** **Bryce Jurkouich**, Mingjie Ying, Daniela Ricci, Romie Azor and Yair Argon

The unfolded protein response (UPR) is a physiological response to accumulation of misfolded proteins where Inositol requiring enzyme 1 (IRE-1) is activated and plays a major role. In parallel to acquisition of enzymatic activity during this activation, the intracellular distribution of IRE1 changes from dispersed throughout the Endoplasmic reticulum (ER) to clustered in distinct molecular foci. IRE-1 clustering has been described mainly in response to ER stress induced by the glycosylation inhibitor Tunicamycin (TM), but we find that it is also induced in response to agents that perturb intracellular calcium.

In our experiments, cells expressing GFP-tagged IRE-1 were exposed to ER-stress plus compounds which impact luminal and cytosolic calcium. When the cells were stressed with Thapsigargin (TG), the inhibitor of the SERCA pump responsible for retrieving Calcium into the ER, IRE1 displayed smaller and more numerous clusters, which developed faster than those induced by TM. These clusters failed to form in stressed cells whose intracellular Ca was chelated. TG-induced small clusters seem to represent an intermediate size in the TM-induced clustering, since in order of addition experiments, TG clusters were dominant.

To understand the previously observed relation of IRE-1 clustering and on-going protein synthesis, we treated the cells with TG plus ES1, an inhibitor of the sec61 complex in the ER membrane, which mediates the translocation of newly synthesized polypeptides into the ER lumen in a calcium-gated process (Bhadra P et al). ES1 treatment inhibited IRE-1 clustering and, depending on the order of addition, also caused dispersal of pre-formed clusters. Five ES1-related derivatives exhibited similar activity to ES1, except ES47, which fails to inhibit the Ca^{2+} pore activity of sec61 (Bhadra P et al).
In a related set of experiments we showed that IRE1 clustering is sensitive to Caffeine, an inhibitor of ryanodine receptors (RyR), which increases calcium release from luminal ER stores. IRE1 clustering was inhibited in dose-dependent manner by Caffeine, indicating that it is dependent on RyR. Remarkably, when caffeine was given to cells at peak clustering time, it inhibited dispersal of TM clusters but not of TG clusters. This highlights another distinction between the two forms of ER stress, whose basis will be pursued in future work.

Taken together, these experiments show that the intracellular Ca^{2+} gradient across the ER membrane is essential for the proper clustering of IRE-1 and support that Sec 61 is a necessary component that links Ca++ gating activity of the ER to entry of newly synthesized polypeptide to the lumen.

18.

Moesin regulates integrin-dependent T cell migration

Marie Juzans, Dong-Hun Lee, Daniel A. Hammer, and Janis K. Burkhardt

T cells migrating through body tissues exhibit a wide range of motile behaviors that allow them to migrate along vessel walls, pass through endothelial barriers, and traverse dense tissues using a pattern of runs and turns that facilitates antigen search. Recently, it has become clear that membrane-cortex attachment controls cell migration as it restricts sites where protrusions can form. In T cells, moesin is the dominant regulator of cortex-membrane attachment. We previously showed that moesin fosters bleb-based motility by biasing bleb formation toward the leading-edge. We now find that moesin also plays a major role in integrin-dependent mesenchymal motility. Moesin-deficient T cells exhibit enhanced adhesion to ICAM-1, and this is linked to increased integrin clustering and cell deformability. Moreover, when migrating on ICAM-1, these cells present larger lamellipodia while often lacking defined uropods. This phenotype results in faster migration over longer distances in 2D, and shear flow settings. We are now exploring the hypothesis that loss of moesin induces constitutive detachment of cortical actin from the membrane, resulting in lower cortical tension and altered levels of Rac1 and Rho A activation at the front and rear of the cell, respectively.

19.

Leveraging Optogenetic Platforms to Study the Role of RNA Granule Transport in Axon Development

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Neuronal axons can traverse vast distances and often exhibit complex, branching geometries to innervate multiple downstream targets. The formation of these structures is spatially and temporally regulated by extracellular guidance cues, which can modulate intracellular processes (e.g. cytoskeletal dynamics and membrane turnover) to regulate growth cone dynamics at the axon tip and/or branching dynamics along the midaxon. More recent work has also revealed a key role for local translation, with guidance factors inducing translation of locally available mRNA species to generate nascent protein and influence local outgrowth dynamics. While studies have demonstrated that trafficking of these mRNA species into the axon relies on their interaction with RNA binding proteins (RBPs) to form RNA granules that can bind to cytoskeletal motors and/or hitchhike on other motile organelles, it is unclear how different signaling cues and molecular mediators govern the positioning of these RNA granules within the axon, regulating when and where nascent protein is synthesized. Furthermore, it is unclear what capacity these RNA granules possess as organizers of local remodeling and if their mobilization is necessary and sufficient to induce local structural responses. These questions have been difficult to answer due to a paucity of tools to manipulate axonal structures and granule transport with sufficient spatial and temporal resolution, but our lab has leveraged novel optogenetic platforms in primary cultured neurons to address these gaps. The first platform, termed the **"Opto-Receptor"** system, leverages the blue light sensitive protein CRY2 and its ability to simultaneously homodimerize and heterodimerize with CIB1 as described in Duan et al., 2018. By fusing the intracellular domain of either the TrkB or PlexinA4 guidance receptor with the CRY2 protein and co-expressing it with membrane bound CIB1 in a bicistronic vector, we have generated a blue light-inducible platform with which to locally trigger these signaling pathways and study various developmental phenomena with exquisite spatiotemporal precision. The Plexin A4 system (iPlex-Opto) induces rapid collapse in illuminated growth cones, while the TrkB system (iTrkB-Opto) induces greater dynamics in illuminated growth cones and is also able to trigger a branching response along illuminated segments of midaxon. We have leveraged the latter tool to study how local growth cues regulate the positioning of RNA granules. The outgrowths induced from local iTrkB-Opto stimulation are translation dependent and correlate with the recruitment of different RNA granule species like FMRP, suggesting that mechanisms exist for guidance signaling to regulate RNA granule positioning and optimize the availability of translational substrates at sites of demand. The second platform, termed the **"Opto-Motor"** system, leverages a separate class of blue light sensitive proteins to recruit cytoskeletal motors to cargos of interest and manipulate their positioning, building on previous work from Nijenhuis et al., 2020. By generating RNA granule constructs that are tagged with a blue light responsive iLID domain (e.g. FMRP-mScarlet-iLID) and co-expressing optogenetic motors tagged with an SSPB domain (e.g. KIF5C(1-560)-GFP-SSPB, SSPB-GFP-GCN4-KinesinVIb(861-1321), etc.) that can reversibly bind iLID following illumination, we have generated a system in which the focal application of light can be used to locally displace RNA granules and study how their enrichment or depletion from different sites along the axon affects growth dynamics. Having completed proof of concept experiments in COS-7 cells, we are performing studies in primary neurons to study the role of different RNA granule species at sites of branching and at the growth cone. Overall, the Opto-Receptor and Opto-Motor systems represent effective platforms for interrogating the cues regulating RNA granule transport and the capacity of said transport for guiding axonal morphogenesis, respectively. Additionally, these systems demonstrate the utility of optogenetics in performing spatiotemporally precise manipulations and represent the kinds of exciting questions that can be tackled in the fields of neurodevelopment and axon biology.

20.

Endurance exercise training partially rescues aged metabolic phenotypes and improves healthspan in mice lacking a functional circadian clock

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The circadian clock, which regulates rhythmic gene expression, is orchestrated by the transcription factor *Bmal1*. Embryonic *Bmal1*$^{-/-}$ knockout mice (eKO) exhibit shortened lifespan, metabolic disorders, and reduced muscle function, while postnatel inducible *Bmal1*$^{-/-}$ knockout mice (iKO) exhibit normal lifespan and muscle mass despite identical behavioral rhythm disruptions. Given exercise's potential to mitigate sarcopenia and metabolic disorders, we examined its impact on young and moderately aged eKO and iKO mice. We investigated the effects of an 8-wk endurance exercise program on skeletal muscle function and metabolism in the eKO, iKO, and aged iKO models using deep phenotyping, transcriptomics, metabolomics, and high resolution mitochondrial respirometry. Sedentary eKO mice have exercise and metabolic deficits (VO_2 and RER) compared to wild type (eWT) controls, which were rescued by endurance training. In contrast, iKO mice show limited deficit in exercise tolerance and minimal metabolic differences compared to iWT. Aged (18-22 months) sedentary iKO mice exhibit greater metabolic deficits than younger iKO mice. Respirometry analysis of the soleus muscle and heart suggests mitochondrial dysfunction in both sedentary eKO and iKO mice, supported by marked alterations in plasma acylcarnitines and the expression of genes related to mitochondrial function in pathway analyses. The eKO mice mitochondrial function appears to be rescued by exercise. In summary, antenatal deletion of *Bmal1* leads to a premature aging that is partially rescued by exercise. Postnatal loss of *Bmal1* has minimal effects on muscle function of young mice but leads to increased functional deficits with age.

21.

In vitro approach to investigate the molecular context-dependent interactions between the Ndc80 complex and its kinetochore receptor CENP-T

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The assembly of mitotic kinetochores involves a hierarchical array of pairwise protein-protein interactions. Structural biologists have successfully reconstructed large kinetochore complexes using highly concentrated purified proteins. However, quantitative studies with human cell extracts and purified proteins have shown that these binding reactions are highly inefficient at physiologically relevant concentrations and timescales. Interestingly, in dividing cells, clustered CENP-T induces the robust assembly of kinetochore-like particles rich in Ndc80 complexes, whereas soluble CENP-T monomers do not efficiently recruit Ndc80. We developed a novel in vitro approach to investigate the molecular context-dependent interactions between Ndc80 and its two binding sites in CENP-T. Our assay utilizes genetically engineered 60-mers, which form a core particle conjugated to the 242 aa N-termini of CENP-T. Binding of soluble Ndc80-GFP Bonsai protein and complex dissociation are monitored in real time to determine kinetic constants. Using this versatile assay, we uncovered that Ndc80 binding to CENP-T is mediated by an unstable but tunable binding interface, which matures slowly in monomeric molecules. However, within macromolecular CENP-T clusters, a larger fraction

	of Ndc80 molecules is retained with stronger affinity. Notably, site 2 matures faster than site 1, which we hypothesize is due to differences in the configurations of their unstructured segments. The poster will present our progress in testing this hypothesis using AlphaFold3 modeling, as well as direct measurements of Ndc80 binding to CENP-T proteins with engineered composite binding sites. The successful completion of these efforts should reveal the molecular determinants of the Ndc80-CENP-T binding interface and the rate of its maturation.
22.	**Breaking Synapses: Defining functional phenotypes of SynGAP1 haploinsufficiency *in vitro* for gene therapy research** **Nicolas Marotta**, Alex Felix PhD, Jennine Dawicki-McKenna PhD , Benjamin L. Prosser PhD *Objective:* Characterize phenotypes of SynGAP1 haploinsufficiency in *in vitro* primary cortical cultures using multielectrode arrays (MEA) and confocal microscopy. *Background*: *SYNGAP1* Related Intellectual Disorder (SRID) is caused by (often) *de novo* and heterozygous mutations in *SYNGAP1*, which encodes SynGAP1 (**Syn**aptic **G**TPase **A**ctivating **P**rotein **1**). The disorder is characterized as a developmental and epileptic encephalopathy and neurodevelopmental disorder (NDD) and has a wide range of clinical presentation. Currently, there are only palliative care options and gene therapy options could lead to potential curative therapies. Mutations in *SYNGAP1* (~85% PTV and ~15% Missense) result in a haploinsufficient disease mechanism. There is no report of homozygous carriers of *SYNGAP1* mutations and *Syngap1* knock out mice are embryonically lethal. SynGAP1 interacts with post-synaptic-density protein-95 (PSD-95), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR, a glutamate receptor), and N-methyl-D-aspartate receptors (NMDARs) to alter synaptic strength. Specifically, SynGAP1 inhibits AMPAR insertion into the membrane and downstream RAS GTPase signaling to weaken synapses or prevent them from forming. Previously published work in cultured neurons established phenotypes of *SYNGAP1* haploinsufficiency and altered dendritic spine development. Further, haploinsufficient mice parallel phenotypes of intellectual disability, memory impairments, and synaptic plasticity defects in patients. However, how SynGAP1 haploinsufficiency impacts neuronal network dynamics remains uncharacterized and we predict it will be negatively impacted. To address this, our lab has recently developed a humanized *SYNGAP1* haploinsufficient mouse model (*SYNGAP1*$^{Hu/-}$). *Methods*: To evaluate gene therapy efficacy *in vitro,* we are utilizing multi-electrode arrays (MEA) and confocal microscopy techniques on primary cortical neurons isolated from E16.5 embryos of the *Syngap1*$^{Hu/Hu}$ x *Syngap1*$^{+/-}$ breeding. MEA allows us to passively measure the extracellular fluctuation in current over the course of life in culture and gain insight into a high throughput measure of network connectivity. Further, confocal microscopy allows us to delve into neuronal morphology, synaptic connectivity, and neuronal population specific effects due to *Syngap1* haploinsufficiency. *Results*: We find that neuronal network metrics are altered in *Syngap1*$^{Hu/-}$ haploinsufficient neurons compared to controls on MEA. Additionally, we observe an increased projection density and abnormal bipolar neuron morphology indicating that the neurons isolated from the haploinsufficient mice parallel the findings in the literature and show potential for use as a screening platform for gene therapy modalities.
23.	**Map7 coordinates motor recruitment to regulate transport selectivity at branch junctions** **E. Moese**[1], S. Tymanskyj[2], L. Ma[2]

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Proper development and function of neurons requires tightly regulated microtubule (MT) -based transport of various cargo from cell bodies to synaptic terminals. Improper transport is implicated in various neurological diseases. Neurons have elaborately branched axons creating complex connections. Intracellular transport at axon branch junctions requires tight regulation of cargos, as suggested by recent discovery of selective transport in cultured dorsal root ganglion (DRG) neurons. Anterograde transport of lysosomes displayed preference for longer branches or branches with a dynamic growth cone. Furthermore, this selectivity is differentially regulated for various cargos and mediated by kinesin-3 motors. However, it is unknown what local mechanisms at branch junctions are mediating selectivity. Interestingly, MAP7 is a MT associated protein enriched at branch junctions in embryonic DRG neurons. *In vitro*, MAP7 recruits kinesin-1 to MTs but inhibits kinesin-3 from MT binding. Transport of cargos such as lysosomes when MAP7 is overexpressed displayed increased run time but decreased velocity indicating kinesin-3 MT binding is blocked. Therefore, we hypothesize that MAP7 coordinates motor recruitment and transport selectivity at branch junctions via phosphoregulation. To test this hypothesis, we first examined MAP7 knockout neurons. Preliminary data showed an increase in the anterograde transport velocity of lysosomes, consistent with the idea that MAP7 normally inhibits kinesin-3 but favors kinesin-1 transport. Currently, we are using these neurons to determine the requirement of MAP7 on transport selectivity. Next, we examined the role of MAP7 phosphorylation by mutating 13 SP/TP sites in the P-domain, a highly unstructured region that is involved in MT binding and axonal localization. Based on fluorescence recovery after photobleaching (FRAP) analysis, we found that that the phospho-mimetic mutant displays decreased MT binding compared to the phospho-null mutant. Intriguingly, the phospho-mimetic mutant is not localized to the branch junctions as the wildtype or the phospho-null mutant, instead it appears in the distal axons as well as the growth cones, which are normally avoided by MAP7. These data suggest phosphorylated MAP7 is readily removed from MTs, which could in turn increases kinesin-3-mediated transport at branch junctions. To test this possible mechanism, we are currently testing the role of the phosphomutants on selective transport. By parsing out the relationship between the regulation of MAPs and intracellular transport we will begin to understand how regulated transport supports axon development and maintenance.

24.

Fibroadipogenic Progenitor-Mediated Skeletal Muscle Regeneration After Injury is Disrupted During Whole-Body Weight Loss

Natalie Moore, Cindy Lu, Sarah Traynor, Carmen Flesher, David Merrick

Skeletal muscle is a highly dynamic and metabolically active organ that is commonly subjected to injury via strain or overuse, but is also uniquely capable of self-regeneration even in the adult. This regenerative capacity is dependent on tissue-resident fibroadipogenic progenitors (FAPs), a fibroblastic cell type that plays a supportive role in directing muscle progenitor satellite cells following injury and maintaining homeostatic muscle health. Through this process, FAPs differentiate into mature adipocytes, forming intramuscular adipose tissue that replaces contractile muscle tissue with non-contractile fat. Given that the role of FAPs in the undifferentiated state is supportive, the metabolic changes accompanying obesity and drastic weight loss pose an interesting lens through which to study FAP biology. As global populations grow increasingly obese, and with the rise of GLP-1 agonist drugs for weight loss, it is crucial to understand how skeletal muscle, responsible for a large proportion of the body's energy expenditure, responds to these dynamic changes, and how injury responses might differ in lean, obese, and weight-loss patients. Whole-body weight loss is commonly complicated by lean mass loss, therefore, understanding how FAPs become permissive of muscle atrophy is of utmost importance. We hypothesize that, during weight loss, skeletal muscle FAPs undergo a maladaptive identity shift to facilitate muscle catabolism, which consequently diminishes their ability to support muscle health and myogenesis, potentially leading to worsened clinical outcomes after injury.

	To investigate FAP transcriptional dynamics during muscle regeneration we generated a single-nucleus RNA-sequencing timecourse of a murine model of glycerol-induced muscle injury. Our results revealed that skeletal muscle FAPs enter a distinct 'activated' transcriptional state in response to injury. Importantly, we discovered that this FAP transcriptional response is perturbed by weight loss. Furthermore, while FAPs from lean animals are highly adipogenic *ex vivo*, FAPs isolated from weight-loss animals display reduced adipogenic capacity. These results suggest that FAPs retain an imprinted, cell-intrinsic memory of the physiologic state of their tissue of origin, which influences their ability to respond to injury. Future studies will aim to quantify differences in regeneration and recovery after injury between lean, obese, and weight-loss animals by measuring *in situ* force generation. Overall, these studies point to FAPs as a key cell type that regulates skeletal muscle health, with implications for preservation of lean mass during weight loss.
25.	**Parkin-dependent mitophagy and NF-κB signaling in neurons** **Neha M. Nataraj**, Erika L.F. Holzbaur Neurodegenerative diseases affect millions worldwide, most with no effective treatments available. Neurons are long-lived with high metabolic requirements, relying on autophagy to degrade damaged organelles or aggregated proteins. Mitochondrial autophagy (mitophagy) is a highly regulated process essential for homeostasis. Disruptions in mitophagy, such as mutations in PINK1, Parkin, and Optineurin, are linked to multiple neurodegenerative diseases, including Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis, emphasizing the critical role of mitophagy in homeostasis and health. Thus, elucidating the molecular regulation of mitophagy and responses to mitophagy disruption will provide insight into the pathogenesis of multiple neurodegenerative diseases. Upon mitochondrial damage, the kinase PINK1 activates the E3 ubiquitin ligase Parkin at the outer mitochondrial membrane (OMM), leading to rapid ubiquitination of OMM proteins and mitochondrial clearance by autophagy. Our lab recently discovered that in parallel, mitochondrial damage leads to Parkin-dependent recruitment of the IKK protein NEMO, which activates NF-κB. This recruitment was observed and characterized in HeLa cells and also observed in murine astrocytes. Additionally, my preliminary data demonstrates recruitment in hiPSC-derived cortical neurons. NF-κB activation is critical for cell survival and for mounting immune responses, but it is also associated with autoinflammatory disease. Thus, NF-κB signaling must be carefully regulated. Critically, Parkin protects multiple cell types from cytotoxicity and inflammasome activation. Dopaminergic (DA) neurons are selectively lost in PD. PINK1/Parkin mitophagy, which is dysregulated in PD, has been relatively unexplored in DA neurons, and the role of NEMO recruitment to the OMM and subsequent NF-κB signaling in neurodegenerative diseases is unknown. Furthermore, how this process is regulated in a neuroinflammatory environment has not been explored. I hypothesize that Parkin-dependent NEMO recruitment to the OMM is required for neuronal survival, and disruption of Parkin signaling leads to cell death. I also hypothesize that inflammatory stimuli combined with mitochondrial stress will induce dysfunctional signaling and increase neuronal susceptibility to cell death. In order to test these hypotheses, I will utilize chemical inhibitors, CRISPR-Cas9 targeting, spinning disk confocal microscopy, and biochemical assays in hiPSC-derived cortical and dopaminergic neurons. By characterizing neuronal molecular responses to mitochondrial stress and inflammation that are critical for homeostasis and are perturbed in multiple neurodegenerative diseases, we aim to shed new light on disease progression to help inform future therapeutic development.
26.	**Skeletal muscle and adipose tissue-specific roles of ActRIIA/B in regulating body composition and metabolic homeostasis during obesity** **Elizabeth Nunn**[1,2], Matthew Gavin[1,2], Joe Baur[1,2], Paul Titchenell[1,2]

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New anti-obesity drugs target the glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptors to reduce food intake, resulting in significant weight loss. Reduction in total body weight is the primary clinical end point for obesity therapeutics, but weight loss is driven by reductions in both fat and skeletal muscle mass. The goal for emerging obesity therapeutics is to mitigate loss of muscle mass during weight loss driven by GLP-1/GIP receptor agonists (GLP-1/GIPRAs). Skeletal muscle is not only vital for physical mobility and strength, but also plays central roles in glucose regulation and systemic energy expenditure. Therefore, a therapeutic approach which improves body composition (amount of lean vs. fat mass) during weight loss may not only be beneficial for overall physical function but may also improve long-term maintenance of weight loss and metabolic outcomes. A central pathway which regulates muscle mass is signaling through the activin type IIA and IIB receptors (ActRIIA/B). ActRIIA/B bind TGFβ-like ligands such as myostatin and activin A, engaging downstream signaling resulting in muscle atrophy. In individuals with obesity, blockade of ActRIIA/B significantly reduces fat mass and increases muscle mass, highlighting that this pathway could be targeted to treat metabolic disease. Our published work demonstrates that in a mouse model of obesity, treatment with an anti-ActRIIA/B antibody during weight loss stimulated by a GLP-1RA mitigates loss of muscle mass and further enhances loss of fat mass. Systemic blockade of ActRIIA/B affects adipose tissue mass, muscle mass, and glucose homeostasis, however, the cell-autonomous mechanisms underlying these effects are incompletely understood.

To detangle the cell autonomous effects of ActRIIA/B signaling, we have generated novel muscle- and fat-specific ActRIIA/B knockout mouse models. We hypothesize that congenital muscle-specific deletion of *Acvr2a/b* (genes for ActRIIA/B) will result in increased muscle mass and decreased fat mass in obesity, improving metabolic homeostasis, and adipocyte-specific deletion of *Acvr2a/b* will reduce adipose tissue mass, resulting in improved glucose regulation due to reduced adiposity. Muscle-specific (Muscle-KO) and adipose-specific (Fat-KO) mice were placed on 60% HFD for 12 weeks, where body composition was measured every 2 weeks via EchoMRI and glucose tolerance assessed at 6 and 12 weeks. After 12 weeks of HFD feeding, Muscle-KO mice had significantly increased muscle mass and decreased fat mass compared to littermate controls, while Fat-KO mice did not exhibit changes in body composition. Muscle-KO mice displayed improved glucose tolerance after 12 weeks of HFD feeding, and there was no strong effect on glucose homeostasis in Fat-KO mice. This data suggests that congenital deletion of *Acvr2a/b* in the skeletal muscle drives both muscle hypertrophy and secondary reductions in fat mass, while blocking ActRIIA/B signaling in adipose tissue does not modulate body composition and glucose regulation during obesity. We have also generated an inducible mouse model where deletion of *Acvr2a/b* in skeletal muscle is induced by tamoxifen injection in adult mice after 12 weeks of HFD feeding (Muscle-ESR-KO). In contrast to the congenital mice, obese Muscle-ESR-KO mice have increased muscle mass but do not experience reductions in fat mass. This data suggests that although inhibition of *Acvr2a/b* signaling in both developing and adult muscle leads to muscle hypertrophy, deletion of *Acvr2a/b* in adult myofibers in an obese state does not exert the same secondary effect on adipose tissue mass.

27.

PAI-1's role in defining the "myospan" of skeletal muscle

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	By mass, skeletal muscle is the largest human organ, and it is a potent mediator of health and longevity. The inevitable and progressive loss of muscle with advanced age negatively impacts movement and mobility, respiration, thermoregulation, and numerous other physiological parameters. The biological mechanisms that cause age-related changes in skeletal muscle remain poorly understood. PAI-1 is a 43 kDa, secretory protein involved in diverse biological processes including inflammation, cell death, and senescence. PAI-1 also regulates several pathophysiological processes (e.g., metabolic syndrome and fibrosis) and is a major contributor to the multimorbidity of aging. The latter is consistent with gradual systemic accumulation of PAI-1, over time. The impact of elevated PAI-1 on aging skeletal muscle, however, is also largely unknown. Here, we tested the hypothesis that PAI-1 and its homologs are evolutionarily-conserved, negative regulators of muscle function and, thereby, directly contribute to its age-related deterioration. To examine the effects of augmented PAI-1 levels on skeletal muscle function, we evaluated activation and relaxation, kinetics and mechanics, of single myofibrils isolated from mice overexpressing (OE) PAI-1, which developed accelerated vascular aging. Relative to age-matched controls, skeletal myofibrils from 7-mo-old PAI-1 OE animals generated ~40% less maximum force, which was roughly the same as that produced by myofibrils from a 56-wk-old control mouse. Owing to its rapid life cycle, well-developed genetics, and highly-conserved factors that dictate lifespan, *Drosophila melanogaster* has been used as a model for aging research for > 100 years. We observed a complete loss of flight ability from one through seven weeks of age. Quantitative proteomics of dissected one-, four-, and seven-week-old indirect flight muscle (IFM) fibers revealed a number of proteins and pathways were significantly up-regulated over time, including several PAI-1 homologs, i.e., members of the serine protease inhibitor family (Spn42D). Muscle-specific knockdown of *Spn42Da* preserved flight ability over seven weeks. Histological analysis revealed intact myocyte structure, whereas both control and wild- type flies displayed age-related morphological deterioration. These results indicate transcriptional repression of *Spn42Da* mitigates age-associated skeletal muscle degeneration and preserves flight ability. In conclusion, our findings imply that PAI-1/Spn42Da contributes to age-related pathologies across phyla, and as a key regulator of skeletal muscle function and longevity, PAI-1 inhibition improves the "myospan" to counter age-related deficits in muscle mass, strength, and function.
28.	**Autophagic stress activates distinct compensatory secretory pathways in neurons** **Sierra D. Palumbos**[1,2], Jacob Popolow[1], Juliet Goldsmith[1,2], Erika L.F. Holzbaur[1,2*] *[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA* Autophagic dysfunction is a hallmark of neurodegenerative disease, leaving neurons vulnerable to the accumulation of damaged organelles and proteins. However, the late onset of diseases suggests that compensatory quality control mechanisms may be engaged to delay the deleterious effects induced by compromised autophagy. Neurons expressing common familial Parkinson's disease (PD)-associated mutations in LRRK2 kinase exhibit defective autophagy. Here, we demonstrate that both primary murine neurons and human iPSC-derived neurons harboring pathogenic LRRK2 upregulate the secretion of extracellular vesicles. We used unbiased proteomics to characterize the secretome of LRRK2^{G2019S} neurons and found that autophagic cargos including mitochondrial proteins were enriched. Based on these observations, we hypothesized that autophagosomes are rerouted toward secretion when cell-autonomous degradation is compromised, likely to mediate clearance of undegraded cellular waste. Immunoblotting confirmed the release of autophagic cargos and immunocytochemistry demonstrated that secretory autophagy was upregulated in LRRK2^{G2019S} neurons. We also found that LRRK2^{G2019S} neurons upregulate the release of exosomes containing miRNAs. Live-cell imaging confirmed that this upregulation of exosomal release was dependent on hyperactive LRRK2 activity, while pharmacological experiments indicate that this release staves off apoptosis. Finally, we show that markers of both vesicle populations are upregulated in plasma from mice expressing pathogenic LRRK2. In sum, we find that neurons expressing pathogenic LRRK2 upregulate the compensatory release of secreted

	autophagosomes and exosomes, to mediate waste disposal and transcellular communication, respectively. We propose that this increased secretion contributes to the maintenance of cellular homeostasis, delaying neurodegenerative disease progression over the short term while potentially contributing to increased neuroinflammation over the longer term.
29.	**Investigating how DISC1 regulates mitochondrial trafficking** **Tania A. Perez**, Gabrielle Glass, Erika L. F. Holzbaur Mitochondria are essential, dynamic organelles that support the function of high-energy demanding neurons by serving as hubs for local energy synthesis, protein translation, and signaling. Proper mitochondrial distribution is necessary for neuron development and function. Mitochondria are trafficked bidirectionally throughout polarized neurons along microtubule tracks by motors of the kinesin-1 family and the dynein-dynactin complex. The disrupted in schizophrenia 1 (DISC1) protein—a risk-factor in schizoaffective and bipolar disorder—is an emerging regulator of mitochondrial trafficking. DISC1 directly interacts with kinesin-1, associates with several mitochondrial transport proteins, and specifically enhances axonal anterograde mitochondrial trafficking in neurons, **yet the mechanism by which DISC1 regulates mitochondrial transport remains unclear**. We hypothesize that DISC1 directly associates with mitochondria to recruit motor proteins at the membrane to regulate trafficking. To address these questions, we have transfected primary hippocampal neurons and HeLa cells with fluorescently tagged DISC1 and mitochondrial markers and utilized a combination of live- and fixed-cell imaging via spinning disk confocal microscopy for visualization. Our preliminary data indicates that DISC1 is highly associated with the mitochondrial network in both primary hippocampal neurons and HeLa cells. Moreover, our data show that DISC1 localizes to axonal mitochondria—both stationary and motile—and can be differentially distributed along mitochondria. We confirmed that DISC1 enhances anterograde mitochondrial trafficking in our system and have shown that DISC1 is specifically associates with the outer mitochondrial membrane. Additionally, we found that DISC1 colocalizes with kinesin-1 on axonal mitochondria and can recruit kinesin-1 to mitochondria in HeLa cells. Overall, our study reveals that DISC1 is highly associated with the mitochondria in both neurons and HeLa cells and can recruit molecular motors to the mitochondrial membrane likely to enhance kinesin-based motility. These initial findings will be used to further dissect the molecular mechanisms by which DISC1 regulates mitochondrial trafficking.
30.	**Active transport of tRNAs facilitates distributed protein synthesis in muscle** **Jennifer M. Petrosino**[1,2]; Vasiliki Courelli[3], Keita Uchida[1]; Barry Cooperman[4], Alexey Bogush[1], Benjamin L. Prosser[2] *[1]Department of Physiology, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.* *[2]Department of Physiology, Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.* *[3]Medical Scientist Training Program, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.* *[4]Department of Chemistry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.* Striated muscle is highly adaptable, and in response to stress and anabolic signaling, muscle cells undergo increases in protein synthesis that subsequently drive increases in their size. For hypertrophy to occur, myocytes coordinate transcription, translation, and proper protein localization to facilitate sarcomere addition. Thus, these densely packed contractile cells rely on long-range active transport mechanisms for RNA and protein localization. Transfer RNAs (tRNA) function as essential regulators of this process by linking transcription and translation. Since their discovery in the 1950s, tRNA localization is believed to occur through passive diffusion. Here, we report that pools of muscle tRNAs

	undergo microtubule-mediated localization and dynamically respond to pro- hypertrophic stimuli. By utilizing fixed and live cell super-resolution imaging techniques, we reveal that these transport-competent tRNA pools are distributed in muscle by hitchhiking on endolysosomal vesicles (ELVs), which concurrently serve as sites for local translation. We establish, through loss-of-function models, that Kinesin-1 is indispensable for the distribution of these tRNA-ELV complexes. Additionally, we identify that leucyl- tRNA synthetase (LeuRS) is essential for tRNA hitchhiking and acts as an adaptor linking tRNAs to ELVs through its interaction with endolysosomal-bound RagD. Performing experiments with a small molecule that specifically inhibits the interactions between LeuRS and ELVs, we demonstrate that disrupting tRNA hitchhiking significantly impairs both local and global translation and ultimately reduces muscle cell size. Together, we identify for the first time that tRNAs undergo microtubule motor-dependent transport to achieve their spatial distribution in muscle. Our findings suggest that tRNA hitchhiking facilitates localized protein synthesis, proposing that local translation is a critical regulator of muscle protein production and size. Collectively, our results provide the first direct evidence that long-range tRNA-ELV trafficking is a mechanism by which pools of tRNAs are distributed to support the maintenance of the muscle proteome.
31.	**Investigating the Role of Rubicon in neuronal LRRK2 mediated secretion** **Jacob Popolow**, Peace Oloko, Sierra Palumbos, Bishal Bisak, and Erika Holzbaur Neurons with strained autophagy are less efficient in their isolation and degradation of misfolded proteins and damaged organelles, which can result in a build up of cellular waste which is harmful to neuronal health. Mutations which impair autophagy are highly associated with neurodegenerative disorders. Hyperactive mutations to the LRRK2 kinase, which phosphorylates targets implicated in vesicular trafficking and autophagy, are the most common cause of familial Parkinson's disease. Hyperactive LRRK2 chronically strains autophagy by delaying autophagosomal axonal trafficking and reducing the efficacy of autophagosome maturation. Recently, our lab determined that neurons with hyperactive LRRK2 upregulate the external secretion of autophagosomes as a compensatory waste disposal mechanism. The protein Rubicon, whose expression increases with age, is an established negative regulator of autophagy which localizes to lysosomes, potentially blocking their fusion to autophagosomes. We ask: can Rubicon alter the fate of autophagosomes between neuronal secretion and lysosomal fusion? To assess this question, we have looked within primary mouse cortical neurons while using shRNA to knockdown Rubicon levels. Using immunohistochemistry, live imaging with dyes, and western blots, we have determined that a knockdown of Rubicon can shift the fate of autophagosomes from secretion toward autophagy. This manipulation is especially pronounced in neurons with hyperactive LRRK2, where a knockdown of Rubicon seemingly "unlocks" lysosomes for autophagosomal fusion, a mechanism known to be strained in LRRK2 neurons. Future studies are needed to define the role of Rubicon in mediating a balance between intracellular metabolism and extracellular secretion.
32.	**Cardiac tissue balance between contractility and extracellular matrix rigidity** **K. Saini**[1], S. Cho[1], M. Tewari[1], S. Belt[1], A. Kumar[2], B. Lee[1], B. Taichman[1], A. Jalil[1], M. Wang[1], A. Kasznel[1], K. Yamamoto[3], N. Kumar[2], D. Chenoweth[1], K. Margulies[1], and D. E. Discher[1] *[1]University of Pennsylvania, Philadelphia, PA 19104, USA; [2] Indian Institute of Technology (IIT) Ropar, India; [3]University of Liverpool, UK* Inhibiting myosin-II not only stops their autonomous beating in embryonic chick hearts but also - based on mass-spectrometry (MS) of extracted lysates - leads to fibrillar collagen degradation by endogenous matrix metalloproteinases (MMPs) unless rescued by a broad-spectrum MMP inhibitor. Collagen, the

	most abundant mammalian tissue extracellular matrix (ECM) protein, controls tissue function during development, homeostasis, and disease via homeostatic a balance between its degradation and synthesis. However, it remains unclear how remodeling fibrillar collagen within complex tissue environments (comprising of many collagen-types and MMP-types) contributes to overall tissue function while it sustains physical forces. Here, we focus on human heart tissue among other samples and assess fibrillar collagen levels via imaging of intact tissues using label-free second harmonic generation (SHG) and immunostaining. We validated fibrillar collagen level changes using a "cell-free" collagen-rich tendon tissue model with a custom-built bioreactor. ECM force perturbation by either pharmacological cellular-contractility inhibition in heart as well as several other tissues or by exogenous loads in cell-free tendons showed forces on ECM not only stabilized both tissue fibrillar collagen against degradation by MMPs and resulting tissue stiffness but also helped preserve both cardiac muscle striation and nuclear lamina morphology. Our observation of fibrillar collagen mechanosensitive nature against enzymatic degradation in the ex-vivo environment agrees with past studies using reductionist models involving acellular gels of purified collagen-I. Interestingly, collagenase activity is strain- rather than stress-suppressed in tissues since physiological strains of ~5% in both beating embryonic hearts and tendons differing by >1000-fold in both collagen levels and stiffness similarly suppressed collagenase activity. Mechanistically, such stabilization of tissue collagen results from strain-dependent collagen molecule triple-helix unfolding but is independent of permeation, tissue mass density, collagenase mobility changes or of tissue (i.e. heterogenous or uniaxial in case of tendon) deformation modes. Such 'use-it-or-lose-it' kinetics for tissue collagen provide insight into microgravity effects, regeneration processes, and scaling of organ-size while suggesting contractility-driven therapeutics.
33.	**NPF binding to Arp2 is allosterically linked to the release of ArpC5's N-terminal tail and conformational changes in Arp2/3 complex** **Andrew J. Saks**, Kyle R. Barrie, Grzegorz Rebowski, and Roberto Dominguez Arp2/3 complex generates branched actin networks essential for numerous motile functions of the cell. It comprises seven subunits: actin-related proteins (Arps) 2 and 3 and five scaffolding subunits (ArpC1-5). The complex adopts two major conformations: inactive, with the Arps interacting end-to-end, and active, with the Arps aligned side-by-side like subunits in the actin filament. Activation involves several cofactors, including ATP, WASP-family nucleation-promoting factors (NPFs), actin monomers, and the mother actin filament. NPFs bind to two sites, one on Arp2ArpC1 and one on Arp3, delivering actin subunits at the barbed end of the Arps to initiate branch elongation. However, the mechanisms by which each NPF drives the equilibrium toward activation remain unclear. We present two cryo-electron microscopy (cryo-EM) structures of Arp2/3 complex at 2.9-Å resolution: one with NPFs bound to Arp3 and ArpC1 but not Arp2 and another with NPFs bound to Arp3 and Arp2–ArpC1. The structures reveal that NPF binding to Arp2 is allosterically linked to the release of ArpC5's N-terminal tail from Arp2 and conformational changes in Arp2, including closure of its ATP-binding cleft and partial rotation and translation toward its position in the active complex at the branch. Previous work identified another allosteric switch linking NPF binding to Arp3 with the release of its inhibitory C-terminal tail, which we also observe. In summary, both NPF-binding sites induce allosteric changes in Arp2/3 complex, collectively shifting the equilibrium toward activation.
34.	**Microtubule dynamics control directional growth in the heart** **Emily A. Scarborough**, Rani M. Randell, Keita Uchida, Benjamin L. Prosser When the adult heart undergoes hypertrophy, each post-mitotic cardiomyocyte increases in size, not number. Cardiomyocytes grow through the addition of discretized contractile units (sarcomeres) either along the cellular short axis or long axis, resulting in widening (concentric growth) or lengthening (eccentric growth), respectively. However, the molecular mechanisms which spatially govern these two types of growth in the heart is opaque. Our group and others revealed that microtubules are necessary

for growth in the heart. Previous work suggests at least two, non-mutually exclusive mechanisms by which microtubules could guide directional growth: 1) microtubule dynamics alter mRNA localization and local translation of new sarcomeres and/or 2) microtubule dynamics alter intercalated disc structure, thereby changing local permissiveness to sarcomere addition. We aimed to test both possibilities.

Using neonatal rat cardiomyocytes, we observed that microtubule destabilizers result in eccentric remodeling whereas microtubule stabilizers result in concentric remodeling. We visualize sarcomeric mRNA (*Actc1*) and translation under these conditions and find that after microtubule stabilization, both are enriched in the direction of growth; though microtubule destabilization shifts translation toward the tip of the cell and increases *Actc1* abundance, it does not meaningfully change mRNA localization. To further explore concentric remodeling, we turn to adult rat cardiomyocytes. We show dynamic microtubules, components of the LINC complex, nucleoporins, microtubule motors and mRNA export are concentrated at the poles of the cardiomyocyte nucleus, aligned along the length of the cell. After modest microtubule stabilization, dynamic microtubules are instead enriched at the nuclear short axis, resulting in nuclear wrinkling and relocalization of the aforementioned components and translation along the width of the cell. We find this relocalization of microtubule motors, nuclear wrinkling, and biased mRNA export is dependent on an intact LINC complex. Additionally, we find that increasing microtubule stability reinforces intercalated disc structure at the end of the cell, whereas microtubule destabilization disrupts it. Disrupting intercalated disc structure directly using an n-cadherin adhesion antagonist peptide is sufficient to cause cardiomyocyte lengthening, and concentric remodeling through microtubule stabilization does not require intercalated disc structure maintenance. Altogether, we find that microtubule dynamics alter mRNA/translation localization and intercalated disc structure, and that these mechanisms work in concert or independently to facilitate bidirectional cardiac growth. In sum, we propose that microtubule stability is a molecular toggle to promote concentric or eccentric growth of heart muscle.

35.

An Osteoarthritis Model in Long-Evans Rats: A time-course of tibialis anterior strength and measures of mechanosensitivity and pain behavior

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Introduction: Osteoarthritis (OA) is a leading cause of disability in the industrialized world and nearly half of patients sustaining significant joint damage will develop post traumatic osteoarthritis (PTOA) within 10 years. OA is a disease of the entire joint, involving synovial inflammation, cartilage/bone damage and damage to other soft tissues leading to pain and disability. OA is characterized by the degeneration of articular cartilage and bone, specifically in osteophytes and subchondral bone sclerosis, as well as decreased joint space. Prevalence of OA is on the rise, estimating 1 in 4 people will have OA by 2040, highlighting the urgent need for enhanced treatment strategies to combat OA. Therefore, by utilizing a surgical induction of PTOA in Long-Evans rats, we are investigating the affected cartilage, bone, muscle and resulting pain behaviors in efforts to identify potential PTOA treatment strategies.

Method: All experiments were conducted in male Long Evans rats (N=16) that were 16 to 18 weeks old at the time of surgery. Animal use protocols were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. To induce PTOA, we used a surgical ACLT (anterior cruciate ligament transection) and DMM (destabilization of the medial meniscus) model, as previously described. Prior to surgery, animals (N=16) were assigned to ACLT+DMM (n=8) or Sham (n=8) groups. At post-surgical timepoints: animals were assessed for mechanosensitivity and pain behavior (4-, 8-, 12-, 16- and 20-weeks) as well as *in vivo* dorsiflexor muscle strength (4-, 8-,14-, and 18-weeks). Statistical analyses were performed using statistical software (JMP Pro 17: SAS Institute

Inc., 2023). We utilized a two-way analysis of variance (ANOVA) to detect group differences between ACLT+DMM and Sham over various timepoints post-surgery. Data were presented as means ± standard deviation (SD), with a significance level set at $p < 0.05$.

Results: Presurgical body mass was significantly greater in ACLT+DMM as compared to Sham ($p<0.001$). At 18-weeks post-surgery group differences in body mass were significant ($p=0.039$), however, weight gain was similar between groups ($p=0.75$). At 8-weeks post-surgery, ACLT+DMM group trended towards greater dorsiflexion torque than Sham animals ($p=0.062$), with a significantly greater twitch torque ($p<0.001$). Torque kinetic data demonstrates a shift towards a type-II fiber with altered rate of torque development and relaxation times ($p<0.001$). At both 14-weeks, and 18-weeks post-surgery, no significant differences in muscle contractility measures were detected ($p>0.05$). Evoked pain mechanosensitivity (von Frey) was longitudinally assessed in rats in both the operated (Sham or ACLT+DMM leg) and the un-operated limb (Contralateral), at 4-, 8-, 12-, 16- and 20-weeks post-surgery. Surgical induction of OA showed an ipsilateral decrease in pain mechanosensitivity of the hindpaw at 4, 8, 12, 16 and 20 weeks relative to the contralateral hindpaw becoming significant by 16 weeks ($p=0.023$). Weight bearing incapacitance data showed an increase in sensitivity and avoidance of weight bearing on the operated side as compared to Sham ($p=0.033$). This increased sensitivity resolved to baseline for both ACLT+DMM and Sham by 20 weeks ($p=0.35$) (dashed line equals 1).

Discussion: ACLT+DMM group demonstrated increased mechanosensitivity and perceived pain during static weight-bearing during early timepoints when compared to Sham. Our data supports a minimal influence of ACLT + DMM on hindlimb muscle strength, specifically the primary ankle dorsiflexor muscle group: Tibialis Anterior, EDL. Surprisingly, ACLT+DMM group showed an early increase in muscle strength (8-weeks post-surgery), likely to be a compensatory mechanism to adapt to increase knee laxity. Currently, our data does not support a muscle deficit about the ankle dorsiflexors following ACLT+DMM surgery, with some evidence of altered mechanosensitive and increased instances of pain.

36.

Investigating platelet activation and aggregation using microfluidic-based experimental approaches

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Proper clot formation is essential for preventing blood loss after vascular injury, as circulating platelets adhere and aggregate to seal the breach. While platelet activation is well studied in static conditions, the regulation of platelet activity under flow within a growing clot remains poorly understood. We addressed this gap in vitro using microfluidic-based systems at two levels: isolated single-platelet analysis and real-time visualization of the growing platelet-rich clot. At the single-cell level, we monitored thrombin-induced activation of human platelets adherent to immobilized fibrinogen under flow. We found that dense granule secretion occurs in discrete bursts, triggered stochastically by calcium spikes, as revealed using the Ca^{2+}-specific fluorescent Calbryte-590^{AM} dye. In platelets from a patient with macrothrombocytopenia, calcium spike frequency was disrupted. Normal-sized patient platelets (<5 μm) exhibited irregular spiking similar to healthy controls (12.3 ± 1.2 vs. 14.2 ± 0.3 spikes/min), while larger patient platelets showed fewer spikes (5.7 ± 1.8 spikes/min) and more regular oscillatory patterns. Ongoing work will determine whether calcium spiking frequency reflects large platelet size alone or associated molecular abnormalities in macrothrombocytopenia. In parallel, we examined clot formation by perfusing whole blood from healthy subjects through a 100-μm microfluidic chamber at 1,000 s^{-1}, mimicking arteriolar flow. Clotting was initiated on a 50-μm strip coated with collagen and tissue factor

and monitored in 3D using differential interference contrast (DIC) microscopy. Clot growth proceeded at 2.5 ± 0.4 μm/min (N = 5) in height until full occlusion, regardless of collagen density or channel width, indicating a robust yet non-physiological occlusive growth pattern. Combined DIC and scanning electron microscopy revealed that while aggregation began at the activation site, it extended downstream nearly threefold, forming an overgrown "tail" with heterogeneous platelet packing, fibrin fibers and entrapped red blood cells. Current efforts aim to identify mechanisms that limit such overgrowth, which may occur *in vivo*, and to explore how calcium signaling in platelet, representing their activation level, affects clot formation outcome and architecture.

37.

Novel *TUBB2A* variant linked to pediatric neurodegeneration leads to hyperstable microtubules and increased mitochondrial respiration

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During early embryogenesis, cells undergo divisions without concurrent growth. Zygotic genome activation (ZGA) is triggered when cell sizes are sufficiently reduced, and ZGA is required for downstream development. In *Xenopus* embryos, ZGA initiation is linked to the reduction to a critical size threshold, with small animal pole cells activating their genomes before large vegetal pole cells. This gradient of cell sizes is conserved among amphibians and lampreys, but the significances of the gradient and the coupled pattern of genome activation remain unclear. To address this, we spatially inverted the pattern of genome activation in *Xenopus* embryos and evaluated its developmental consequences. Using a temperature controller, we applied a temperature gradient to *Xenopus* embryos during the blastula stage. By specifically cooling the top, we slowed down the cell division in the animal pole, resulting in an inverted cell size gradient and delayed genome activation in this region. Strikingly, embryos with

38.

Investigating the protective functions of stress granules in the heart

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Stress granules are phase-separated assemblies of RNA and RNA binding proteins that form after stress-induced translation arrest. In non-muscle cell types, it is known that stress granules can protect cells from death during and after ISR activation. The heart is largely made up of post-mitotic, nonregenerative cardiomyocytes (CMs), increasing the necessity for CMs to recover from stress without inducing cell death. Stress granule assembly can result from activation of the integrated stress response (ISR). Previous work suggests CMs experience ISR activation during pathology, such as ischemia-reperfusion injury. However, if stress granules assemble, and what function they might serve, is not known in CMs. We hypothesize that stress granules assemble in CMs after ISR activation to maintain CM viability. We observe formation of stress granules in murine hearts after ischemia-reperfusion injury suggesting pathological relevance and were motivated to more carefully dissect underlying mechanisms in vitro. We find that arsenite induces oxidative stress and activates the ISR in neonatal CMs. Arsenite also assembles granules containing RNA-binding proteins G3BP1, PABPC1 and FXR1, as well as mRNA. Granule formation is promoted by G3BP1 overexpression and prevented by pretreatment with an ISR inhibitor. Stress granules can also assemble in adult CMs with heat stress, which are similarly prevented by an ISR inhibitor. Together, these data suggest stress granules assemble in response to stress in CMs both in vivo and in vitro. Stress granule assembly may function to protect CMs from cell death in

	response to stress, which we will directly test in the future by measuring cell viability during and after stress as stress granule formation is either promoted or prevented.
39.	**Tropomyosin 3.1 inhibits Myosin-19 and is localized to mitochondrially-associated actin** **Cameron P. Thompson**, Luther W. Pollard, Erika L.F. Holzbaur, E. Michael Ostap Myosin-19 (Myo19) is the mitochondria-specific, actin-based motor important for regulating many aspects of mitochondrial function through the regulation of organelle fission, motility, and metabolism. Given its crucial role in maintaining mitochondrial health, and therefore the health of the cell in general, proper regulation of the motor is pivotal. Non-muscle tropomyosins are known to differentially regulate members of the myosin superfamiliy. Notably, some myosins are activated by tropomyosin while others are inhibited. Here, we show that the interaction of Myo19 with actin is inhibited in the presence of Tpm1.7 or Tpm3.1. Interestingly, this inhibition appears to be highly cooperative, with an all-or-none style inhibition of filament sliding. Initial experiments suggest a mechanism by which tropomyosin and Myo19 compete for actin binding. These biochemical findings point toward a tropomyosin-based regulation system that ensures the crucial functions of Myo19 are tightly coordinated to maintain both organelle and cellular health.
40.	**Sites of translation initiation relocalize following hypertrophic stimulation in cardiomyocytes** **Keita Uchida**, Emily Scarborough, Ben Prosser Cardiomyocytes are terminally-differentiated, binucleated cells that must balance the energetic demands of proteostasis with constant contraction. Myocytes display the lowest rates of protein synthesis of any cell type, but can rapidly activate translation to undergo hypertrophic growth in response to stress. We recently demonstrated that 1) protein synthesis in cardiomyocytes is highly localized, 2) mRNA and ribosome localization strongly depend on microtubule-based transport, and 3) localized translation is necessary for productive hypertrophic growth of the heart. Yet how translational machinery is locally regulated in cardiomyocytes remains unclear. Translation initiation is canonically controlled by the mTORC1 kinase pathway through phosphorylation of the translation initiation repressor 4EBP1. Hyperphosphorylated 4EBP1 releases eIF4E to recruit initiation factors eIF4G and eIF4A to form the eIF4F complex to recruit ribosomes to mRNA. To examine where translation is initiated, cardiomyocytes were labeled for eIF4A and eIF4G. Formation of the eIF4F complex was measured by colocalization of the two antibodies. Under basal conditions, eIF4A and eIF4G form puncta localized near Z-disks of the sarcomere. mTOR inhibition with torin1 strongly suppressed eIF4F formation while pharmacological tethering of eIF4A with rocaglamide A led to a strong increase in the abundance of eIF4F puncta. Surprisingly, hypertrophic stimulation of cardiomyocytes restricted the formation of eIF4F to a narrow strip between the two cardiomyocyte nuclei. To determine the the signaling pathway regulating localized translation initiation, translation was measured in single cardiomyocytes and co-labeled with phospho-specific antibodies. At baseline, protein synthesis was heterogeneous across cardiomyocytes driven primarily through differential mTORC1 phosphorylation of 4EBP1. Hypertrophic stimulation robustly increased 4EBP1 phosphorylation at Ser64, which was unexpectedly MEK-ERK dependent and mTORC1 independent, to augment translation. Experiments are ongoing to determine whether the novel ERK signaling pathway underlies the relocalization of translation initiation sites during hypertrophic stimulation. The data demonstrate that, concurrent with the canonical mTORC1-dependent pathway, translation initiation in cardiomyocytes also relies on non-canonical, ERK-dependent phosphorylation of 4EBP1 that may be spatially regulated during myocyte growth.

41.

Pseudo-acetylation of Lysine 326 and 328 on cardiac actin impairs relaxation and enhances force production in murine cardiomyocytes

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Thin filament-mediated contractile regulation involves a pivoting of tropomyosin (Tpm) along F-actin from the 'Blocked' (B)- to the 'Closed' (C)-state as Ca^{2+} binds troponin C, followed by its displacement to the Open (M)-state during myosin binding to actin. In the B-state Tpm sterically blocks actomyosin associations, a fundamental requirement for proper relaxation. F-actin-Tpm interactions are largely electrostatic in nature and involve a cluster of highly-conserved, positively-charged residues on actin, including lysines 326 and 328. Proteomic analyses have revealed that these residues can be acetylated *in vivo*, in mammalian hearts. Since lysine acetylation neutralizes its positive charge, this post translational modification may play an important role in modulating F-actin-Tpm interactions and thus, thin filament-mediated contractile regulation. We therefore tested this hypothesis in both mouse and *in silico* models. A myocardial-targeting adeno-associated virus (AAV) vector was used to deliver a Myc-tagged *mActc1* wildtype (WT) gene or a mutant construct that encoded Myc-tagged *mActc1* with K326 and K328 replaced by polar, but uncharged glutamines. Therefore, our animals expressed either WT (*Myc-mActc1*^{WT}) or 'acetyl-mimetic' (*Myc-mActc1*^{K326,328Q}) actin exclusively in the heart. Epifluorescence imaging revealed efficient cardiomyocyte (CM) transfection throughout all chambers. Immunohistochemistry of isolated CMs with anti-Myc antibodies showed that the virally-expressed WT and acetyl-mimetic actins discretely incorporated into the CM myofibrillar apparatus, and co-polymerized with endogenous actin. CMs were isolated, subjected to electrical pacing, and Ca^{2+} and contractility dynamics examined. Ca^{2+} transients from individual *AAV-Myc-mActc1*^{WT} (control) vs. *AAV-Myc-mActc1*^{K326,328Q} (test) CMs showed no significant differences. However, myocytes from test hearts showed significantly slower relaxation kinetics when compared to controls. We are currently using molecular dynamics simulations to examine the movement of Tpm back to its B-state configuration following Ca^{2+} removal, to determine if re-establishing the thin filament B-state is compromised by K326/328 acetylation, and thus retarded, *in silico*. Additionally, single myocyte, steady-state force measurements showed that test CMs generated significantly higher maximum tension, with no change in Ca^{2+} sensitivity vs. controls. Thus, acetylation of K326 and K328 of cardiac actin could destabilize the inhibitory B-state of Tpm along thin filaments, impairing force inhibition, and leading to a hypercontractile CM phenotype.

42.

Relating tenocyte chromatin states to native tendon physiology using Expansion Microscopy

Marcus Woodworth, Tristan McDonnell, and Melike Lakadamyali

Tendinopathy is a widely occurring and costly clinical problem, with few strategies for tendon repair and limited knowledge on what mechanisms lead to proper tissue regeneration. One highly under-studied aspect of tendinopathies is how chromatin, the native form of genomic DNA, is organized and epigenetically marked to allow for proper gene regulation within tenocytes, the resident tendon cells that secrete and build up the extracellular matrix (ECM). This is because there are no techniques available to visualize tenocyte chromatin architecture in intact tissues within the context of the native ECM. As a result, tenocyte identity remains poorly-defined, often established only in extracted cells lacking context of the native tendon environment, or by RNA profiling that obscures potential epigenetic states that bias tenocyte response to biomechanical cues. Therefore, it is imperative to develop new tools capable of revealing how ECM relates to tenocyte chromatin organization within healthy and diseased tissue. To

	address this need, I have applied the novel method Fluorescent Labeling of Abundant Reactive Entities (FLARE) along with Expansion Microscopy (ExM) to visualize immunolabeled chromatin features within tendon ECM. FLARE provides details of both ECM matrix uniformity and composition, and is compatible with histone mark staining, establishing a relationship between ECM matrix heterogeneity and chromatin state distributions. To compliment this technique, I will adapt the method Single Cell Evaluation of Post-TRanslational Epigenetic Encoding (SCEPTRE), which I previously developed, to determine the density of active and repressed histone marks across disease related genes. Application of these novel methods will uncover the epigenetic changes that happen at the global and gene specific level in healthy and diseased tenocytes within tissue, allowing for a broader understanding of tendinopathy progression, while creating the possibility for future therapeutics that target epigenetic states for better patient outcomes.
43.	**Myosin-I Synergizes with Arp2/3 Complex to Enhance Pushing Forces of Branched Actin Networks** **Mengqi Xu**[1, 3]†, David M. Rutkowski[2]†, Grzegorz Rebowski[1], Malgorzata Boczkowska[1], Luther W. Pollard[1]*, Roberto Dominguez[1]*, Dimitrios Vavylonis[2]*, E. Michael Ostap[1, 3]* *Author Contributions: † These authors contributed equally.* Branched actin polymerization mediated by Arp2/3 complex provides pushing forces for a variety of cellular processes (e.g. membrane protrusion and invagination). Myosin-Is, which frequently colocalize alongside such branched actin networks, have also been shown to participate in these processes and contribute to force generation. Yet, the molecular mechanism of how myosin-Is interact with the actin assembly to modulate the force generation remain largely unknown. Using a highly tunable 'actin comet tail' bead motility system, we mimicked the interplay between myosin-I, actin and Arp2/3 complex at the membrane interface on micro-sized bead, where branched actin polymerization mediated by Arp2/3 complex was initiated by nucleation promoting factor coated on bead surface and directly interact with the surface-bound myosin-I. We found that myosin-I enhances force generation by regulating the dynamics and architecture of branched actin network through its force-generating power strokes, promoting a more efficient network elongation, and triggering network fracture. Computational modeling recapitulated these observations suggesting myosin-I exerts a repulsive force that pushes actin filaments away from the surface reshaping the network architecture and boosting its force-generating capacity. These findings reveal a synergy between myosin-I motor activity and actin polymerization in driving morphological changes at the cell membrane.
44.	**INF2 regulates IP3-mediated ER calcium release** **Maite R Zavala**, Amrapali Ghosh and Rajarshi Chakrabarti[#] [#]*MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA* Timely and regulated actin filament turnover regulates various cellular functions including inter-organelle interaction and homeostasis. INF2 (Inverted formin 2) is a member of the formin family of actin assembly factors that promote both actin nucleation and filament elongation. In cells, INF2 exists in two distinct isomeric forms, INF2-caax and INF2-noncaax. INF2-caax localizes to Endoplasmic Reticulum (ER) while INF2-noncaax exists in the cytosol. Gain-of-function dominant mutation of INF2 has been associated with FSGS and CMTD. Previous reports have shown that INF2-caax generated actin filaments on the ER acutely induces ER-mitochondrional contacts (ERMC) and facilitates ER to mitochondrial calcium transfer that promotes mitochondrial division (PMID: 29142021). While most of the cellular roles of INF2 have been attributed to mitochondrial dynamics including its ability to promote ER to mitochondrial calcium transfer, its role on ER physiology and dynamics have remain elusive. In

	the present study we provide evidence that INF2 regulates agonist-induced ER calcium release through IP3-receptors. Acute knockdown of total INF2 in HeLa cells results in reduced histamine-induced ER calcium release measured both through cytosolic and ER calcium probes. INF2 depleted HeLa cells also show an increase in cytosolic calcium oscillations when using sub-maximal dose of histamine. Interestingly SERCA inhibition showed comparable ER calcium in both WT and INF2-KO cells. Rescue experiments carried out on INF2-KO cells showed that both the ER- bound and cytosolic INF2 isoforms could significantly rescue histamine-induced ER calcium release suggesting ER-residency was not required for this function. Further we examined whether INF2 activity affects all the IP3R isoforms equally. Acute knock-down of INF2 in HEK- TKO cells expressing either of the IP3R receptors showed that INF2 affects the IP3R2 and IP3R3 functions more significantly than IP3R1. Further we show that carbachol stimulation in INF2 depleted (KD and KO) HEK-IP3R2 (R1/R3 DKO) and IP3R3 (R/R2 DKO) cells result in significantly reduced ER calcium release compared to the respective control cells. These results indicate that INF2 play a significant role in shaping IP3R-mediated ER calcium dynamics. Future experiments will unravel the specific mechanisms through which INF2 regulate agonist-mediated ER calcium release.