Talk Abstracts:

Morning Session I:

Multiplexed DNA-PAINT imaging of the heterogeneity of late endosome/lysosome protein composition

Charles Bond, Siewert Hugelier, Jiazheng Xing, Elena M. Sorokina, and Melike Lakadamyali

Late endosomes/lysosomes (LELs) are crucial for numerous physiological processes and their dysfunction is linked to many diseases. Proteomic analyses have identified hundreds of LEL proteins, however, whether these proteins are uniformly present on each LEL, or if there are cell-type dependent LEL sub-populations with unique protein compositions is unclear. We employed a quantitative, multiplexed DNA-PAINT super-resolution approach to examine the distribution of seven key LEL proteins (LAMP1, LAMP2, CD63, Cathepsin D, TMEM192, NPC1 and LAMTOR4) on individual LELs. While LAMP1, LAMP2 and Cathepsin D were abundant across LELs, marking a common population, other analyzed proteins were associated with specific LEL subpopulations. Our multiplexed imaging approach identified up to eight different LEL subpopulations based on their unique membrane protein composition. Additionally, our analysis of the spatial relationships between these subpopulations and mitochondria revealed a
cell-type specific tendency for NPC1-positive LELs to be closely positioned to mitochondria. Our approach will be broadly applicable to determining organelle heterogeneity with single organelle resolution in many biological contexts.

Microtubules couple mRNA export, transport and local translation to direct growth in the cardiomyocyte

Emily A. Scarborough, Rani M. Randell, Keita Uchida, and Benjamin L. Prosser

In response to physiological or pathological stressors, the heart remodels, which is dependent on growth of individual post-mitotic cardiomyocytes (CMs). This occurs by the addition of contractile units (sarcomeres) either along the length of the cell (eccentric growth) or width of the cell (concentric growth). How the CM spatially couples transcription, translation, and sarcomere addition to enable growth in a specific axis is unknown. Our lab's previous work has indicated the necessity of microtubules (MTs) for proper localization of mRNA, translation, and general growth of the CM, but it is unknown if MTs play a role in directing growth. To test this, we first used drugs or genetic tools to alter MT stability in neonatal rat CMs. Strikingly, we observed that MT destabilizers result in eccentric remodeling whereas MT stabilizers result in concentric remodeling, leading us to hypothesize that MT stability dictates mRNA localization and local translation to promote site-specific growth in the CM. We visualize sarcomeric mRNA (Actc1) and translation under these conditions and find that both are enriched in the direction of growth. In adult rat CMs, we show dynamic MTs, components of the LINC complex, nucleoporins, MT motors and mRNA export are concentrated at the poles of the CM nucleus, aligned along the length of the cell. After modest MT stabilization, dynamic MTs 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 MT motors, nuclear wrinkling, and biased mRNA export is dependent on an intact LINC complex. Consistently, we observe that sarcomeric mRNA and rRNA are redistributed to the nuclear short axis in both an acute and chronic mouse model of concentric hypertrophy.
In sum, we propose that microtubule stability is a molecular toggle to promote concentric or eccentric growth of heart muscle. In adult CMs, mRNA export and transport machinery are inherently polarized along the length of the cell; modest MT stabilization, as occurs early in concentric remodeling, results in preserved dynamic MTs at the nuclear short axis, revealing a role for dynamic MTs at the nucleus to direct and couple mRNA export, transport and growth. We are presently exploring how MTs may also regulate cell-cell junctions to control CM growth.

Compression Stiffening in Adipose Tissues

Xuechen Shi, Carmen Flesher, David Merrick, and Paul Janmey

Fat tissues in the human body are constantly subjected to mechanical forces, whether while sitting, lying on the back, or lying on the front, especially in individuals with obesity, where gravity significantly contributes to these forces. From a physiological perspective, these forces, primarily compressive, can impact the cells within adipose tissues directly by deforming them and/or indirectly by altering the tissue characteristics, particularly its mechanical properties. This study employs rheology to investigate how mechanical compressions affect the mechanics of adipose tissues, including subcutaneous and visceral types, collected from male mice on normal or high-fat diets. We first observed the relaxation of normal stress under compression, indicating that adipose tissues undergo significant structural rearrangements when subjected to mechanical forces, potentially affecting their function and interaction with surrounding tissues. Notably, we also observed a substantial 10-fold increase in the shear modulus of the tissues under compression that is comparable to the forces experienced when individuals sit. Additionally, this compression-induced stiffening varies with the normal stress throughout the entire course of compression. It also depends on the type of adipose tissue, and tissues from mice on high-fat diets display greater stiffening, suggesting that dietary factors influence the mechanical responses of adipose tissues. The degree of compression-induced stiffening was further correlated with mouse body weight, adipose tissue weight, and liver weight. In summary, this study demonstrates that mechanical compression induces significant alterations in the mechanics of adipose tissue. Given that individuals with obesity experience elevated levels of
compression on their adipose tissues, due to both increased body weight and greater adipose tissue gravity, it is likely that their tissues exhibit higher stiffness. Our ongoing research aims to elucidate the underlying mechanisms of these modifications and their impact on metabolic disorders associated with adipose tissue. By understanding these processes, we hope to provide insights into the physiological and pathological roles of compressive forces, uncover new therapeutic targets for obesity-related complications, and ultimately contribute to better health outcomes.

Morning Session II:

Mechanisms of actin filament severing and elongation by formins

Nicholas Palmer, Kyle Barrie, and Roberto Dominguez

Humans express fifteen formins, playing crucial roles in actin-based processes, such as cytokinesis, cell motility, and mechanotransduction 1,2 . However, the lack of structures bound to the actin filament (F-actin) has been a major impediment to understanding formin function. While formins are known for their ability to nucleate and elongate F- actin 3-7 , some formins can additionally depolymerize, sever, or bundle F-actin. Two mammalian formins, inverted formin-2 (INF2) and diaphanous-1 (Dia1), exemplify this diversity. INF2 displays potent severing activity but elongates weakly 8-11 , whereas Dia1 has potent elongation activity but does not sever 4,8 . Using cryo-electron microscopy (cryo-EM), we reveal five structural states of INF2 and two of Dia1 bound to the middle and barbed end of F-actin. INF2 and Dia1 bind differently to these sites, consistent with their distinct activities. The FH2 and WH2 domains of INF2 are positioned to sever F-actin, whereas Dia1 appears unsuited for severing. Structures also show how profilin-actin is delivered to the fast-growing barbed end, and how this is followed by a transition of the incoming monomer into the F-actin conformation and the release of profilin. Combined, the seven structures presented here provide step-by-step visualization of the mechanisms of F-actin severing and elongation by formins.

Role of ciliopathy-associated protein TMEM138 in skin pigmentation

Brice Magne, Chloe E. Snider, Yuanging Feng, Sarah A. Tishkoff, and Michael S. Marks

The primary cilium is an essential organelle for cell polarity and signaling in most mammalian cells. Defects in its assembly or function underlie the pathogenesis of developmental disorders known as ciliopathies. Expression of ciliopathy-associated protein TMEM138 is regulated by non-pathogenic alleles that are found in African populations with diverse skin phototypes, suggesting a role in skin pigmentation. TMEM138 supports primary cilia biogenesis in eye and kidney cells, but whether it regulates pigmentation via signaling from the primary cilium, melanogenic cargo trafficking, and/ or pigment dissemination across the epidermis is unknown. To test for a role in melanogenesis (i.e. melanin synthesis), we assessed the impact of altered TMEM138 expression in pigment cells. Thus, we generated immortalized C57BL/6-derived mouse melanocytes stably overexpressing epitope-tagged human TMEM138, or depleted of endogenous mouse TMEM138 by shRNA expression. Repeated experiments (n=5 to 10) showed that knockdown of endogenous TMEM138 caused a marked loss of pigmentation, which was restored by re-expression of epitope-tagged human TMEM138. Expression of melanogenesis genes including TYRP1, PMEL, OCA2 and MITF was reduced (n=3) and residual TYRP1 was mislocalized to lysosomes in TMEM138-depleted cells (n=3), suggesting that TMEM138 may function in both signaling and trafficking during melanogenesis. Ongoing experiments are testing the underlying mechanism by which TMEM138 impacts both processes in melanocytes, whether the other ciliopathy gene products play a similar role, and whether TMEM138 functions in melanin transfer to keratinocytes.

Mitochondrial damage in astrocytes triggers PINK1/Parkin mitophagy and NF-κB-mediated inflammation

Julia F. Riley, Charity V. Robbins, and Erika L.F. Holzbaur

Astrocytes are the most abundant cells in the brain, and their ability to promote neuroinflammation is a key aspect of Parkinson’s disease (PD) pathology. Accumulating data suggest a link between neuroinflammation and mitochondrial dysfunction, another hallmark of PD. Mutations in the enzymes PTEN-Induced Kinase 1 (PINK1) and Parkin, which facilitate clearance of damaged mitochondria via mitophagy, are sufficient to cause

PD. However, the clearance mechanism for damaged mitochondria in astrocytes and the inflammatory signaling that is initiated by mitochondrial damage require further investigation. Here, we used OXPHOS inhibitors Antimycin A and Oligomycin A to induce mitochondrial damage in murine cortical astrocytes. Damaged mitochondria accumulate phospho-ubiquitin and Parkin, targeting them for clearance via PINK1/Parkin mitophagy. Parkin recruitment to mitochondria and subsequent mitochondrial degradation are both PINK1-dependent. We then sought to identify autophagy receptors that participate in this process. We observed upregulation of p62 expression, and
colocalization of p62 with phospho-ubiquitin on damaged mitochondria, suggesting involvement of this receptor in astrocytic mitophagy. Next, we examined a link between mitophagy initiation in astrocytes and inflammatory signaling. Previous work from our group has shown that mitochondria targeted for mitophagy recruit the NF-κB effector molecule (NEMO), promoting activation of the NF-κB pathway for innate immunity. Using the same damage paradigm that induces mitophagy, we observed NEMO recruitment to damaged mitochondria in astrocytes. Using qPCR, we determined that levels of NF-κB-associated cytokines TNF-α and IL6 are elevated following mitochondrial damage; this response does not occur in PINK1-/- astrocytes. Further, inhibition of NF-κB ameliorates mitochondrial damage-induced upregulation of TNF-α expression. Ongoing experiments using RNA-sequencing will more fully define the inflammatory pathways induced by mitochondrial damage in astrocytes, which may be neuro-protective, neuroinflammatory, or both. These results provide new insights into cell non-autonomous mechanisms linking mitochondrial stress to neuroinflammation and neurodegeneration.

Cardiac myosin with Hypertrophic Cardiomyopathy mutation M493I alters motor kinetics and SRX equilibrium but preserves the working stroke

Robert C. Cail, Faviolla A. Baez-Cruz, Bipasha Barua, Donald A. Winkelmann, Yale E. Goldman, and E. Michael Ostap

Hypertrophic cardiomyopathy (HCM) is a common genetic disorder and a leading cause of sudden cardiac death. HCM-causing mutations are most commonly found in the gene MYH7, which encodes the principal ventricular myosin paralog, β-cardiac myosin. HCM is thought to be a gain-of-function disease; however, mutations in MYH7 can lead to both gain-of-function and loss-of-function phenotypes for individual myosins. How these opposite effects lead to the same disease state is not clear. Here, we have used purified myosin constructs to characterize the mechanical and kinetic effects of one severe and highly penetrant MYH7 mutation, M493I. M493I slows actin gliding velocity by 70% relative to WT, from 1.45 μm/s to 0.40 μm/s. Transient kinetic analysis reveals that M493I slows ADP release 5-fold (13 s^{-1} vs 70 s^{-1}) while preserving ATP binding (5.5 μM $^{-1}$*s^{-1}), causing the slowed gliding velocity. Optical trap experiments reveal that M493I preserves the 5-nanometer, two-substep working stroke of myosin, but with a significantly lengthened attachment duration. The per-head steady-state ATPase V max is doubled by M493I relative to WT from 1.2 s -1 to 2.35 s -1 , despite longer actin attachment and slowed ADP release; we report a novel method to estimate the equilibrium between closed (super-relaxed, SRX) and open (disordered relaxed, DRX) myosin conformations, and we find that M493I doubles the proportion of DRX heads. Optical trap assays reveal that M493I interacts with actin filaments twice as frequently as WT, indicating that the increased DRX conformation leads to more active myosin heads. Together, these data explain the hyper-contractility and septal restriction found in M493I patients, and help to resolve a debate about myosin's equilibrium state between SRX and DRX conformations.

Afternoon Session:

NAD + metabolism in endothelial cells prevents ROS accumulation specifically during the transition from proliferation to quiescence and is essential for Angiogenesis

Wencao Zhao, Ioana Soaita, Boa Kim, Wenkai Zhu, Jae Woo Jung, Michael Noji, Huajun Bai, Yansen Xiao, Yifan Yang, Jessie Axsom, Kristina Li, Ivan Kuznetsov, Yijun Yang, Jonathan Edwards, Jian Li, Joseph Baur, and Zoltan Arany

Angiogenesis, a process that requires the coordination of endothelial cell (EC) activities such as
cell division, migration, and proper quiescence establishment, is impaired with age. Nicotinamide adenine dinucleotide (NAD +), a redox cofactor, has a critical role in energy metabolism and longevity. NAD + levels decrease with age in vascular endothelium. However, it is not known that if the drop of NAD + level has an impact on EC behaviors and vascular growth. Here we report that endothelial NAD + is essential to angiogenesis specifically at the critical EC transition from proliferation to quiescence (but not during proliferation or once quiescence is established). Deprivation of NAD + in ECs by inactivating NAMPT, the rate-limiting enzyme of NAD + salvage pathway, does not affect cell proliferation and migration but completely blocks the transition to a fully formed quiescent monolayer. Supplementation of NAD + precursors nicotinamide riboside or nicotinamide mononucleotide (NMN) which bypasses NAMPT enzymatic activity normalizes the failed quiescence transition of ECs with NAD + deprivation. NAD + -deprived ECs display defective angiogenic activity in a 3D sprouting model in vitro or ex vivo, which are rescued by NMN treatment. Mice with endothelial Nampt deletion show defective retinal angiogenesis and wound healing in vivo. Mechanistically, we find that EC transition to quiescence triggers a reactive oxygen species (ROS) burst, the suppression of which requires a supply of NAD + . ROS is necessary and sufficient for this effect: exogenous ROS blocks EC transition to quiescence, while inhibition of ROS restores EC
transition to quiescence even when NAD + is deprived. We further find critical roles for glucose metabolism and NADPH in the generation and control of ROS production, respectively. In summary, we identify a unique and indispensable role of NAD production during the EC transition from proliferation to quiescence, a time when transitioning angiogenic ECs form newly
lumenized vessels that are newly exposed to blood and ROS-inducing high oxygen tension.

A Novel Cytoskeleton-based Pathway Required for Maintenance of Mitochondria Dynamics and Energetics in Skeletal Muscle

Kayleigh M. Voos, Joyce Tzeng, Priya Patel, Trevor Pharr, Grace Choi, Sophie Rubinsky, and Damaris N. Lorenzo

Mitochondria undergoes morphological remodeling via fusion and fission events in response to changes in the cellular environment and energy needs to maintain bioenergetic homeostasis. In skeletal muscle (SKM), mitochondria dynamics facilitate its remarkable adaptability to increases in exercise load and strength, and other physiological stressors, such as aging, muscle weakness, and metabolic disorders. These physiological changes impose energetic and oxidative stresses that can damage the mitochondrial network and impair their energetic capacity. The cytoskeleton is a key modulator of mitochondria dynamics, but our knowledge of the cytoskeletal factors and mechanisms mediating these processes is largely incomplete. Here, we report a novel pathway that depends on the cytoskeleton scaffolding protein ankryin-B (AnkB), which promotes mitochondrial dynamics in SKM under energetic stress.
Variants in AnkB, encoded by ANK2, increase risk for cardio-metabolic diseases in humans and cause age- or diet-dependent metabolic syndrome in mice. However, the metabolic roles of AnkB in SKM have not been characterized. We found that young conditional knockout mice selectively lacking AnkB in SKM (SKM-AnkB-KO) exhibit reduced endurance exercise capacity without alterations in muscle strength, body weight, or systemic glucose regulation. Structurally, AnkB-deficient muscle fibers display loss of transverse microtubules and enlarged and hyper-connected mitochondria. Supporting a previously unappreciated role of AnkB in bioenergetic regulation, muscle fibers from SKM-AnkB-KO mice are unable to maintain aerobic

respiration under exhaustion conditions. Super-resolution imaging revealed mitochondria in AnkB-KO muscle fibers are uncoupled from the endoplasmic reticulum. Notably, we found that AnkB forms a complex with key modulators of mitochondria dynamics, and AnkB deficiency results in downregulation of these factors. Additionally, live, and super-resolution imaging reveals that AnkB-deficient muscle cells fail to properly remodel their microtubule and mitochondrial networks under cellular stress, which results in reduced fission and removal of damaged mitochondria. Thus, we propose that AnkB is required to maintain bioenergetic homeostasis under energetic stress by promoting efficient mitochondrial fission.

Active transport of tRNAs facilitates distributed protein synthesis in terminally differentiated cells.

Jennifer M. Petrosino, Vasiliki Courelli, Keita Uchida, Barry Cooperman, and Benjamin L. Prosser

In response to stress, cells have evolved structural and functional adaptations that can be physiological or pathological in nature. These adaptations can be broken down into hypertrophy (increased cell size), atrophy (decreased cell size), hyperplasia (increased cell number), or metaplasia (conversion in cell type). Terminally differentiated cells, such as cardiac myocytes, skeletal myofibers, and neurons, are limited to adaptations that promote changes in size. Thus, these cells coordinate transcription, translation, and long-range transport dynamics to facilitate proper protein localization and subsequent growth. 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. However, using super-resolution microscopy, we demonstrate that tRNA localization in terminally differentiated cells relies on the microtubule network and occurs via active transport. Through live and fixed cell imaging, we show that tRNAs are distributed along microtubule tracks and undergo long-range transport by Kinesin motors.Perturbations to the microtubule network or adenoviral delivery of a dominant-negative Kinesin construct caused tRNA perinuclear accumulation, preventing their distribution in cardiomyocytes, skeletal muscle cells, and primary neurons. We next asked if tRNAs could co-opt established mechanisms of microtubule-mediated RNA trafficking. Additional investigation revealed that actively transporting tRNAs hitchhike along components of the endo-lysosomal system, and this process was halted by the knockdown of key adaptors linking lysosomes to kinesin motors. Further mechanistic studies revealed that tRNA-lysosomal hitchhiking also depends on interactions between tRNAs and the synthetases that charge them; specifically, Leucyl-tRNA synthetase (LARS). Knockdown of LARS led to perinuclear tRNA accumulation, impaired long-range tRNA transport, and overall reductions in protein synthesis. Prevention of LARS-lysosomal docking with a small molecule inhibitor also recapitulated this phenotype. Together, our findings reveal, for the first time, that tRNAs in terminally differentiated cells are distributed by hitchhiking on lysosomes undergoing active transport along the microtubule network.

Keynote:

Cell Physiology Through the Lens of Membrane Lipid Homeostasis

Christopher Burd, Ph.D.
Professor, Interim Chair: Department of Cell Biology
Yale University School of Medicine

Organelle membranes compartmentalize biochemical reactions that are metabolically linked or that compete with biochemical reactions in the cytosol. Hence, cell physiology depends critically on lipid and protein homeostasis pathways that modulate metabolism and inter-organelle sorting of macromolecules to sustain organelle biogenesis and maintenance. Whereas the pathways that sort and traffic integral membrane proteins from their site of synthesis to different organelles are well defined, far less is known about the pathways and mechanisms of membrane lipid homeostasis. Recent advances in lipid analysis and *in situ* imaging have provided new opportunities for investigating lipid metabolism and trafficking in cells. We discovered a metabolic pathway that regulates the production of sphingomyelin, an abundant 'structural' lipid of the plasma membrane, in response to changes in cellular cholesterol content. Upon cholesterol depletion, the rate of sphingomyelin synthesis increased by a surprising mechanism: the rate of ceramide (the precursor to sphingomyelin) trafficking to the Golgi apparatus, where sphingomyelin is produced, is increased. There is no net increase in sphingolipid content. The newly synthesized sphingomyelin is then rapidly trafficked to the plasma membrane where it is vital for maintaining the physical properties (viscosity) and physiological functions of the plasma membrane. Our study identifies a new mode of lipid metabolic regulation involving inter-organelle trafficking of sphingolipids.

Poster Abstracts:

Morning Session:

M1	Adriana N. Santiago-Ruiz	Super-Resolution Imaging Uncovers Nanoscale Tau Aggregate Hyperphosphorylation Patterns in Human Alzheimer's Disease Brain Tissue

Super-Resolution Imaging Uncovers Nanoscale Tau Aggregate Hyperphosphorylation Patterns in Human Alzheimer's Disease Brain Tissue

Adriana N. Santiago-Ruiz, Siewert Hugelier, Charles R. Bond, Edward B. Lee, and Melike Lakadamyali

Tau aggregation plays a critical role in Alzheimer's Disease (AD), where tau neurofibrillary tangles (NFTs) are a key pathological hallmark. While much attention has been given to NFTs, emerging evidence underscores nano-sized pre-NFT tau aggregates as potentially toxic entities in AD. By leveraging DNA-PAINT super-resolution microscopy, we visualized and quantified nanoscale tau aggregates (nano-aggregates) in human postmortem brain tissues from intermediate and advanced AD, and Primary Age-Related Tauopathy (PART). Nano-aggregates were predominant across cases, with AD exhibiting a higher burden compared to PART. Hyperphosphorylated tau residues (p-T231, p-T181, and p-S202/T205) were present within nano-aggregates across all AD Braak stages and PART. Moreover, nano-aggregates displayed morphological differences between PART and AD, and exhibited distinct hyperphosphorylation patterns in advanced AD. These findings suggest that changes in nano-aggregate morphology and hyperphosphorylation patterns may exacerbate tau aggregation and AD progression. The ability to detect and profile nanoscale tau aggregates in human brain tissue opens new avenues for studying the molecular underpinnings of tauopathies.

M2	Cameron Thompson	Tropomyosin 1.7 and 3.1 inhibit Myosin-19 motile activity in vitro

Tropomyosin 1.7 and 3.1 inhibit Myosin-19 motile activity in vitro

Cameron P. Thompson, Luther W. Pollard, Erika L. F. Holzbaur, and 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 superfamily. 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.

M3	Elana Baltrusaitis	Structure and regulation of the MIRO-TRAK complex

Structure and regulation of the MIRO-TRAK complex

Elana E. Baltrusaitis, Erika E. Ravitch, Tania A. Perez, Kyle R. Barrie, Erika L.F. Holzbaur, and Roberto Dominguez

MIRO (mitochondrial Rho GTPase) is a master scaffolding protein involved in mitochondrial dynamics and motility. MIRO recruits the activating adaptor TRAK (trafficking kinesin-binding protein) to mitochondria, and

TRAK coordinates the microtubule motors dynein-dynactin and kinesin-1 for mitochondrial motility. MIRO consists of two GTPase domains flanking two EF-hand ligand mimic (ELM) domains, which each contain one Ca^{2+} binding EF-hand. The presence of these domains suggests that nucleotide and cation cofactors may regulate the MIRO-TRAK interaction. Previously, we characterized and mapped a cofactor-independent interaction (site #1) between a conserved region of TRAK1 ($TRAK1_{401-431}$) and MIRO1's ELMs and C-terminal GTPase. Here, we describe a 3.75Å resolution cryo-EM structure of a second conserved TRAK1 region ($TRAK1_{569-623}$) bound to cytoplasmic MIRO1 ($MIRO1_{1-591}$), constituting an additional interaction site (site #2). The structure reveals a MIRO1-TRAK1 heterotetramer, comprised of two MIRO1 molecules that are dimerized through their ELM2 and C-terminal GTPase domains and two TRAK1 site #2 peptides. Each TRAK1 site #2 peptide forms an extensive binding interface along MIRO1's N-terminal GTPase and ELM1 domains. In vitro binding assays confirmed that this interaction can occur in all cofactor conditions (Ca^{2+}/EGTA and GTP/GDP), although Ca^{2+} and GTP cofactors are preferred. Structure-informed mutagenesis confirmed that binding of site #2 requires TRAK1's interaction with both the N-terminal GTPase and ELM1 domains. In a TRAK1 construct containing both site #1 and site #2, site #1 compensates for loss of TRAK1's interaction with MIRO1's ELM1 but not loss of TRAK1's interaction with MIRO1's N-terminal GTPase. In cells, expression of a TRAK1 construct containing both site #1 and site #2 showed significant MIRO1-dependent localization to mitochondria. Similarly, disruption of TRAK1 binding to MIRO1's N-terminal GTPase significantly decreased this localization while disruption of TRAK1 binding to MIRO1's ELM1 did not. Taken together, these data suggest that (1) MIRO1 binds two independent and conserved regions of TRAK1, (2) neither site #1 nor site #2 is regulated by Ca^{2+} or nucleotide cofactors, (3) MIRO1 can exist as a dimer on the outer mitochondrial membrane and can simultaneously bind two or more TRAK1 molecules.

M4	Katey Stone	Investigating protective functions of stress granules in the heart

Investigating protective functions of stress granules in the heart

Katey Stone, Emily A. Scarborough, and Benjamin L. Prosser

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 frequently follows 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.

M5	Libby Nunn	Impact of ActRIIA/B blockade on systemic metabolism and body composition during weight loss with a GLP-1/GIP-RA and weight regain

Impact of ActRIIA/B blockade on systemic metabolism and body composition during weight loss with a GLP-1/GIP-RA and weight regain

Elizabeth Nunn, Matthew Gavin, Joseph Brozinick, Joe Baur, and Paul Titchenell

Obesity is a chronic, multifactorial disease predicted to affect over 25% of the global population by 2035. 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 weightloss. GLP-1 receptor agonists (GLP-1RAs) and dual GLP-1/GIP receptor agonists (GLP-1/GIPRAs) induce up to 15-20% 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 GLP-1/GIPRA-driven weight loss. Skeletal muscle contributes to resting energy expenditure, and maintenance of muscle mass during weight loss could slow weight regain after cessation of GLP-1/GIPRA treatment. TGFβ-like ligands such as myostatin and activin A are negative regulators of skeletal muscle mass which signal via the activin type IIA and IIB receptors (ActRIIA/B) to stimulate atrophy. Our recently published data show that obese mice treated systemically with an antibody to block ActRIIA/B are protected from loss of muscle mass during weight loss and lose additional fat mass compared to mice treated with a GLP-1RA alone. We hypothesize that treatment with an anti-ActRIIA/B antibody alone to stimulate muscle hypertrophy will elevate energy expenditure, and that ActRIIA/B blockade during weight loss will reduce weight regain after GLP-1/GIPRA treatment is discontinued.
We placed a cohort of diet induced obese (DIO) mice inside metabolic cages during 2 weeks of treatment with a GLP-1/GIPRA (tirzepatide), an anti-ActRIIA/B antibody (CDD866), or a combination, monitoring body weight, body composition (lean vs. fat mass), and energy expenditure (EE) throughout. We find that treatment with CDD866 alone and combined with tirzepatide treatment elevates systemic EE. A separate cohort of DIO mice were treated with tirzepatide with or without CDD866 for 2 weeks before treatment was discontinued and animals were allowed to regain weight for 3 weeks. We find that combined treatment leads to reduced fat regain compared to mice treated with a GLP-1/GIPRA alone. These results suggest that targeting ActRIIA/B during weight loss with anti-obesity drugs can alter systemic metabolism and improve body composition during weight regain if treatment with a weight loss drug is discontinued.

M6	Tania Perez	Investigating how DISC1 regulates mitochondrial trafficking

Investigating how DISC1 regulates mitochondrial trafficking

Tania A. Perez, Gabrielle Glass, and 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. Defects in mitochondrial transport are linked to neurodegenerative and developmental disorders. 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 have also shown 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 mitochondria in both neurons and HeLa cells and can recruit molecular motors to the mitochondrial membrane. These initial findings will be used to further dissect the molecular mechanisms by which DISC1 regulates mitochondrial trafficking.

M7	Alex Simon	MS4A8B is a novel regulator of store-operated Ca^{2+} entry in airway epithelial cells

MS4A8B is a novel regulator of store-operated Ca^{2+} entry in airway epithelial cells

Alexander A. Simon, Ray Ma, Arielle Muller, and Robert J. Lee

The MS4A gene family encodes for 18 different transmembrane proteins with widespread expression throughout the body. Clinical data and genetic analysis suggest the MS4A family is involved in cell death/survival, inflammation, and various cancers. However, their molecular function is poorly resolved. MS4A8B is one family member identified as an important cell differentiation marker in airway epithelial cells, preferentially localized to the apical membrane. The apical side of the airway epithelium lines the nasal passages, sinuses, and lungs at the critical host-environment interface. It is in direct contact with inhaled irritants and is accessible to therapeutics delivered by inhalation. Our recent studies have begun to elucidate the molecular basis for which MS4A8B can regulate Ca^{2+} signaling. Using a combination of live-cell Ca^{2+} imaging, FRET microscopy, and biochemistry, we show MS4A8B potentiates store-operated Ca^{2+} entry and directly interacts with the pore-forming Ca^{2+} channel subunit Orai1. An improved mechanistic dissection of Ca^{2+} signaling regulated by MS4A8B in airway physiology represents a new dimension to define the mechanisms that may control gene expression for airway health or pathogenesis.

M8	Brianna Hill	Bitter agonists increase macrophage phagocytosis of head and neck squamous cell carcinoma cells

Bitter agonists increase macrophage phagocytosis of head and neck squamous cell carcinoma cells

Brianna L. Hill, Sarah M. Sywanycz, Zoey A. Miller, Robert J. Lee, and Ryan M. Carey

Head and neck squamous cell carcinoma (HNSCC) is the 7 th most commonly diagnosed cancer worldwide and is expected to increase in incidence by 30% by 2030. The HNSCC microenvironment is characterized by substantial macrophage infiltration. However, tumor-associated macrophages often cultivate an immunosuppressive environment that aids tumor progression. Previous work has shown that macrophages express bitter taste receptors (T2Rs), a class of GPCRs that can be activated with bitter compounds. Previous work has also demonstrated that stimulation of T2Rs in macrophages with bitter agonists, such as denatonium benzoate, increases phagocytosis of bacteria, but the impact of bitterants on macrophage phagocytosis of tumor cells is unknown. The goal of this study was to assess the impact of bitter compounds on macrophage phagocytosis of HNSCC cells in vitro. Human peripheral blood mononuclear cells obtained from healthy donors were used to generate monocyte-derived macrophages (MDMs). To investigate the effects of bitter agonist stimulation on macrophage phagocytosis, CFSE-labeled MDMs and DAPI-labeled HNSCC cell line SCC47, were co-stimulated with denatonium benzoate, and macrophage phagocytosis was quantified. This revealed that treatment with denatonium benzoate led to a 70% increase in macrophage phagocytosis compared to controls. Additional analysis revealed a 1.8-fold increase in F-actin in MDMs in response to denatonium benzoate measured via CellMask™ Actin staining, and that macrophages had generated F-actin positive tunneling nanotubes (TNTs). Further characterization with live cell imaging showed that these TNTs were being utilized as a means of mitochondrial transfer as observed by MitoTracker™ and CellMask™ Actin staining. Furthermore, there was a 2.5-fold decrease in mitochondrial ATP production in MDMs stimulated with denatonium benzoate, possibly indicating metabolic reprogramming. Timelapse imaging of MDMs demonstrated a reduction in mitochondrial ATP production, with a concomitant increase in F-actin production. These observations were confirmed in Phorbol 12-myristate 13-acetate differentiated THP-1 cells. Taken together, these results indicate that bitter taste receptor stimulation may contribute to a more activated macrophage phenotype denoted by a decrease in mitochondrial ATP production, increased F-actin staining, and increased phagocytosis of tumor cells.

M9	Devasmita Chakravarty	Actin isoform specificity in platelets and red blood cells is functionally independent of their amino acid sequence

Actin isoform specificity in platelets and red blood cells is functionally independent of their amino acid sequence

Devasmita Chakravarty, Pavan Vedula, Li Chen, Stephanie Sterling, Alina D. Peshkova, Aae Suzuki, Liang Zhao, N. Adrian Leu, Rustem I. Litvinov, Charles Abrams, and Anna Kashina

Actin is an essential component of the cytoskeleton in every eukaryotic cell. Cytoplasmic β-and γ-actin isoforms are over 99% identical to each other in their amino acid sequence, with only four conservative substitutions within their N-termini, yet they play vastly different biological roles in embryogenesis and cell migration. Certain cell types – prominently, red blood cells (RBC) and platelets – contain predominantly b-actin (an estimated 80-90%). It has always been assumed that b-actin protein, specifically, its N-terminal amino acid sequence, is uniquely required for its selective functionality in these cell types. Here we tested this hypothesis by analyzing the gene-edited mouse model, in which β-actin is completely replaced by γ-actin, so that γ-actin is expressed both from the native γ-actin gene and also from the nearly intact β-actin gene. These mice preserve all the nucleotide sequence-mediated actin properties, but have the entire b- actin protein pool replaced by g-actin. Thus, impairment of any physiological processes in these mice can be uniquely linked to the specific amino acid sequence of b- actin. Analysis of these mice revealed that, despite complete lack of b- actin protein, they had normal blood properties, including hemoglobin and blood cell counts, cell morphology, platelet aggregation, clotting, and molecular composition of the actin cytoskeletons. No physiological changes related to RBC or platelet function could be detected in these mice. In contrast to what has previously been thought, these results definitively show that the individual differences in amino acid sequence of cytoplasmic actin isoforms do not contribute to unique biologic functions in RBCs and platelets.

M10	Jaclyn Welles	Reduction in hepatic PC biosynthesis augments copper metabolism and drives MASH pathogenesis

Reduction in hepatic PC biosynthesis augments copper metabolism and drives MASH pathogenesis

Jaclyn E. Welles, Michael V. Gonzalez, James P. Garifallou, David Li, Rebecca G. Wells, Donita C. Brady, and Paul M. Titchenell

PCYT1A encodes for CTP: phosphocholine cytidyltransferase, alpha (CCT-α), rate-limiting enzyme required for ~70% of Phosphatidylcholine (PC) production via the Kennedy pathway. The reduction of dietary PC intake has been observed in several human and animal studies, to contribute to the onset of metabolic dysfunction-associated nonalcoholic fatty liver disease (MAFLD). Hepatic PC production is also significantly decreased in patients with metabolic dysfunction-associated steatohepatitis (MASH), an advanced disorder which affects a subset of patients with MAFLD and T2D. To date however, It remains unknown how the reduction of hepatic PC biosynthesis, promotes the fibrosis and inflammation associated with MASH. Thus, in this study, we used a diet-focused model, the low methionine, choline deficient, 42% kcal/fat high-fat diet (LMCD-HFD), and a genetic model lacking PCYT1A expression in the liver, to address our hypothesis which was that the absence of PC directly contributes to the onset of inflammation and fibrosis observed in MASH.

Given the complexity of MASH, as well as the heterogeneity of the liver, we used 10X single-nuclei RNA-Seq for this study. Interestingly, transcriptomics analysis of DE of genes found in hepatocytes in our MASH models associated with Metal Ion Binding and Signaling. Additional cluster analysis showed decreases in Cu-dependent proteins (ex., SLC31A1/CTR1; MT1; SLC25A3), cu-dependent antioxidant proteins (ex., SOD1/2; GSS; GSTA3; CLU) and increase in cu-dependent ubiquitin-degraded protein CCS. Notably, ICP-MS analysis of MASH serum and livers showed significant decrease in copper content. Pro-fibrotic or activated stellate cell (aHSCs) clusters were also enriched for genes associated with Cu-associated ECM organization, TGFB-mediated signaling, and fibrosis (ex., SMAD3; COL3A1; CD44). From these data we hypothesize that PC-dependent impairments in hepatocyte-dependent copper import, caused by PC-dependent changes in membrane plasticity copper transporter function/activity, leads to increases in circulating copper, that acts on aHSCs, promoting inflammation and fibrosis in MASH.

Notably, in patients with Menkes disease, a genetic loss-of-function disorder that affects the function of ATP7A, copper exporter from the gut, leading to decreases in hepatic copper, reduction in copper-dependent enzymes, and tissue-specific copper accumulation, phenotypes similar to what we observed in our MASH mouse models. Preliminary results from pilot in vivo studies using cell impermeable copper chelator BCS, demonstrates partial protection against diet-induced MASH through reductions in fibrosis as observed by reductions in Sirius red and decreases in pro-fibrogenic gene expression of PDGFRB and SAA1. In in vitro studies using human hepatoma cells HUH-7, CuSO4 can be seen inducing the expression of several cu-dependent genes (ex., CTR1; MT1A; MT1B; CLU) in a dose-dependent manner, a finding not observed in HUH-7 cells deprived of copper using serum-free media or treated with BCS. Interestingly, in HUH-7 cells treated with PCYT1A siRNA, CuSO4 was unable to promote significant induction in copper-dependent genes similar to our observations in our in vivo MASH models. Given the FDA-approval of copper chelators such as D-Penicillamine (DPA), and Tetrathiomolybdate (TTM), for diseases such as cancer, and Wilson's disease, our work may highlight an alternative use for these therapeutics against MASH in the future.

M11	Jillian Weissenkampen	Mcu^{KO} induces a metabolic switch in murine pancreatic adenocarcinoma which can be reversed by Snail expression

Mcu^{KO} induces a metabolic switch in murine pancreatic adenocarcinoma which can be reversed by Snail expression

Jillian S. Weissenrieder, Usha Paudel, Yonathan Janka, Ioana Soaita, Jessie Axsom, Zoltan Arany, and J. Kevin Foskett

The mitochondrial Ca^{2+} uniporter (MCU), is the pore forming subunit of an inner mitochondrial membrane complex which provides the main pathway by which Ca^{2+} enters the mitochondrial matrix. High MCU expression associates with poorer survival in patients with pancreatic ductal adenocarcinoma (PDAC), a highly malignant form of cancer known for early metastasis, rapid progression, and poor treatment responses. We previously showed that MCU deletion reduces tumorigenicity and metastatic capability in isogenic murine xenograft models derived from the ***K**ras*$^{LSL\text{-}G12D}$*; T**p**53*$^{LSL\text{-}R172H}$*; Pdx1-**C**re; Rosa26*$^{LSL\text{-}\mathbf{Y}FP}$ (or KPCY) murine model of PDAC in a manner associated with reduced epithelial-to-mesenchymal transition (EMT), a process believed to contribute to metastatic competency in PDAC. As mitochondrial [Ca^{2+}] is known to regulate the activities of multiple key matrix dehydrogenases, we hypothesized that MCU deletion may adversely affect cellular metabolism, contributing to the reduced apparent malignancy of MCU^{KO} cells. Here, we report that MCU deletion induces profound changes in the expression of metabolic genes. These alterations associate with increased levels of pyruvate and reduced levels of citrate. Notably, mitochondrial Ca^{2+} promotes the activity of pyruvate dehydrogenase, which serves as a gatekeeper enzyme for the tricarboxylic acid (TCA) cycle by converting pyruvate into acetyl-CoA. Our findings suggest that MCU^{KO} may reduce PDH activity, thus causing a backlog of pyruvate and relative reductions in TCA cycle carbons. Indeed, supplementation with acetate (which can be converted to acetyl-CoA) increased proliferation of MCU^{KO} cells but had no effect in MCU^{WT} cells. No significant alterations were seen in glucose or glutamine uptake rates, though acid production was reduced by MCU^{KO}. Surprisingly, in MCU^{KO} cells labeled with ^{13}C-glucose, increased carbon utilization is observed in TCA cycle intermediates. However, ^{13}C-glutamine-associated carbons are diminished. These alterations are associated with a switch from glutamine to glucose dependency for cellular survival in reduced nutrient media (2.5% FBS ± 25 mM glucose ± 2 mM glutamine). MCU^{WT} cells largely relied on glutamine availability to fuel wound healing behaviors, whereas MCU^{KO} cells required both glucose and glutamine in the media. The induction of EMT by expression of the key transcription factor, Snail, reduced the dependency of MCU^{KO} cells on glucose, inducing them to utilize glutamine-derived carbons and reducing the glutamate secretion. Snail expression also improved clonogenicity over empty vector controls in both MCU^{WT} and MCU^{KO} paradigms. These findings suggest that the reinstatement of malignant phenotypes in Mcu^{KO} cells by the stable overexpression of Snail is at least partially induced by metabolic plasticity, which enables a switch from glucose to glutamine dependency.

M12	Kaya Matson	Investigating convergent molecular and cellular signatures of microtubule instability in Amyotrophic Lateral Sclerosis

Investigating convergent molecular and cellular signatures of microtubule instability in Amyotrophic Lateral Sclerosis

Kaya J.E. Matson and Erika L.F. Holzbaur

Amyotrophic lateral sclerosis (ALS) is a devastating disease that affects motor neurons in the brain and spinal cord, leading to progressive muscle weakness and paralysis. While there are over 50 genes that have been linked to ALS, it is unclear how these genes with different roles in the cell all contribute to ALS. A shared feature across ALS patients is the aggregation of proteins, with TDP-43 aggregates present in 97% of patients. TDP-43 dysfunction has been linked to microtubule instability and presynaptic loss at the neuromuscular junction through alternative splicing and decreased expression of the microtubule-binding protein, Stathmin-2 (STMN2). This nervous system-specific protein binds to tubulin dimers to regulate microtubule stability and influences growth cone dynamics to regulate axon outgrowth. The decrease in STMN2 may hinder axonal resilience by disrupting the readily releasable reserve of tubulin. I hypothesize that different ALS-related mutations with decreased STMN2 have depleted readily releasable pools of tubulin which normally serve as a buffer to repair the cytoskeleton under stress, leading to impaired axonal resilience particularly in distal axons. Using isogenic human iPSC-derived motor neurons (iMNs) with ALS-related mutations, I have used live cell imaging to examine microtubule dynamics and found no differences at baseline between revertant wildtype, TARDBP (TDP-43) homozygous SNV, KIF5A homozygous SNV or FUS homozygous SNV iMNs. However, the microtubule polymerization rate for TARDBP homozygous SNV iMNs did not recover after depolymerization by nocodazole, suggesting an impeded cytoskeletal repair under stress. Next, I will examine additional microtubule dynamics and trafficking to determine whether these ALS-related mutations share the same vulnerability to cellular stressors. Additionally, I will compare RNA expression across several ALS-related mutations as well as datasets from patients with sporadic ALS. Across multiple published datasets, STMN2 expression was reduced, both in TARDBP SNV mutants and from donor samples from patients with sporadic ALS. Overall, this proposal addresses the multigenic nature of ALS, seeking to identify convergent molecular or cellular signatures related to microtubule dynamics that lead to neurodegeneration.

M13	Marcus Woodworth	Relating tenocyte chromatin states to native tendon physiology using Expansion Microscopy

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.

M14	Mengqi Xu	Myosin-I synergizes with Arp2/3 Complex to enhance pushing forces of branched actin networks

Myosin-I synergizes with Arp2/3 Complex to enhance pushing forces of branched actin networks

Mengqi Xu, David M. Rutkowski, Grzegorz Rebowski, Malgorzata Boczkowska, Luther W. Pollard, Roberto Dominguez, Dimitrios Vavylonis, and E. Michael Ostap

Myosin-Is colocalize with Arp2/3 complex-nucleated actin networks at sites of membrane protrusion and invagination, but the mechanisms by which myosin-I motor activity coordinates with branched actin assembly to generate force are unknown. We mimicked the interplay of these proteins using the “comet tail” bead motility assay, where branched actin networks are nucleated by Arp2/3 complex on the surface of beads coated with myosin-I and the WCA domain of N-WASP. We observed that myosin-I increased bead movement efficiency by thinning actin networks without affecting growth rates. Remarkably, myosin-I triggered symmetry breaking and comet-tail formation in dense actin networks that resist spontaneous fracturing. Even with arrested actin assembly, myosin-I alone could break the network. Computational modeling recapitulated these observations suggesting myosin-I acts as a repulsive force shaping the network's architecture and boosting its force-generating capacity. We propose that myosin-I leverages its power stroke to amplify the forces generated by Arp2/3 complex-nucleated actin networks.

M15	Paul Mollenkopf	Fluid permeability and poroelasticity of polymer scaffolds at cellular dimensions

Fluid permeability and poroelasticity of polymer scaffolds at cellular dimensions

Paul Mollenkopf and Paul Jamney

Soft biopolymer networks, intrinsic to living cells [1], the extracellular matrix (ECM) [2], and processes like blood coagulation [3], significantly shape the mechanical properties of biological structures. Governed by hydraulic permeability and (visco)elastic properties, these networks play vital roles in cellular shape regulation, force generation for cell migration, material flow facilitation, and protection against physical stresses via the ECM [4]. Their permeability is a key determinant for mass transport in soft tissues, influencing cell behavior and impacting blood clot functionality [5].
While there is advanced understanding of the viscoelastic properties of polymer networks, their dynamic responses to compressive deformations, crucial for physiological scenarios like solid tumor growth and collective cell migration, remain underexplored. Poroelastic effects, particularly in volume-changing deformations, significantly affect rheological behavior, possibly explaining the ECM's stability against compressive loads. Conventional techniques face challenges in evaluating the permeability of adhesive biopolymers [6]. This study aims to experimentally explore the elastic characteristics of biopolymer gels under compression, employing a poroelastic hybrid approach consisting of rheometer-based compression rheology and camera facilitated sample shape detection. The setup presented here allows for the direct measurement of fluid flux and network permeability. Providing insights into how network stress and fluid pressure are coupled this project sheds light on a long-standing question in the field of soft matter physics.

1. F. Huber, A. Boire, M. P. López and G. H. Koenderink, Curr. Opin. Cell Biol., 2015, 32, 39–47
2. V. Vogel, Annu. Rev. Physiol., 2018, 80, 353–387
3. J. W. Weisel and R. I. Litvinov, Springer International Publishing, 2017, vol. 82, pp. 405–456
4. Huber, F. et al., Advances in physics, 2013, vol. 62, 1–112
5. Swartz, M. A. & Fleury, M. E., Annual review of biomedical engineering, 2007, vol. 9, 229–256
6. Punter, M. T. J. J. M., Vos, B. E., Mulder, B. M. & Koenderink, G. H. Soft matter, 2020, vol. 16, 1298– 1305

M16	Siewert Hugelier	Robust quantification and classification of 2D and 3D single-molecule localization microscopy data with ECLiPSE

Robust quantification and classification of 2D and 3D single-molecule localization microscopy data with ECLiPSE

Siewert Hugelier, Qing Tang, Hannah Kim, Melina Theoni Gyparaki, Charles Bond, Adriana Naomi Santiago-Ruiz, Sílvia Porta, and Melike Lakadamyali

The recent advancements in super-resolution microscopy have unlocked the potential to visualize sub-cellular compartments and organelles at nanoscale spatial resolution in both two and three dimensions. Yet, analytical tools to quantify these changes and accurately classify for example organelles or protein aggregates into distinct categories have not kept pace.

To bridge this gap, we developed Enhanced Classification of Localized Pointclouds by Shape Extraction (ECLiPSE), a pipeline that leverages a comprehensive range of shape descriptors to accurately characterize the morphology of individual structures imaged using single-molecule localization microscopy. With this approach, we achieved exceptionally high classification accuracies nearing 100% on five distinct sub-cellular structures including organelles, cytoskeletal filaments, and protein aggregates. Additionally, the automatic feature selection process included in the pipeline further enhances results by prioritizing the most informative descriptors, while discarding the ones that are not. We then also demonstrated the versatility of ECLiPSE through several novel biological applications: quantifying the clearance of Tau protein aggregates, a critical marker for neurodegenerative diseases, and differentiating between two distinct strains of TAR DNA-binding protein 43 proteinopathy, each exhibiting unique seeding and spreading properties. Additionally, 3D-ECLiPSE extends this capibility to three-dimensional structures, and was able to provide a robust quantification of complex 3D structures. This was illustrated by identifying significant morphological differences between healthy and diseased mitochondria.

To sum up, ECLiPSE has proven to be highly effective, and we anticipate that this versatile approach will significantly enhance the study of cellular structures in 2D and 3D across various biological contexts to advance biomedical research and therapeutic developments.

M17	Yobouet Inès Kouakou	Antibacterial bitter β-acid extracted from hops induces sinonasal cilia and mitochondrial dysfunction

Antibacterial bitter β-acid extracted from hops induces sinonasal cilia and mitochondrial dysfunction

Yobouet Ines Kouakou, Joel C. Thompson, Li Hui Tan, Zoey A. Miller, Ray Z. Ma, Nithin D. Adappa, James N. Palmer, Noam A. Cohen, and Robert J. Lee

Routine prescription of antibiotics to treat chronic rhinosinusitis (CRS) exacerbations may contribute to antibiotic resistance. Lupulone, a hops bitter β-acid, possesses potent antibacterial properties and, as a T2R1 and T2R14 agonist, may improve the impaired mucociliary clearance (MCC) described in CRS patients. We investigated lupulone as an alternative treatment to antibiotics in CRS management based on its antibacterial and T2Rs agonist properties.

Human nasal primary cells (NECs) and RPMI 2650 cells cultures were used as study models. Relevant T2Rs expression in cell culture models and human nasal tissue was assessed using immunofluorescence and qPCR. We performed calcium imaging and cilia beat frequency experiments to investigate T2Rs activation in response to lupulone stimulation. Finally, we assessed lupulone's cytotoxicity on cells using immunofluorescence, cell viability and cell death assays.

We report T2R1 and T2R14 expression in NECs and RPMI 2650 cell cultures. Lupulone induced an increase in cytosolic calcium that appeared dependent on T2Rs signaling. This response was accompanied by

mitochondrial membrane depolarization, decreased cell proliferation, ciliostasis, and cell remodeling after a single exposure to lupulone at micromolar concentrations.

Our data suggest that lupulone may not be beneficial as treatment in CRS patients and may instead contribute to the disease by impairing cell health and further deteriorating the MCC.

M18	Leah Simpson	Electrophysiological characterization of the h-ITPR3 T1424M mutation associated with demyelinating Charcot-Marie-Tooth Disease

Electrophysiological characterization of the h-ITPR3 T1424M mutation associated with demyelinating Charcot-Marie-Tooth Disease

Leah Simpson, Sourajit Mukherjee, Xiaonan Cui, Horia Vais, and Kevin Foskett

Charcot-Marie-Tooth (CMT) disease, also known as hereditary motor and sensory neuropathy, results in damage to the peripheral nerves. Although this pathology is most often a result of the PMP22 gene mutation, a recurrent heterozygous missense variant p.Thr1424Met in the IP3R3 gene, encoding for the h-IP3R3, was identified in 33 individuals from 9 unrelated families with CMT. This IP3R3 mutation causes a supposed Ca2+ leak, resulting in increased levels of extracellular calcium. Using nuclear patch-clamp electrophysiology, the gating of the WT and mutant channels in various ligand (IP3 and Ca) conditions were characterized. The results presented herein indicate that Po of the h-IP3R exhibits a sigmoidal dependence on IP3 and a bi-phasic calcium dependence, similar to that of the r-IP3R3. The mutant channel showed an increased sensitivity to IP3 and, surprisingly, complete loss of calcium - induced inactivation, irrespective of IP3 concentrations. Further experiments are being conducted to fully characterize the effect of the mutation on channel gating in a range of IP3 ligand and calcium concentrations. Additional mutations involving the salt bridge neighboring the T1424M are also underway, with the aim of determining if altering this salt bridge sustains the same calcium leak observed in the T1424M.

M19	Sarah Sywanycz	Bitter taste receptor, T2R5, regulates cell viability and apoptosis in head and neck squamous cell carcinomas

Bitter taste receptor, T2R5, regulates cell viability and apoptosis in head and neck squamous cell carcinomas

Sarah M Sywanycz , Brianna Hill , Zoey A Miller, Robert J Lee, and Ryan M Carey

Bitter taste receptors (T2Rs) are a large family of G-protein coupled receptors (GPCRs) first identified for their role in detecting bitter stimuli. T2Rs have been shown to be expressed in benign and malignant extra-oral tissue, including head and neck squamous cell carcinomas (HNSCC), a cancer with high morbidity and mortality. In HNSCC, certain T2Rs appear to regulate proliferation and apoptosis. Bitter taste receptor, T2R5, may pose a novel therapeutic target for HNSCC due to its higher relative expression in cancerous tissue compared to normal tissue and high specificity for agonists. 1,10-phenanthroline-5,6-dione (phenanthroline) is a high potency T2R5 agonist yet to be studied for potential anti-cancer effects. We hypothesized that phenanthroline will act on endogenously expressed T2R5 in HNSCC lines to stimulate a GPCR-mediated calcium response, decrease cell viability, and increase caspase 3/7-mediated apoptosis. We found that three HNSCC cell lines (SCC 47, RPMI 2650, FaDu) express T2R5 but to varying degrees measured by qPCR (n= 6). There was a significant dose-dependent calcium response to phenanthroline measured by calcium fluorescent indicator dye, Fluo-4 AM, for all three cell lines. Calcium responses were significantly dampened with Gα protein inhibitors, YM254890 and FR900359, suggesting a GPCR mediated calcium response consistent with established T2R functionality. Treatment with phenanthroline showed a dose-dependent decrease on cell viability at 24 hours measured by crystal violet assay. Individual IC50 values from crystal violet were roughly inversely correlated to level of T2R5 expression. Stimulation with phenanthroline increased

apoptosis measured by a caspase 3/7 activity dye (CellEvent) at 24 hours. Pretreatment with phenanthroline prior to treatment with cisplatin showed decreased overall cell viability compared to each treatment alone ($p = 0.0062$), which may suggest a role as a sensitizing agent. Comparison of HNSCC patients with high or low expression of T2R5 using The Cancer Genome Atlas showed greater long-term overall survival for the high versus low expression group ($p= 0.04$), possibly supporting T2R5's role in cancer regulation. In summary, T2R5 and its high potency agonist phenanthroline may be important regulators of apoptosis in HNSCC warranting further exploration. Future direction will continue to explore the mechanisms and roles of T2R5 in HNSCC and potential sensitization effects when used in combination with other agents like cisplatin.

M20	Taisia Shepeliuk	Dynamics of clot growth investigated using microfluidic system in vitro

Dynamics of clot growth investigated using microfluidic system in vitro

Taisia Shepeliuk, Roman Kerimov, Ekaterina Molotkova, Fazoil I. Ataullakhanov, and Ekaterina L. Grishchuk

Proper clotting is crucial for preventing blood loss following a vessel wall injury, which exposes circulating platelets to collagen and tissue factor (TF). Clot growth starts after platelets adhere to collagen and become activated by the TF-induced thrombin, which is generated locally at the injury site. As the incoming platelets continue to aggregate to seal the wound, excessive clot growth must be prevented to avoid vessel occlusion, which during pathology may lead to stroke or ischemia. Although platelet activation and aggregation have been studied extensively, the mechanisms that limit clot growth are not well understood. Moreover, prior efforts to reconstruct clot growth in microfluidic chambers in vitro have been reported to result in occlusion and normal clotting dynamics remain to be reconstructed. We study clot growth using a microfluidic flow chamber mimicking blood flow through an arteriole (50 μm diameter) at the physiological shear rate (1,000 s-1). The freshly collected citrated blood is mixed with calcium and magnesium ions directly in the chamber, to minimize time between the recalcification and clotting initiation. The uniform and stable recalcification in a microfluidic channel was confirmed by directly measuring calcium ion concentration in real-time using fluorescent Rhod-5N indicator. Blood clotting is induced at the 50 μm strip containing collagen type I and the TF-containing vesicles. Clot growth is measured in 3D in real-time using differential interference contrast (DIC) microscopy, allowing monitoring changes in the clot height. Using this system, we observed initial adhesion of the platelets followed by their steady aggregation, confirming prior reports of the prevalent occlusion outcome (88%, N=17). The initial adhesion was accelerated by about 2.5 mins by incorporating collagen type III (N=8), which is present together with collagen type I at a typical vessel injury site, however, this addition did not reduce the occurrence of occlusions. Our lead hypothesis about the lack of limited clot formation in the microfluidic systems is that blood withdrawn from human veins lacks some natural platelet inhibitors, such as endothelial-derived nitric oxide (NO), which is avidly scavenged by red blood cells. Our poster will describe the ongoing experiments to modulate the extent of platelet activation and clot growth in the collected blood by controlling the levels of NO. The success of our approach will be measured by our ability to reconstruct non-occlusive clot growth, and in revealing the factors that control its dynamics.

Afternoon Session:

A1	Ashley Aguillard	Loss of ankyrin-B in BAT affects lipid handling and protects mice from high fat diet-induced obesity

Loss of ankyrin-B in BAT affects lipid handling and protects mice from high fat diet-induced obesity

Ashley Aguillard, Jiamin Chen, Kyla Manja, Joyce Tzeng, and Damaris Lorenzo

Genetic variants in ANK2, encoding the scaffold protein ankyrin-B (AnkB) leads to AnkB deficiency in metabolic tissues and are associated with an elevated risk for type 2 diabetes. We previously found that the conditional loss of AnkB in white (WAT) and brown (BAT) adipose tissues in mice (AT-AnkB KO) led to age-dependent fat accumulation and insulin resistance. We uncovered that AnkB modulates Glut4 endocytosis in white adipocytes, and its deficiency increases intracellular glucose levels and lipogenesis. Notably, AT-AnkB KO mice also exhibited decreased energy expenditure and BAT lipid accumulation, suggesting additional roles of AnkB in BAT. To explore this premise, we developed a mouse model with selective loss of AnkB in BAT (BAT-AnkB KO). Young (4-month-old) BAT-AnkB KO display increased lipid content in BAT and in primary brown adipocytes indicative of a cell-autonomous role of AnkB in BAT lipid homeostasis. Through proteomic-MS analysis, we confirmed that AnkB forms complexes with Glut4 in BAT and leads to increases in glucose uptake into brown adipocytes without modulating levels of lipogenesis proteins. Our interactome data also identified complexes between AnkB and lipolytic enzymes Atgl and Hsl in BAT. Interestingly, loss of AnkB leads to decreases in hormone-sensitive lipase (Hsl) and slower basal lipolysis in brown adipocytes. BAT-AnkB KO mice also exhibited age-dependent increases in fat mass and BAT lipid deposition. This phenotype often correlates with impaired BAT function, yet, 10-month-old BAT-AnkB KO mice were able to maintain thermogenesis and energy expenditure during a cold challenge, likely due to compensatory enhancement of BAT lipid oxidation. Notably, BAT-AnkB KO mice are resistant to diet-induced obesity and insulin resistance. These findings suggest that although BAT-AnkB KO mice have enhanced lipid accumulation, compensatory mechanisms exist that prevent BAT dysfunction and metabolic disease.

A2	Carris Borland	Investigating the role of KIF1A in neuronal autophagy

Investigating the role of KIF1A in neuronal autophagy

Carris Borland, Jayne Aiken, Jacob Popolow, and Erika L.F. 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, wellconserved 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.

A3	Hannah Kim	SNAP: Characterizing and Classifying Chromatin Single-molecule Localization Microscopy Data to Reveal Cell Type-Specific Patterns

SNAP: Characterizing and Classifying Chromatin Single-molecule Localization Microscopy Data to Reveal Cell Type-Specific Patterns

Hannah H. Kim, Jose A. Martínez Sarmiento,, Yujia Zhang, Su Chin Heo, Robert L. Mauck, and Melike Lakadamyali

Chromatin, a dynamic DNA-protein complex, modulates DNA accessibility to transcriptional elements, playing a critical role in gene expression regulation and ultimately cell behavior. Advancements in microscopy and sequencing technologies have revealed that chromatin is spatially organized within the nucleus across multiple length scales. Single Molecule Localization Microscopy (SMLM), a super-resolution technique, has been crucial in elucidating the relationship between nanoscale and mesoscale chromatin organization and cell state. However, current analyses largely rely on basic spatial features, such as heterochromatin domain size or overall chromatin compaction, limiting the scope at which chromatin architecture is examined. To address this gap, we developed SNAP (Super-resolution Nuclear Architecture Profiler), a novel computational tool that automates the comprehensive characterization of chromatin-related targets (e.g., DNA or histone proteins or modifications) visualized via SMLM. SNAP extracts 150+ quantitative spatial features from chromatin localization data and identifies those that best discriminate different cell types, including human fibroblasts, embryonic stem cells, tenocytes, and chondrocytes. Using these features, machine learning models achieve 70-85% classification accuracy depending on the complexity of the system, demonstrating the capability of nucleosome localization features to distinguish cell types efficiently. The pipeline also employs volcano plots and clustering algorithms to reveal the most differentially altered features, paralleling single-cell sequencing analyses and offering insights into the biological pathways associated with specific cell states. Overall, this study establishes SNAP as a powerful tool that maximizes the use of SMLM data, enabling a deeper understanding of the relationship between chromatin architecture and cell phenotype, with implications to manipulate epigenetic processes in developmental and disease contexts.

A4	Kyle Barrie	Mechanism of actin filament severing and capping by Gelsolin

Mechanism of actin filament severing and capping by Gelsolin

Kyle R. Barrie, Grzegorz Rebowski, and Roberto Dominguez

Gelsolin is the prototypical member of a family of Ca 2+ -activated F-actin severing and capping proteins. A structure of Ca 2+ -bound full-length gelsolin at the barbed end shows domains G1G6 and the inter-domain linkers wrapping around F-actin. Another structure shows domains G1G3, a fragment produced during apoptosis, on both sides of F-actin. Conformational changes that trigger severing occur on one side of F-actin with G1G6 and on both sides with G1G3. Gelsolin remains bound after severing, blocking subunit exchange.

A5	Megan Stefkovich	Investigating PFK2 as a mediator of insulin’s acute control of hepatic glucose metabolism

Investigating PFK2 as a mediator of insulin’s acute control of hepatic glucose metabolism

Megan Stefkovich, Won Dong Lee, Talia Coopersmith, Dominic Santoleri, Joshua Rabinowitz, and Paul Titchenell

Liver glucose uptake is significantly decreased in patients with insulin resistance and T2DM, which may contribute to hyperglycemia and progression of insulin resistance. Recent studies show that insulin rapidly shifts hepatic glucose balance from glucose production to uptake postprandially, on a time scale inconsistent with a transcriptional mechanism. Our lab and others demonstrated that insulin directly controls liver glucose production through AKT, a serine/threonine kinase and an obligate insulin signaling intermediate in hepatocytes. However, how AKT signaling controls hepatic glucose balance is unclear. This led to the hypothesis that insulin acutely controls hepatic glucose balance through activation of an AKT-PFK2 signaling pathway, resulting in allosteric activation of glycolysis and inhibition of gluconeogenesis. Using stable isotope tracers, we found that AKT rapidly increases glucose contribution to glycolytic intermediates and lipogenic precursors within five minutes, independent of changes to glycogen metabolism. Moreover, insulin stimulated phosphorylation of an allosteric regulator of glycolysis, PFK2/FBPase2, in hepatocytes at Ser486 in an AKT-dependent manner. Adeno-associated mediated expression of a constitutively phosphorylated PFK2 (S469D/S486D) or constitutively dephosphorylated PFK2 (S469A/S486A) in hepatocytes did not alter glucose homeostasis in normal chow-fed mice. However, in response 12 weeks of high fat diet feeding, expressing S469A/S486A PFK2 had improved glucose tolerance compared to wildtype or S469D/S486D PFK2 and reduced fat mass. Future studies utilizing liver-specific PFK2 approaches and gene replacement of PFK2 phosphorylation mutants at these AKT-sensitive residues are ongoing. Overall, these experiments uncover an acute mechanism by which insulin directly controls hepatic glucose balance, which may reveal novel therapeutic targets for treating insulin resistance and maintaining glucose homeostasis.

A6	Zoey Miller	Quorum sensing molecule 3-oxo-C12HSL from *Psuedomonas aeruginosa* activates T2R14 and kills head and neck squamous cell carcinoma

Quorum sensing molecule 3-oxo-C12HSL from *Psuedomonas aeruginosa* activates T2R14 and kills head and neck squamous cell carcinoma

Zoey Miller, Arielle Mueller, Brianna Hill, Sarah Sywancyz, Ryan Carey, and Robert J Lee

Head and neck squamous cell carcinomas (HNSCCs) are cancers that arise in the oral, nasal, larynx and pharynx mucosa. Patients face a 50% 5-year survival rate and an overall decline in quality of life (QOL) due to morbidities of current treatments. Novel targeted therapies are needed to prolong survival and to maintain QOL. We recently discovered that bitter taste receptor 14 (T2R14), a G-protein coupled receptor, induces apoptosis in HNSCC cells when activated with bitter tasting compounds, such as lidocaine. We are now investigating bitter agonists that are endogenously present in the HNSCC tumor microenvironment (TME). The TME consists of different cell types, signaling molecules, and microbes, which can greatly influence pro- or anti-tumor environments. Pseudomonas aeruginosa is a gram-negative bacteria species that often colonizes or infects HNSCC patients. N-3-oxo-dodecanoyl-L-acylhomoserine lactone (3-oxo-C12HSL) is a quorum-sensing molecule secreted by P. aeruginosa that is classified as a bitter compound. 3-oxo-C12HSL also induces apoptosis, however the underlying mechanism is not well defined. We hypothesized that 3-oxo-C12HSL induces apoptosis in HNSCC cells through activation of T2R14. Here, we show that 3-oxo- C12HSL activates an initial intracellular Ca 2+ response in HNSCC cells. This response is inhibited in the presence of T2R14 antagonists. When T2Rs are activated, intracellular cAMP decreases. However, we found that 3-oxo-C12HSL increases cAMP. Nonetheless, 3-oxo-C12HSL inhibits cell viability, decreases NADH production, depolarizes the mitochondrial membrane, and causes production of superoxide species. This leads to induction of apoptosis via caspase-3 and -7 cleavage and inhibition of HNSCC tumor spheroid formation. There are several quorum sensing acylhomoserine lactones from other bacteria. In a screen of lactones, 3-oxo-C12HSL was the only molecule that induced a Ca 2+ response and apoptosis. P. aeruginosa specifically may play an important role in regulating the HNSCC TME. Furthermore, 3-oxoC12HSL could serve as a new higher-affinity bitter agonist therapeutic for HNSCCs as pseudomonas-derived toxins have been proposed as cancer therapeutics. More work is warranted to discover and understand the mechanisms of other endogenous T2R agonists present in the TME.

A7	Corey Holman	UPenn Rodent Metabolic Phenotyping Core

UPenn Rodent Metabolic Phenotyping Core

Corey Holman, Michelle Lee, Jane Touch, Sam Tomlinson, Jim Davis, Qingwei Chu, Paul Titchenell, and Joe Baur

The Rodent Metabolic Phenotyping Core (RMPC), directed by Joe Baur, PhD and Paul Titchenell, PhD, is housed the Institute for Diabetes, Obesity and Metabolism (IDOM) at the Perelman School of Medicine at the University of Pennsylvania. The RMPC's mission is to provide the necessary resources and expertise to allow investigators to perform state-of-the-art studies of metabolism in rodent models. Rodents serve as important models for human diseases and our understanding of diabetes, obesity and other metabolic disorders has increased tremendously as a result of dietary and genetic manipulations in rodent models. Essential services offered by the RMPC include hyperinsulinemic euglycemic clamps, the gold standard for assessing insulin sensitivity, whole body energy balance assessment in metabolic cages through indirect calorimetry, and continuous blood glucose or heart rate/EKG monitoring with implantable DSI Telemetry. In these and many other cases, the standard techniques for phenotyping rodents are not accessible to individual investigators because they require expert surgical and mouse-handling skills, specialized facilities, and/or prohibitively expensive equipment. The RMPC houses the necessary expertise, facilities (e.g., infusions space for clamps using radiolabeled isotopes), and specialized equipment (e.g., indirect calorimetry, DSI telemetry). We also provide guidance as to the selection of appropriate assays within the suite of services offered by the core. By providing these services, the RMPC reduces these barriers and enables investigators at UPenn and beyond to perform high-level metabolic and phenotyping studies. The RMPC is continuously expanding capabilities to advance metabolic phenotyping and offers a dynamic service that pioneers the field by working with investigators to answer their specific scientific questions. To this end, the RMPC has been developing methods of running stable isotope infusions with concurrent 13C gas analysis to measure 13C exhaled in the breath using the Promethion metabolic cage system and during exercise with metabolic treadmills. We are also developing a technique to run clamp studies with continuous blood glucose monitoring using the DSI telemetry system to enable unparalleled glycemic measurements. We welcome any and all inquiries, so please reach out to our Technical Director, Corey Holman, PhD at holmanc@pennmedicne.upenn.edu if you would like to schedule an experiment. The RMPC (RRID:SCR_022427) is supported in part by NIH grant S10-OD025098 and the Cox Institute.

A8	Bishal Basak	Mitochondrial damage induces concerted degradation of negative regulators of neuronal autophagy

Mitochondrial damage induces concerted degradation of negative regulators of neuronal autophagy

Bishal Basak and Erika L.F. Holzbaur

Maintaining mitochondrial quality is critical for neuronal health and function, with Pink1/Parkin-mediated mitophagy playing an essential role in regulating mitochondrial homeostasis. Mutations in genes involved in this process are linked to neurological disorders like Parkinson's disease. In this study, we identify a novel pathway, termed the Mitophagic Stress Response (MitoSR), which complements Pink1/Parkin-mediated mitochondrial quality control in neurons. Triggered by acute mitochondrial stress, MitoSR enhances mitochondrial clearance by selectively degrading proteins that inhibit autophagy. Our findings reveal that MitoSR orchestrates the coordinated and graded degradation of Myotubularin-related phosphatase 5 (MTMR5), MTMR2, and Rubicon in response to oxidative stress, specifically in neurons. These proteins are ubiquitinated and subsequently targeted to the proteasome for degradation. The MTMR2-MTMR5 complex is known to inhibit autophagosome biogenesis; consistent with this we find that depletion of MTMR2 results in a marked increase in the number of autophagosomes. However, the molecular function of Rubicon in neurons remains less characterized. Our work reveals that Rubicon localizes primarily to lysosomes in the neuronal soma in a Rab7-dependent manner. Knockdown of Rubicon increases the number of mature and functional lysosomes, enhancing autophagosome-lysosome fusion in neurons, while Rubicon overexpression impairs this

process. We show that the targeted removal of these negative regulators during MitoSR activation alleviates autophagy inhibition by upregulating autophagosome biogenesis, promoting lysosomal maturation, and enhancing the fusion of autophagosomes carrying damaged mitochondria with lysosomes. These results suggest that targeting Rubicon and MTMR5/2 can provide new therapeutic strategies for improving mitochondrial clearance and neuronal health in Parkinson's disease.

A9	Fedor Balabin and Volodya Demidov	Biophysical investigation of the gliding of kinetochore protein NDC80 along microtubule under dragging force

Biophysical investigation of the gliding of kinetochore protein NDC80 along microtubule under dragging force

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

During cell division, the kinetochore-localized microtubule-associated proteins maintain mobile bonds with spindle microtubules. The key player in this process is the Ndc80 protein complex, which exhibits Brownian diffusion along microtubules in vitro. However, how NDC80 glides along the microtubule under dragging force is not known. We use a laser trapping instrument to move a microtubule "dumbbell" near the "pedestal" bead coated with human NDC80 Bonsai protein. With an ultrafast force clamp, Ndc80 is dragged along the microtubule surface under a constant force ranging from 2 to 15 pN, and single molecule gliding events are detected and analyzed. Strikingly, we found that the NDC80 translocates with different velocities under the same force depending on its direction. Specifically, when NDC80 is pulled toward the microtubule plus-end, the velocity is lower than expected based on the force-free diffusion coefficient of NDC80, which we refer to as the mobile catch-bond translocation mechanism. When multiple NDC80 molecules are gliding together in the absence of the force clamp, the continuous translocation consists of periods of gliding with constant velocity ranging from 1 to 10 μm/s, interrupted by the short intervals during which the velocity is much higher. Strikingly, the constant velocity gliding occurs under the linearly increasing force, which exceeds the initial force by 2 – 25 pN. The constant translocation velocity under the increasing dragging force suggests a velocity-limiting mechanism in the ensemble of gliding NDC80 molecules. We are currently investigating the underlying mechanisms and whether they are specific to NDC80.

A10	Jayne Aiken	Spastin locally amplifies microtubule dynamics to pattern the axon for presynaptic cargo delivery

Spastin locally amplifies microtubule dynamics to pattern the axon for presynaptic cargo delivery

Jayne Aiken and Erika L. F. Holzbaur

Neurons rely on long-range trafficking of synaptic components to form and maintain the complex neural networks that encode the human experience. With a single neuron capable of forming thousands of distinct en passant synapses along its axon, spatially precise delivery of the necessary synaptic components is paramount. How these synapses are patterned, and how efficient delivery of synaptic components is regulated, remains largely unknown. Here, we reveal a novel role for the microtubule severing enzyme spastin in locally enhancing microtubule polymerization to influence presynaptic cargo pausing and retention along the axon. In human neurons derived from induced pluripotent stem cells (iPSCs), we identify sites stably enriched for presynaptic components, termed 'protosynapses', which are distributed along the axon prior to the robust assembly of mature presynapses apposed by postsynaptic contacts. These sites are capable of cycling synaptic vesicles, are enriched with spastin, and are hotspots for new microtubule growth and synaptic vesicle precursor (SVP) pausing/retention. Disruption of neuronal spastin, either by CRISPRi-mediated depletion or transient overexpression, interrupts the localized enrichment of dynamic microtubule plus ends and diminishes SVP accumulation. Using an innovative human heterologous synapse model, where microfluidically isolated human axons recognize and form presynaptic connections with neuroligin-expressing non-neuronal cells, we reveal that neurons deficient for spastin do not achieve the same level of presynaptic component accumulation

as control neurons. We propose a model where spastin acts locally as an amplifier of microtubule polymerization to pattern specific regions of the axon for synaptogenesis and guide synaptic cargo delivery.

A11	Kahkashan Rashid	Metabolic and cognitive challenges in Down Syndrome: Evaluation of chemical chaperone therapy in Ts65Dn mouse model

Metabolic and cognitive challenges in Down Syndrome: Evaluation of chemical chaperone therapy in Ts65Dn mouse model

Kahkashan Rashid, David Frederick, Will Duncan, James Davis, Thato Tsolo, Nirinjini Naidoo, and Joseph Baur

Introduction: Down syndrome (DS), caused by trisomy 21, is characterized by metabolic dysregulation and intellectual disability due to dosage imbalance. Very limited studies have been conducted to explore the effect of trisomy in whole body metabolism and no unified mechanism has yet been discovered for the treatment of these co-occurring conditions. The Ts65Dn mouse model of DS, which recapitulates many features of human DS, has not been extensively studied in terms of age-dependent metabolic changes under basal state. Disruptions in protein quality control (PQC) and the unfolded protein response (UPR) are implicated in these conditions. We hypothesize that the reduction of chronic UPR will restore metabolic phenotype and improve cognition in an age-dependent manner. Hence, in this study, key metabolic parameters and cognitive behavior studies had been conducted.

Methods: A comprehensive detailed analysis of metabolic and cognitive phenotypes in Ts65Dn mice of varying ages and both sexes had been conducted. Mice were maintained on a standard chow diet and treated with PBA (4-phenyl butyrate), a chemical chaperone. Metabolic phenotyping included indirect calorimetry, body composition analysis, and glucose profiling. Beta-hydroxy-butyrate, IL-6 and leptin were also measured. Cognition was assessed using spatial object recognition tests.

Results: The study revealed sex-specific metabolic differences in Ts65Dn mice. Young Ts65Dnmale mice exhibited lowered body weight and refed leptin levels than age-matched WT littermates while female mice showed no alteration in body weight but displayed elevated leptin. Despite these differences, both sexes had no change in body mass composition, food and water intake, respiratory quotient and energy expenditure. No change in fasting-refeeding blood glucose and plasma insulin was observed in young mice. Young Ts65Dn mice displayed heightened locomotor activity and mild glucose intolerance, whereas older female mice demonstrated pronounced glucose intolerance. Two hours of refeeding significantly increased blood glucose in the aged male; no similar alterations were observed in female. PBA showed a trend to reduce glucose phenotype in younger and older mice. Cognitive testing revealed both young and aged had substantial cognitive impairments relative to controls. However, PBA treatment for 12 weeks effectively improved cognition in younger females.

Conclusions: Our findings indicate that metabolic dysregulation intensifies with age in DS mice, while cognitive deficits are present across all ages. PBA treatment shows promise in mitigating cognitive deficits and potentially improving metabolic profiles in DS mice. Further long-term studies are underway to explore the effects of PBA on metabolism and cognitive function over extended periods.

A12	Khanh Doan	Investigating a heart-liver crosstalk underlying cardiac benefits of NAD^+ precursor supplementation

Investigating a heart-liver crosstalk underlying cardiac benefits of NAD^+ precursor supplementation

Khanh V. Doan, Thato T. Ts'olo, Ryan B. Gaspar, Ricardo A. Velazquez Aponte, Gabriel Komla Adzika, David W. Frederick, and Joseph A. Baur

Nicotinamide adenine dinucleotide (NAD^+) is a co-factor for numerous metabolic pathways as well as a co-substrate for key signaling enzymes such as histone deacetylase sirtuins and poly(ADP-ribose) polymerases, which regulates almost every aspect of cell physiology including the heart. We found that depletion of cardiac NAD^+ by targeting the major enzyme of NAD^+ biosynthesis nicotinamide phosphoribosyltransferase (Nampt) specifically in cardiomyocytes alters cardiac metabolism and drives hypertrophic remodeling in adult mouse heart, resulting in severe functional consequences including high risk of lethal arrhythmias and sudden death. Supplementation of high doses of NAD^+ precursors, but not low doses, corrected cardiac NAD^+ metabolism and phenotypes. Unexpectedly, these low doses still reduced cardiac hypertrophy and sudden death despite not sufficient to restore NAD^+ levels in the heart. We hypothesize that systemic supplementation of low-dose NAD^+ precursor acts on the liver to indirectly benefit the heart and test whether enhanced hepatic ketogenesis is an underlying mechanism.

A13	Olesia Lunko	Kinetic modeling of nuclear large-conductance cationic channels

Kinetic modeling of nuclear large-conductance cationic channels

Olesia Lunko, Oleksii Lunko, and J Kevin Foskett

Large-conductance cationic (LCC) channels are nuclear membrane ion channels selective for monovalent cations with preferential permeability to K+ ions. In symmetrical [K+] these channels exhibit a large single-channel open-channel conductance (~200 pS). They are common in the nuclear membranes of Purkinje neurons and pyramidal neurons of the hippocampus. The channels are not sensitive to Ca2+ and their activation depends on voltage, reaching a maximum at positive transmembrane potentials. The physiological roles and molecular identity of LCC channels have not yet been determined. LCC channels demonstrate slow activity dynamics and complex voltage-dependent kinetics featuring multiple timescales; however, a kinetic model is not yet available. This significantly complicates the modeling of processes in which these channels may be implicated, limiting methodological approaches for examining mechanisms and modulatory actions on LCC channels. In this study, we employed a simulation-based high-resolution analytical approach using single-channel data to perform a detailed evaluation of the voltage-dependent kinetic features of LCC channels. Our results indicate that channel activity is best fitted by a kinetic model consisting of five states: one open state, three closed states (slow, medium and fast) and one fast sublevel. Analyses of the kinetics of non-stationary activation and deactivation revealed relaxation hysteresis, which manifests itself in different temporal characteristics (time asymmetry) of activity stabilization depending on the direction of change of the transmembrane potential. Our detailed kinetic model provides a framework for further investigation of LCC channel gating and modulation mechanisms, as well as a better understanding of the general principles of ion transport through LCC channel's pore.

A14	Sierra Palumbos	Autophagic stress activates two distinct compensatory secretory pathways in neurons

Autophagic stress activates two distinct compensatory secretory pathways in neurons

Sierra Palumbos, Jacop Popolow, Juliet Goldsmith, and Erika LF Holzbaur

Neurons are reliant on autophagy to constitutively degrade damaged proteins and organelles in order to maintain cellular homeostasis. Autophagic dysfunction is associated with neurodegenerative diseases including Parkinson's Disease. Mutations in the kinase LRRK2 are one of the most common causes of familial Parkinson's disease; neurons expressing pathogenic LRRK2 mutations exhibit impaired autophagosome maturation. Here, we use biochemical, live cell imaging and unbiased proteomic approaches to demonstrate that LRRK2 mutant neurons engage two distinct secretory autophagy pathways as compensatory quality control mechanisms. First, we find that LRRK2 mutant neurons upregulate secretory autophagy, releasing cargos usually degraded by basal autophagy such as synaptic proteins and mitochondria. Second, we find that LRRK2 mutant neurons exhibit increased release of MVB-derived exosomes. We propose that these two secretory pathways act in tandem to dispose of cellular waste and to induce transcellular communication,

respectively. We demonstrate that both pathways are upregulated in a knockin mouse model expressing pathogenic LRRK2 and that exosomal release prevents the apoptosis-mediated cell death of neurons from this mouse. These findings identify compensatory pathways that are upregulated by expression of pathogenic mutations disrupting neuronal autophagy. These pathways contribute to the maintenance of cellular homeostasis and thus may delay neurodegenerative disease progression over the short term, but have the potential to exacerbate degeneration over the longer term through the release of proinflammatory components such as mitochondrial DNA.

A15	Wei Zhou	The tumor suppressor folliculin maintains genome stability via stabilizing p53 in response to high-fat diet

The tumor suppressor folliculin maintains genome stability via stabilizing p53 in response to high-fat diet

Wei Zhou, Chelsea Thorsheim, Bridget Gosis, Gabe Durso, Jian Li, and Zoltan Arany

Metabolic alterations induced by a high-fat diet increase the risk of tumorigenesis, including hepatocellular carcinoma. However, the mechanisms underlying metabolism-associated genome instability remain poorly understood. Our previous study demonstrated that hepatocyte-specific deletion of Folliculin (Flcn) protects the liver from metabolic dysfunction-associated fatty liver disease (MAFLD) that caused by high-fat diet. Specifically, the Rag C/D GTPase Flcn selectively activates mTORC1 to control transcription factor E3 (TFE3) activity, which in turn regulates multiple hepatic lipid pathways. Surprisingly, loss function of Flcn accelerates the progression of MAFLD-induced hepatocellular carcinoma. Both wild-type and Flcn depleted mice exhibit elevated DNA damage induced by different types of high-fat diets. Comprehensive bioinformatic analyses indicate that the p53 signaling pathway is compromised in Flcn-depleted mice compared to controls. Flcn knockout suppresses p53 protein expression but does not affect its transcription level. Cycloheximide treatment shows that FLCN is required for the stability of the p53 protein via proteasome-mediated degradation. Notably, Flcn knockout sensitizes hepatocytes to Etoposide or Olaparib treatment, revealing a diminished DNA damage response due to reduced p53 activity. Our findings uncover a novel mechanism by which Folliculin regulates p53 protein abundance and highlight the potential of using DNA damage inducers or repair inhibitors to enhance therapeutic efficacy for cancers associated with Folliculin mutations.

A16	Joel Thompson	Effects of *Pseuomdonas pyocyanin* and 1-hydroxyphenazine on intracellular Ca^{2+} signaling in nasal epithelial cells

Effects of *Pseuomdonas pyocyanin* and 1-hydroxyphenazine on intracellular Ca^{2+} signaling in nasal epithelial cells

Joel C. Thompson, Yobouet Ines Kouakou, Zoey A. Miller, Nithin D. Adappa, James N. Palmer, and Robert J. Lee

Pseudomonas aeruginosa is an opportunistic pathogen in immunocompromised individuals,burn victims, and cystic fibrosis patients which can lead to serious infection and even death. Pyocyanin is a secondary metabolite produced by P. aeruginosa which acts as a virulence factor and quorum sensing molecule. Additionally, pyocyanin can diffuse through cell membranes and produce superoxide, leading to cell stress, and eventual apoptosis. 1-Hydroxyphenazine is another structurally-related secondary metabolite produced by *P. aeruginosa* which may also act as a virulence factor and is produced instead of pyocyanin in the absence of the gene *phzM*. While pyocyanin has been suggested to alter Ca^{2+} signaling, both pyocyanin and 1-hydroxyphenazine have not been properly explored, especially in the context of the upper airway and nasal epithelium. Human primary nasal epithelial cells (HNECs) and RPMI 2650 nasal squamous carcinoma cells were used as experimental models in this study. Calcium imaging and localized calcium biosensors were used to assess pyocyanin and 1-hydroxyphenazine effects on cell signaling. Crystal violet and tetramethylrhodamine ethyl ester (TMRE) assays were used to assess pyocyanin and 1-hydroxyphenazine effects on cell viability and mitochondrial function. We found that Pyocyanin induced both a cytosolic and mitochondrial calcium response.

In contrast, 1-Hydroxyphenazine slightly dampened calcium responses in both RPMIs and HNECs. Both pyocyanin and 1-hydroxyphenazine depolarized mitochondria and decreased cell proliferation in RPMI 2650 cancer cells. However primary HNECs were less sensitive to the antiproliferative effects of both pyocyanin and 1-hydroxyphenazine. Future studies will be directed to understanding the role of Ca^{2+} signaling in host innate immunity responses to pyocyanin and 1-hydroxyphenazine.

A17	Li Chen	Amino acid-level differences in alpha tubulin sequences are uniquely required for meiosis

Amino acid-level differences in alpha tubulin sequences are uniquely required for meiosis

Li Chen, Xi Chen, and Anna Kashina

Tubulin is the major structural constituent of the microtubule cytoskeleton that plays an essential role in intracellular transport, cell division, and cellular homeostasis. In cells, obligatory ∝/β- tubulin heterodimers serve as building blocks for microtubule assembly. Different eukaryotic species contain a variety of ∝- and β-tubulin genes that encode proteins with high sequence similarity, and the functional reasons for such diversity are unknown. Here, we tested this question using yeast Schizosaccharomyces pombe as a model system. The genome of S. pombe encodes one single β- tubulin and two ∝-tubulin genes, Nda2 and Atb2, that are functionally non-redundant: Nda2 is essential for viability, while Atb2 deletion does not lead to any strong phenotypes. This limited complexity makes S. pombe an ideal organism to identify the determinants of differential tubulin function. Using CRISPR-Cas9 gene editing, we generated a yeast strain expressing Nda2-coded Atb2, by editing the Nda2 coding sequence to encode Atb2 protein without strongly altering the Nda2 codon usage. Strikingly, unlike Nda2 deletion, this yeast strain, termed NcA, was viable and displayed no visible abnormalities in cell morphology, cell division. However, NcA cells showed strong impairments of meiosis, with high rates of meiotic chromosome mis-segregation and severe abnormalities in meiotic spindle dynamics and horsetail movement of the nucleus — a hallmark microtubule-dependent process essential for chromosome pairing in meiotic prophase. These defects resulted in extremely poor ability of NcA mutants to form proper spores. Furthermore, NcA meiotic spindles showed altered binding of the microtubule crosslinker Ase1 and two spindle kinesis, Klp2 and Klp9, suggesting that the differential ability of Nda2/Atb2 tubulins to bind these proteins modulates in meiosis. Our data indicate that the amino acid sequence of Nda2 is uniquely required for normal meiosis and identify a novel determinant that underlies functional distinction between closely related tubulin isoforms.

A18	Sasha Maiorov	Evolution-guided approach to reveal molecular determinants of the tunable binding interface for CENP-T and Ndc80 kinetochore proteins

Evolution-guided approach to reveal molecular determinants of the tunable binding interface for CENP-T and Ndc80 kinetochore proteins

Aleksandr S. Maiorov, Ekaterina V. Tarasovetc, Adarsh Ramamurthy, Evie Stambler, and Ekaterina L. Grishchuk

During mitosis, the core kinetochore component Ndc80 must bind strongly to the unstructured N-terminus of its receptor CENP-T, which is present in multiple copies within the kinetochore meshwork. We have previously reconstructed human Ndc80-CENP-T interaction in vitro using quantitative fluorescence-based assay, revealing that it is mediated by an unstable but tunable interface. Specifically, the fraction of stably bound CENP-T/Ndc80 complexes increases over time, which we refer to as the binding site “maturation”. Using AlphaFold3 models of the N-terminus of CENP-T complexed with the Spc24/25 globular domain of the Ndc80 complex, we have determined binding configurations for two different human CENP-T sites. In both sites, the central α-helix of CENP-T snugs into the grove of the Spc24/25 globule. However, the flanking unstructured regions show different configurations for the two binding sites. Interestingly, the more extended interface of site 2 correlates with its faster rate of maturation, as determined using fluorescence-based binding assay in vitro. We therefore hypothesized that flanking regions of the CENP-T binding site tune maturation rate of the

corresponding site. To test, we replaced the flanking regions of site 2 with those from site 1 and observed a significant decrease in the maturation rate, as expected. To gain more insight into the molecular mechanisms of maturation, we are also using an evolution-guided approach. Sequence alignment of the CENP-T site 2 sequences across the vertebrates revealed its high degree of conservation. The prominent feature of this sequence is that it contains a conserved phosphorylation motif for mitotic CDK1 kinase, TPR, which is located at the junction between the N-terminal flanking regions and central α-helix. Curiously, mouse and pangolin CENP-T sequences lack the TPR motif, suggesting that they may have different maturation rates. The poster will describe our ongoing efforts to examine binding configurations for the CENP-T binding sites in these species using the AlphaFold3, as well as to measure the maturation rates directly. The successful completion of this project should reveal molecular determinants of the Ndc80-CENP-T binding interface and the rate of its maturation.

A19	Thato Ts'olo	High throughput screen identification of novel compounds that increase NAD^+ in skeletal muscle disorders

High throughput screen identification of novel compounds that increase NAD^+ in skeletal muscle disorders

Thato Ts'olo, David Frederick, Caroline Consoli, Rishith Ramamurthy, Hannah Vanblarcom, Khanh V. Doan, Donna Huryn, Tejvir Khurana, and Joseph A. Baur

Nicotinamide adenine dinucleotide is an essential redox co-factor involved in metabolism roles and exists as both the oxidized (NAD^+) and reduced (NADH) forms. The need for maintaining the optimal redox ratio between the oxidized and reduced forms of NAD is necessary for cellular homeostasis provided that this co-factor partakes in the tricarboxylic acid cycle, glycolysis, fatty acid oxidation, oxidative phosphorylation (OXPHOS) and maintenance of mitochondrial health. Given its role in cellular respiration and energy homeostasis, the decline in NAD is a hallmark of multiple diseases and disorders including in aging. Previously, the Baur lab has shown that depletion of Nampt (a rate-limiting enzyme in the synthesis of NAD involved in the salvage pathway) can promote muscle degeneration and dysfunction which could be alleviated with the application of nicotinamide riboside (NR). Given this ability to promote NAD synthesis in Nampt knockout models via precursors and the need for this essential co-factor in both health and disease, we hypothesize that using small novel molecules can boost NAD either via activation of enzymes involved in synthesis or through inhibition of NAD consumption. After testing all the compounds in vitro, the most promising molecules will be tested once again in vivo with the ability to monitor pharmacodynamics. The end goal is to find molecules that can increase or shift towards NAD in a dose-dependent manner.

A20	Xiangyu Zou	Therapeutic effect of NAD precursors in Acetaminophen-induced liver toxicity

Therapeutic effect of NAD precursors in Acetaminophen-induced liver toxicity

Xiangyu Zou, Sarmistha Mukherjee, Beishan Chen, Nicole Bye, and Joseph Baur

Acetaminophen (APAP) is a widely used antipyretic and analgesic drug and is safe to use at a therapeutic dose. However, the overdose of APAP is often associated with drug-induced liver injury resulting in increased liver failure and death following surgical resection. The prevalence of APAP overdose in the United States translates to more than 50,000 annual emergency visits, hospitalizations, and fatalities. Unfortunately, the treatment options for drug-induced liver toxicity are limited. The only FDA-approved antidote treatment for APAP toxicity is N-acetylcysteine (NAC), but the disadvantage of this drug is the time efficacy. Therefore, to address this critical gap, we examined the potential therapeutic role of a NAD precursor, nicotinamide riboside (NR) supplementation in APAP-induced toxicity in mice. Previous studies have shown that the concentration of hepatic NAD+ decreases in models of acute liver failure (Shi et al., 2012). Mice were intraperitoneally injected with APAP at a dose of 400mg/kg and treated with NAD precursors like NR and NAC 30 minutes post-APAP poisoning. Blood was collected at 0h, 4h, 6h, 8h, and 24h after the insult, and liver tissues were harvested at

24h. Plasma Alanine Transaminase (ALT) assay significantly increases in the APAP-induced mice compared to the controls. Our findings reveal that NR supplementation following APAP poisoning led to notable improvements in both hepatic and mitochondrial function. Hepatic NAD+ and NADH content was significantly lowered in APAP-poisoned mice, however posttreatment with NR improved hepatic NAD and NADH content in APAP mice. Histological evaluation demonstrated extensive zone 3 necrosis, immune cell infiltration, and inflammation, at 24h post-APAP poisoning. Conversely, NR supplementation significantly reduced zone 3 necrosis compared to NAC-treated mice. NR significantly improved the mitochondrial respiratory capacity and mitochondrial NADH content in the APAP-induced liver. These results highlight the potential therapeutic efficacy of NR in mitigating APAP-induced hepatotoxicity. Further studies are warranted to evaluate whether NR is more effective than NAC in reducing APAP-induced toxicity and to explore the underlying mechanisms and the benefits of these interventions in paracetamol overdose.