Mitochondria & Metabolism Research Day

October 29th 2026

Poster Abstracts

1

Characterization of TGFβ-inducible global-proteomic and phosphor-proteomic reprogramming in activated human cardiac fibroblasts

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Myocardial fibrosis represents a common end-stage pathway in diverse cardiac diseases. However, the mechanisms underlying protein phosphorylation and canonical RNA-binding proteins (cRBPs) that regulate cardiac fibrosis remain unclear. By normalizing phosphor mass spectrometry (MS) data to the global MS data, I have identified 1792 proteins with significantly changed phosphorylated peptides across 467 cRBPs across the proteome in TGFβ-activated human immortalized human cardiac fibroblasts (IHCF) compared to controls. Among these, I observed that phosphorylation of the Ser63 (S63) residue of a cRBP and splicing factor, HNRNPH1, was significantly upregulated, whereas the total protein level of HNRNPH1 was slightly downregulated after TGFβ treatment of IHCF. HNRNPH1 phosphorylation site S63 is conserved across evolution, from zebrafish to humans. To investigate the function of phosphorylation of HNRNPH1 at S63, I generated recombinant HNRNPH1 wild-type (WT), “phosphor-dead” S63A, and “phosphor-on” S63D proteins with an N-terminal Flag-HA tag via molecular cloning. When transiently overexpressed (OE) in HEK293T, HNRNPH1 WT, S63A, and S63D protein levels are comparable. Using confocal IF microscopy with manual quantification, I observed that approximately 95% of HNRNPH1 WT, S63A, and S63D were localized to the nucleus and ~5% to the cytoplasm after OE in HEK293T. I generated stable KD cell lines of HNRNPH1 in HEK293T via shRNA, and validated KD of HNRNPH1 at both mRNA and protein levels. I then overexpressed HNRNPH1 WT, S63A, or S63D plasmids in HNRNPH1 KD cells and performed RNA sequencing. ROCK2, DHX36, DICER, and AGO2 were among the significantly downregulated genes when comparing S63A with WT OE groups.

2

Electron transport chain and tricarboxylic cycle form a functional unit

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Background: Cardiac maturation requires a metabolic transition from glycolysis to mitochondrial oxidative phosphorylation. Pyruvate enters the tricarboxylic acid (TCA) cycle, generating NADH and FADH2 for the electron transport chain (ETC). TCA enzymes, including MDH and IDH, may form metabolons that channel NADH to Complex I. Cyclophilin D (CypD), a regulator of the mitochondrial permeability transition pore, has been implicated in mitochondrial maturation and Complex I activation.
Methods: Mitochondria were isolated from wild-type (WT) and CypD knockout (KO) mouse hearts at eight developmental time points from postnatal day 0 to adulthood. Native gel in-gel activity assays evaluated MDH and Complex I activity. IDH2, IDH3, and MDH2 expression was assessed by SDS-PAGE and Western blotting.
Results: Complex I labeling was consistent across development in WT and KO hearts. MDH activity colocalized with the Complex I monomer. Additional 230- and 700-kDa MDH-reactive bands diminished with maturation in WT hearts but were largely absent in KO hearts. MDH2 expression was unchanged with age. IDH2 was elevated early postnatally versus adulthood in both groups, while IDH3 was unchanged in WT but elevated at younger ages in KO hearts.
Conclusion: In-gel assays suggest a functional association between TCA cycle MDH and ETC Complex I in WT and CypD KO heart mitochondria. Postnatal cardiac maturation involves remodeling of TCA cycle enzyme organization and expression, with age-dependent changes in IDH2 and IDH3 but stable MDH2 expression. Our data support a role for CypD in regulating mitochondrial TCA cycle enzyme organization during postnatal cardiac maturation. Ongoing analyses will further define CypD's role in postnatal cardiac metabolic maturation.

3

Mitochondrial Metabolite Supplementation Reduces Site-Specific Phosphorylated Tau

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by neuronal cell death and protein aggregation, with tau protein being a key driver. While the mechanisms initiating aggregation remain unclear, neurons' reliance on mitochondrial metabolism highlights the role of energy deficits in AD. Interestingly, metabolite supplementation, particularly glutamine, has been shown to reduce phospho-tau and improve memory and cognition, suggesting a direct link between pathological tau and mitochondrial metabolism. However, the mechanisms underlying this relationship remain undefined. Here, we examined the effects of metabolite supplementation on phospho-tau clearance in primary neurons. Exogenous succinate supplementation reduced phospho-tau levels. Interestingly, we identified tau phosphorylated by proline-directed kinases was reduced, while sites phosphorylated by non-proline directed kinases were unaffected. However, inhibiting the proline-directed kinases, such as glycogen synthase kinase 3 beta (GSK-3β), p38, and extracellular signal-regulated kinase (ERK) did not decrease phospho-tau levels at the proline-directed sites. In addition, we assessed the role of phosphatase activity by inhibiting the primary phosphatase that phosphorylates tau, protein phosphatase 2A (PP2A). Inhibition of PP2A with succinate treatment did not block the succinate-induced effect on phospho-tau. We also examined the role of the mitochondria and succinate receptor, SUCNR1, in mediating the effect of succinate supplementation on phospho-tau levels, and found that neither pathway was a mediator. We will continue to explore other potential pathways and mechanisms in future studies.

4

Intermittent hypoxia treatment enhances vitality and extends lifespan in C. elegans.

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Aging impairs cellular function and serves as a primary risk factor for numerous human pathologies. Mitochondria, essential for energy production, accumulate progressive damage with age, resulting in diminished bioenergetics, altered oxygen consumption, and elevated reactive oxygen species production. While reducing mitochondrial function during development extends lifespan—often at the expense of healthspan—recent work shows that restoring mitochondrial bioenergetics in adulthood can enhance longevity, demonstrating that bioenergetic and metabolic contexts shape distinct longevity outcomes, though underlying mechanisms remain unclear. Since mitochondria rely on oxygen to generate energy, hypoxia signaling pathways may drive these age-dependent outcomes. To investigate whether hypoxia influences lifespan, healthspan, and vitality beyond development, we applied intermittent hypoxia treatment (IHT) to Caenorhabditis elegans at various life stages. Our results revealed that IHT initiated at the end of development, mid-life, or late-life significantly increased median lifespan, indicating that longevity pathways remain active in aging organisms. While lifespan was extended regardless of intervention timing, healthspan improvements depended on the starting point and were absent for late-life treatments. Worms subjected to a single hypoxia event exhibited increased thrashing rates in liquid media across all tested life stages, reflecting enhanced vitality. This increased swimming velocity persisted for days post-exposure, independently of lifespan extension. These findings highlight the interplay between hypoxia signaling, mitochondrial function, and aging, emphasizing that intervention timing is critical for optimizing outcomes. Understanding how hypoxia signaling and mitochondrial function impact aging can advance treatments for age-related diseases and strategies to enhance longevity through improved mitochondrial performance.

5

Compartmentalized mitochondrial bioenergetics in neurons.

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Neurons are among the most energy-demanding cells in the body, relying on mitochondria to support electrical activity, calcium handling, redox homeostasis, and survival. Mitochondria generate energy by maintaining the mitochondrial membrane potential (Δψ_m), which drives ATP synthesis, contributes to calcium buffering, and helps maintain cellular redox balance. Because neurons are highly polarized, with distinct compartments including the soma, dendrites, axons, and synaptic boutons each region faces unique metabolic demands and may rely on locally specialized mitochondrial function. However, how Δψ_mvaries across neuronal compartments and how this heterogeneity shapes local metabolism remains poorly defined. In this study, we investigated how Δψ_mis differentially regulated across neuronal compartments and examined the metabolic consequences of this spatial heterogeneity. We also assessed how mitochondrial metabolism influences the surrounding cytosolic environment. Using high-resolution live-cell imaging and genetically encoded biosensors, we measured mitochondrial morphology, mitochondrial and cytosolic NADH, and glucose metabolism across neuronal compartments. Automated image segmentation and analysis enabled single-mitochondrion measurements, providing spatially resolved quantification of Δψ_mand metabolic activity. By integrating high-resolution imaging, functional biosensors, and single-organelle analysis, we generated a detailed spatial map of compartment-specific Δψ_munder physiological conditions and during acute metabolic stress. Our findings reveal that Δψ_mdiffers across neuronal compartments both at baseline and in response to changes in substrate availability. These results advance our understanding of mitochondrial diversity and metabolic specialization within neurons, with the long-term goal of identifying mechanisms and therapeutic opportunities relevant to brain aging and age-related neurological disorders.

6

Metabolic labeling in vivo reveals unique metabolic profile of osteocytes: implications for systemic regulation of glucose and aging

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Osteocytes are terminally differentiated cells that reside in highly mineralized bone matrix. Historically, osteocytes were deemed metabolically quiescent, but insights into their local and systemic regulatory functions reignited interest in their metabolism. Recent studies in vitro show that glucose metabolism is necessary in the early differentiation of osteocytes, but that glycolytic genes are downregulated upon maturation. A separate in vitro study also shows that physiological glucose is sufficient to impede osteocyte differentiation. Despite these results, the metabolic profile of osteocytes in vivo, their role in systemic metabolism, and how it is affected by aging remain unknown.

To address these questions, we administered U-13C- labeled glucose to young and aged female mice as a metabolic tracer. We then harvested the long bones and separated matrix-embedded osteocytes from endosteal cells through centrifugation. Metabolites were analyzed by liquid chromatography-mass spectrometry (LC-MS). Results from the in vivo 13C-glucose tracing experiment suggest that osteocytes do not metabolize glucose, evidenced by the lack of labeled and unlabeled intermediates in all glucose-dependent metabolic pathways. Instead, 13C-lactate, likely produced and exported by other tissues, is imported into osteocytes, resulting in labeling of pyruvate and citrate isotopologues. 13C-labeling of glutamate and glutamine further suggest utilization of lactate-derived metabolites in the TCA cycle. Fractional saturation of 13C-lactate and 13C-pyruvate was significantly reduced in aged vs young mouse bones, suggesting that lactate metabolism in osteocytes is impaired in aged mice. Finally, our results also suggest that osteocytes may play a role in systemic regulation of glucose acting as a glucose depot.

7

Characterizing the role of pentose phosphate pathway in osteogenic lineage

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Metabolic and mitochondrial dysfunction are increasingly recognized as key drivers of age-related decline in tissue regeneration, including impaired bone formation in osteoporosis (OP). Osteoblasts (OBs) are highly metabolically active cells that require coordinated glucose metabolism, mitochondrial function, and redox homeostasis to support proliferation, differentiation, and extracellular matrix synthesis. However, how age-associated metabolic alterations contribute to OB dysfunction remains incompletely understood. Recent data from our group and others demonstrate that aging is associated with altered OB metabolism and increased oxidative stress, characterized by a glycolytic shift and decreased NADPH and pentose phosphate pathway (PPP) metabolites, suggesting reduced glucose flux through the PPP. As a major cytosolic source of NADPH, the PPP is critical for both biosynthesis and antioxidant defense. We hypothesize that preferential glucose flux through the PPP supports bone marrow stromal/stem cell (BMSC) differentiation into OBs and maintains OB proliferation, biosynthetic function, mitochondrial homeostasis, and redox balance. To test this hypothesis, we will use murine models of BMSC-specific and OB-specific PPP loss-of-function (LOF) through expression of an unstable human G6PDS188F mutant (mtG6PDKI), driven by Prx1Cre and inducible Col1CreERt2, respectively. Using complementary in vitro and in vivo approaches, we will determine how impaired PPP activity alters cellular metabolism, mitochondrial function, oxidative stress responses, and the bone-forming capacity of BMSCs and OBs. These studies will define the cellular bioenergetics of the osteogenic lineages and may identify metabolic vulnerabilities that can be targeted to restore bone formation during aging, osteoporosis, and fracture repair.

8

Interplay between lipid droplets and ketone bodies during the Drosophila starvation response

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When animals are starving, most tissues deplete their lipid droplets (LDs) to support survival, yet the mammalian liver and the liver-like oenocytes in Drosophila accumulate LDs (starvation-induced LDs or si-LDs). We discovered that during Drosophila oogenesis certain follicle stages also generate si-LDs. By knocking down various metabolic enzymes in the follicles, we found that the fatty acids (FAs) for si-LDs are biosynthesized from ketone bodies (KBs). We also discovered a role for three other tissues in this response: oenocytes, fat body (adipose tissue), and the corpora cardiaca (pancreatic α cell-like). Our data suggest the following pathway: upon starvation, corpora cardiaca release the glucagon-like hormone Akh which in the fat body triggers lipolysis and release of FAs. In oenocytes, these FAs are transiently stored in LDs, released, and then used for KB production. KBs presumably travel through the hemolymph, the fly blood, to follicles, where they are broken down to power de-novo synthesis of FAs and si-LD production. We are now using these insights to probe the biological function of follicle si-LDs. In starved females, young follicles accumulate si-LDs and arrest development. When the flies are fed again, si-LDs are consumed and follicle development restarts. This restart is delayed in flies impaired in generating follicle si-LDs. Thus, si-LDs may allow starved flies to quickly restart oogenesis. Our studies reveal a new energy flux pathway in starved Drosophila and suggest that KBs are used to funnel limited energy resources to follicles, in order to support reproductive success when food becomes available again.

9

Mitochondria-to-Lysosome Proteostatic Crosstalk Contributes to Skeletal Muscle Atrophy

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Diverse forms of mitochondrial damage can impair the import of proteins into mitochondria, leading to mitochondrial Precursor Over-accumulation Stress (mPOS). To determine the physiological consequences of mPOS, we generated a transgenic mouse model by moderately increasing (two-fold) the protein level of the nuclear-encoded ANT1. In parallel, we established a yeast model carrying an additional genomic copy of AAC2 or GAL10-induced AAC2, the yeast ortholog of Ant1. Disrupting the balance between mitochondrial protein loading and import had little effect on OXPHOS in either mice or yeast. We show that mitochondrial protein import stress causes vacuolar damage in yeast, manifested by vacuolar deacidification and a synergistic interaction with V-ATPase mutants that inhibits yeast cell growth. Ant1Tg/+ mice develop progressive skeletal muscle atrophy, characterized by a marked reduction in myofiber size. Ant1Tg/+ muscle exhibits cytosolic accumulation of mitochondrial proteins, consistent with mPOS. Transcriptomic analysis revealed profound remodeling of the nuclear transcriptome in Ant1Tg/+ skeletal muscle. Differentially expressed genes include those involved in amino acid uptake and biosynthesis/starvation responses, one-carbon and nucleotide metabolism, the integrated stress response (ISR), autophagy and lysosomal function. Ant1Tg/+ muscle accumulates glycogen, lipofuscin, and poorly processed multivesicular bodies, accompanied by activation of multiple lysosomal repair pathways. During aging, lysosomal hydrolytic enzymes, including multiple proteolytic cathepsins, are progressively released from the lysosomal lumen into the cytosol. This lysosomal leakage likely contributes to proteostatic imbalance, reduced myofiber size, and progressive muscle atrophy. Together, our findings uncover an evolutionarily conserved mitochondria-to-lysosome proteostatic axis that plays a critical role in regulating tissue mass homeostasis.

10

Low-Doses of Metals Mixture Reduces Healthspan via Epigenetic Modification and Mitochondrial Dysfunction in C. elegans

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Exposure to low levels of heavy metals such as cadmium (Cd) and lead (Pb) is a continued public health concern. Using C. elegans we established a dose-response of Cd and Pb on pharyngeal pumping rate. We found the no observable adverse effect level (NOAEL) for Cd and Pb to be 1 µM and lowest observable adverse effect level (LOAEL) to be 10 µM. When combined, the Cd and Pb NOAEL significantly reduced pumping. Whole-worm metal burden confirmed uptake with no impact on other metal homeostasis. We used pharmacologic means to modulate Pb and Cd toxicity via a chelator, an antioxidant, and a divalent metal transporter 1 (DMT-1) inhibitor. All modulators rescued the deficit, so did genetic knockout of DMT1. Brood size analysis showed delays in egg laying as well as reduced total brood size with Pb or Cd. Both phenotypes are signs of energetic deficit, suggesting energy production machinery is impacted. Mitochondrial proteomics analysis, post-exposure, showed strong downregulation in proteins and pathways associated with epigenetic patterning. Acutely exposed isolated mitochondria had decreased state 3 respiration, RCR, and ETC and TCA enzyme activity, and increased state 4 respiration. Mitochondrial metal burden confirmed uptake of the metals. Overall, our mixture causes greater than additive responses in behavior, and these phenotypes are partially driven by mitochondrial dysfunction at both the enzyme kinetic and protein abundance levels and altered epigenetic machinery function.

11

Chemogenetic generation and detection of mitochondrial hydrogen peroxide in astrocytes

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The impaired ability of astrocytes to take up tau released by surrounding neurons into the extracellular space contributes to the accumulation of tau in the brain, a critical hallmark of Alzheimer's disease (AD). While the specific mechanisms underlying this process are still under active investigation, recent findings suggest that mitochondrial defects likely precede the onset of tauopathy, accompanied by increased oxidative stress. Given that defective mitochondria are a major source of intracellular reactive oxygen species (ROS), we hypothesize that dysregulated mitochondrial ROS (mtROS) contributes to impaired clearance of tau by astrocytes. To achieve spatiotemporal control of ROS production in vitro, we utilized chemogenetic tools targeted to the mitochondrial matrix. Specifically, we coupled HyPer7, an ultrasensitive ratiometric hydrogen peroxide (H2O2) biosensor, with yeast D-amino acid oxidase (DAAO), a D-amino acid-specific H2O2 producer. Using live-cell imaging, we observed dose-dependent increases in HyPer7 readouts following D-Alanine treatments in Hyper7+DAAO+ cells, indicating successful H2O2 production within the mitochondrial matrix of astrocytes. This system provides a key in vitro platform to study tau uptake by astrocytes in the context of AD, as well as to investigate fundamental mitochondrial dynamics in astrocytes in response to ROS.

12

MIX23 PHB/AFG3L2 Regulatory Axis: A Novel Regulatory Core for Respiratory Chain Proteostasis

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MIX23, a small mitochondrial intermembrane space (IMS) protein, has been associated with motor deficits and white matter tract abnormalities. However, there is limited characterization of MIX23's function both within the mitochondria and the nervous system. Proteomic studies have identified MIX23 interacts with several critical mitochondrial proteins, including the prohibitins (PHB1 and PHB2), the m-AAA protease (AFG3L2), and components of the PAM motor complex, which mediate protein import through the TIM23 channel. These proteins form a network to coordinate protein import, quality control, and respiratory chain assembly. We hypothesize MIX23 forms a novel regulatory axis with PHB/AFG3L2 to regulate OXPHOS assembly. Herein, we utilize MIX23 knockout HeLa cell line and a Mix23 mutant mouse line (Mix23-E4) to investigate MIX23 impact on respiratory chain function. Co-IP and mass spectrometry analysis revealed MIX23 and its strongest binding partner, SMIM12, associate with the Prohibitins (PHB)/AFG3L2 complex in WT cells. However, loss of MIX23 prevents SMIM12's binding with the PHB/AFG3L2 complex but increases its binding to Complex IV assembly factors. Western blot analyses of respiratory chain complexes show decreased Complex I and IV levels in MIX23 KO cells along with PHB destabilization, highlighting a potential loss in regulation. Seahorse MitoStress assay revealed KO cells display inefficient increase in respiration compared to WT. Similarly, high resolution respirometry of brain mitochondria revealed Mix23-E4 mice have increased Complex I and II-linked respiration. Additionally, brain lysates from Mix23-E4 mice display increased S-OPA1 levels, indicating mitochondrial stress. In conclusion, MIX23 and SMIM12 associate with the PHB/AFG3L2 complex to regulate protein quality control pathways of the mitochondria. Loss of this MIX23 function triggers motor impairments in mice and bioenergetic deficits. These findings aid in elucidating the molecular consequences of MIX23 loss to provide foundational information for diagnostics and therapeutic intervention.

13

tRNA and Modification Dependency of Mitochondrial Encoded Genes: Predicting Limits to Translation and Function

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The mitochondrial genome contains 37 genes broken up into 13 protein coding genes, 22 tRNAs and 2 rRNAs. The 22 mt-tRNAs are more fragile than cytoplasmic tRNAs and need to decode 60 codons, making them highly reliant on proper modification for stability and codon-anticodon interactions. With some myopathies and diseases being caused my mutations in mt-tRNA or writers of mt-tRNA modifications. Transcription produces a polycistronic transcript meaning, for every round of transcription only 22 tRNAs are produced for the 13 mRNAs that are hundreds of codons long. Making tRNA a vital and potentially limiting factor to production of mitochondrial proteins and mitochondrial function. Analysis of total codon frequency (TCF) and downstream calculation of tRNA, modification and enzyme dependency reveal specific dependencies of mt-genes, particularly MT-ATP8 and MT-ND6. This tool can assess which mt-genes and electron transport chain complexes (ETC) have the potential to be affected under loss of tRNA or modification, given tRNA or modification profiles.

14

Understanding mitochondrial dysfunction and oxidative stress in a tauopathy model

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Mitochondrial dysfunction and oxidative stress are central characteristics of tauopathies such as Alzheimer's Disease. The C-terminal truncation of tau protein results in the species TauC3, which exhibits multiple toxic gains-of-function, including an aggregation-prone character and the ability to disrupt transport of cargo along microtubules. TauC3 has been associated with several modes of inducing mitochondrial dysfunction, either directly by association or indirectly by slowing axonal transport of mitochondria in neurons. As this truncation product is produced by caspase cleavage, which is an apoptotic pathway governed by mitochondrial signaling, its generation is also tightly linked to mitochondrial health. We propose the relationship between mitochondrial dysfunction and TauC3 is therefore of central importance in tauopathies due to the potential for a pathological feedback loop. This relationship is explored by assessing mitochondrial morphology and the presence of reactive oxygen species in cultured mouse neuronal cell lines stably expressing recombinant tau. Cells expressing TauC3 were observed to be both hardier against mitochondrial stressors and have a unique oxidative stress profile.

15

Impaired CoA Salvage in a C. elegans Model of TANGO2 Deficiency Disorder

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TANGO2 Deficiency Disorder (TDD) is a devastating neurometabolic condition characterized by progressive neurological symptoms and metabolic crises triggered by febrile illness or fasting. The function of the TANGO2 protein remains unknown, though patients with TDD benefit from supplemental pantothenic acid, a substrate of Coenzyme A (CoA) synthesis. Using a double knockout (DKO) C. elegans model lacking TANGO2 homologs hrg-9 and hrg-10, we have observed a robust TDD-like phenotype in worms involving poor motility (47% reduction in distance traveled and 28% slower thrashing in isotonic buffer), reduced brood sizes (56% fewer offspring), and shortened lifespan (62% reduction in lifespan), compared to wildtype (N2). DKO worms harbor reduced CoA pools after fasting (2.6-fold reduction vs. N2) along with decreased pantothenic acid (8x decrease vs. N2) undetectable hypotaurine, and increased pantetheine (5x increase vs. N2). Supplementation with the byproducts of pantetheine hydrolysis (pantothenate and cysteamine) partially rescue phenotypic defects in worms, as does treatment with multiple compounds involved in mitochondrial function and CoA metabolism. In summary, our results support a role for TANGO2 in the salvage of CoA through the breakdown of pantetheine. Work is ongoing to directly test the enzymatic function of human TANGO2 and assess metabolic changes in human cells. This work will be critical for developing diagnostic biomarkers and therapies for patients with TDD.

16

Effects of IL-1α on Mitochondrial Function and Tau Uptake in Astrocytes

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Reactive astrocytes are a hallmark of Alzheimer's Disease pathology, often driven by pro-inflammatory cytokines released by activated microglia. These cytokines, such as Interleukin-1-alpha (IL-1α), can induce astrocyte reactivity, leading to increased reactive oxygen species (ROS) accumulation and impaired mitochondrial function. This study examines the effects of IL-1α on ROS production, mitochondrial function, and tau uptake and processing in primary wild-type (WT) mouse astrocytes. Astrocytes were treated with 0, 3, and 6 ng/mL IL-1α, and ROS accumulation was quantified using CellROX, with MitoTracker Green used to assess mitochondrial ROS localization. Mitochondrial membrane potential and ATP levels were measured using TMRM and ATPred, respectively, while cell viability was evaluated with Calcein AM. IL-1α treatment significantly increased mitochondrial ROS at 3 ng/mL, with no further increase at 6 ng/mL, at both 3- and 6-hour timepoints. While TMRM and ATPred results suggest potential effects on mitochondrial membrane potential and ATP levels, these changes were not statistically significant. Calcein AM assays confirmed that IL-1α did not affect cell viability. Preliminary data on EGF and tau uptake suggest IL-1α may enhance uptake, though further analysis is needed to confirm significance and processing efficiency. These findings indicate that IL-1α induces mitochondrial oxidative stress in astrocytes, potentially impacting mitochondrial function. Ongoing studies will explore whether these IL-1α-induced changes are linked to alterations in endosomal and lysosomal pathways involved in tau processing.

17

N-acetyl Cysteine Attenuates Iron-induced Ferroptosis and Energetic Dysfunction in Presenilin Mutant C. elegans

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Iron is critical for cellular function including mitochondrial health but may be involved in neurodegeneration via ferroptosis. Using isolated mitochondria from C. elegans, we conducted a dose-response of acute iron exposures and assessed respiratory function. We observed a biphasic dose-response on maximum respiration (state 3) wherein the low (1µM) dose inhibited while higher doses (10 and 50µM) stimulated respiration. The leak (state 4) respiration showed a monotonic dose-dependent increase in leak respiration, and the respiratory control ratio was impaired at all doses. Mitochondrial iron burden had a strong positive correlation with leak respiration. We used pharyngeal pumping rate (PR) as an energetic behavior readout. We observed a dose-dependent (0-10mM iron) dose-dependent decrease in pumping rate on WT. We selected 2mM iron dose and tested the hypothesis that presenilin mutant worms (hop-1 and sel-12) would have high mortality and PR deficits. The hop-1 mutant had severe PR deficits and moderate mortality while sel-12 had the highest mortality. Worm iron burden was highest in hop-1 and sel-12 the least. Treating worms with n-acetyl cysteine (NAC) returns the PR to control levels. RSL3 a ferroptosis inducing drug induced similar decline in pumping rate as iron. RSL3 related decline was rescued by NAC. Overall, our data suggests a role for NAC possibly through glutathione regeneration in attenuating ferroptosis and energetic deficit in presenilin mutant AD-like models

18

Optical control of protonmotive force in mammalian cells

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Mitochondria play a central role in ATP production through the process of oxidative phosphorylation. This process is driven by an electrochemical gradient across the inner mitochondrial membrane known as the protonmotive force (PMF). Alteration of this gradient has been directly associated with metabolic dysfunctions in neurological and cardiovascular disorders. Advances in optogenetics have shown that the use of old mtON to increase mitochondrial PMF can increase ATP production in C. elegans and isolated mitochondria. However, this tool has demonstrated limited mitochondrial localization and low efficiency in mammalian cells (HeLa cells), resulting in inconclusive outcomes. To address these limitations, we conducted a comparative study between a newly developed mitochondria-targeted light-activated proton pump, called “mito-glow,” to enhance the PMF in HeLa cells. We showed that mitoGlow exhibits better mitochondrial localisation than mtON and that mitoGlow selectively increases PMF upon light activation. Overall, we demonstrated that the newly developed light-activated proton pump exhibits high mitochondrial localization, and their photoactivation supports the generation of the PMF compared to mtON. This improvement enables optical control of mitochondrial PMF dynamics in mammalian cells, opening new avenues for understanding mitochondrial energy regulation and dysfunction.

19

Imaging fluorescent mitochondria in the living mouse retina

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The retina has high mitochondrial density due to its elevated energy demands. In-vivo imaging of retinal mitochondria at cellular resolution has been limited by insufficient resolution and available transgenic reporters. Here, we used mito-mKate2 transgenic mice to image retinal mitochondria in vivo with adaptive optics scanning light ophthalmoscopy (AOSLO). The mitochondrial distribution was imaged in four mito-mKate2 mice (Jax #32188) using a custom-built AOSLO. mKate2 fluorescence was captured with 561 nm excitation and 630Δ92 nm emission (25–50 µm pinhole; 1.3 - 2.7 Airy disc diameters). Retinal layers were imaged 5–20° from the optic disc and structures were measured in ImageJ. Findings were validated in a single ex-vivo retinal whole mount using confocal microscopy with comparable excitation/emission conditions (28 µm pinhole; 1.5 Airy disc diameters). In vivo, ganglion cell somas showed cytoplasmic fluorescence with exclusion from the nucleus. A bright OPL band contained small puncta (0.8 ± 0.08 µm) and larger aggregations (2.0 ± 0.4 µm), consistent with rod spherules and cone pedicles. The ONL showed sparse bright puncta (2.2 ± 0.6 µm) against low background fluorescence. Dense mitochondrial labeling in photoreceptor inner segments enabled resolution of individual cells (1.7 ± 0.2 µm). In-vivo findings matched the corresponding anatomy observed ex vivo. We provide, to our knowledge, the first images of mitochondria at cellular scale in the living retina. This approach enables visualization of mitochondrial distribution in living retinal cells and may provide a means to study mitochondrial changes associated with retinal health and disease.

20

Oxidative Stress in TauC3 Mouse Cells as a Marker for Mitochondrial Dysfunction

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Alzheimer's disease (AD), the worlds foremost neurodegenerative disease, has two key hallmarks in it’s progression, Amyloid Beta plaques, and Tau Neurofibulary tangles (NFT). Healthy tau proteins reach this NFT state through a physiological disruption, primarily through apoptotic signaling; this process activates the protease caspase-3, which truncates the tau protein into a pathological form known as TauC3. It is this pathological form that aggregates into the NFTs that we see in AD. In relation to this, apoptotic signaling occurs whenever the mitochondria undergoes enough stress to warrant apoptosis, and one of the key stressors of the mitochondria are reactive oxidative species (ROS) such as hydrogen peroxide. Should the mitochondria experience sufficient oxidative stress, it will trigger the apoptotic pathway and the activation of caspase-3. Assuming the cell survives this trigger, active caspase-3 will cleave tau to form TauC3, which has been shown to slow axonal transport of mitochondria, disrupting the mitochondrial regulatory network and generating further stress via the tau aggregates that are likewise formed from TauC3. This loop of TauC3 stressing the mitochondria, which in turn activates caspase-3 and truncates full-length tau, is hypothesized to contribute to the pathogenesis of AD. Through the use of three ROS probs, We sought to determine if cells containing different tau states (both full-length and truncated tau) would have different responses to mitochondrial stressors and what role internal ROS/ROS production potentially plays in AD pathology.

21

A targeted Sorafenib chemical-genetic screen identifies potential synthetic-lethal interactions in several S.cervisiae mitochondrial mutants.

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Sorafenib is an FDA approved drug to treat advanced hepatocellular carcinoma. Sorafenib is a multikinase inhibitor that exerts its effects via mechanisms that include oxidative stress and mitochondrial dysfunction. The molecular structure of Sorafenib includes a diarylurea moiety, which is a potential scaffold that can be utilized as a backbone for the synthesis of other potential therapeutic molecules. Our work is focused on elucidating the target(s) of Sorafenib action in fungal cells, using S.cervisiae to examine the effects following Sorafenib exposure - which will then be tested on a series of nine diarylurea analogues that have been synthesized in collaboration. We performed a targeted genetic screen, evaluating the effect of Sorafenib on several mitochondrial mutants, including: cyc1Δ (cytochrome c), ctp1Δ (inner membrane citrate transporter), grx5Δ (subunit of an iron-sulfur assembly complex), and atp2Δ (component of the mitochondrial F1F0 ATP synthase). Our screen used increasing concentrations of Sorafenib using DMSO as a vehicle for drug delivery. The growth of the mitochondrial mutants was observed over a 120 hour time course and strains were assayed for both stressed and unstressed growth conditions. Our preliminary results have identified Sorafenib as conferring hypersensitivity to H2O2 induced ROS in the atp2Δ genetic background and potential resistance to DMSO induced stress is observed in the ctp1Δ genetic background.

22

Optimizing a Live-Imaging Chamber to Model Hypoxic-Ischemic Injury in Primary Neurons
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Background: Perioperative neuronal injury from intraoperative stroke, cardiac arrest, or hypoperfusion remains a major source of postoperative morbidity. However, standard cell cultures models impose uniform hypoxia conditions, failing to capture the spatial ischemic gradient that neurons experience in vivo.
Aims: We adapted a metabolic microenvironment chamber, originally developed to model tumor microenvironments, to enable live imaging of neurons under graded ischemic stress.
Methods: We designed and 3D-printed cell culture chambers compatible with live-cell microscopy, using a glass coverslip over 75% of the chamber to restrict O2/nutrient diffusion. We validated this system in 5HRE-GFP transfected HeLa cells before application to primary rat neurons. We used SnapTag and TMRM labeling to measure mitochondrial mass and viability, respectively, and acquired images from three chamber regions.
Results: In HeLa cells incubated at defined O2 concentrations (1%, 5%, 10%, 21%), GFP increased with decreasing O2 in a dose-dependent fashion. 8, 12, and 24-hour chamber incubations induced significant increases in GFP intensity ($p<0.05$) between proximal and distal chamber regions. This same comparison revealed significant decreases in SnapTag and TMRM signal ($p<0.01$) distal from the chamber opening. Primary rat neurons showed significant GFP induction following 4- and 24-hour incubations at 1% O2. Data collection for neurons cultured in the gradient chambers is ongoing.
Conclusion: These results validate a microenvironment chamber that recapitulates graded neuronal oxygen deprivation within a controlled, imageable system. This platform may help identify determinants of neuronal survival during ischemia, with the ultimate goal of developing targeted neuroprotective strategies.

23

Development of a Regulatory-Grade, Patient-Reported Outcome Measure for Therapeutic Trials Involving Individuals with Mitochondrial Disease: The Mitochondrial Disease-Health Index (MITD-HI).

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Introduction: It is vital to have a validated disease-specific, patient-reported outcome measure (PRO) to quantify disease burden and measure therapeutic gain in mitochondrial disease research.
Methods: Qualitative interviews and a national cross-sectional survey of individuals with mitochondrial disease identified symptoms that are most important to the population. Symptoms were selected for the Mitochondrial Disease-Health Index (MITD-HI) based on frequency, impact, and potential responsiveness to therapeutic intervention. Subscale internal consistency was evaluated, followed by beta testing to examine usability and relevance. Test-retest reliability was assessed for individual symptom items and subscales.
Results: Twenty-four individuals with mitochondrial disease provided 4,365 quotes identifying potential symptoms of importance, and 145 individuals with mitochondrial disease participated in the national cross-sectional survey generating over 23,000 responses. The most prevalent symptoms were fatigue (97.9%), impaired sleep or daytime sleepiness (95.2%), muscle weakness (94.5%), and pain (90.3%). Beta interviews with 15 individuals with mitochondrial disease indicated that the MITD-HI was understandable and relevant. Test-retest reliability with 27 participants demonstrated high reliability. The MITD-HI measures symptomatic health across 19 subscales and takes an average of 7 minutes to complete. Known groups analysis determined the MITD-HI can distinguish between individuals with varying levels of disease severity.
Conclusions: The MITD-HI quantifies point-in-time disease burden of symptoms reported as the most impactful and prevalent to individuals with mitochondrial disease. The MITD-HI can provide a sensitive and accurate measure of clinically meaningful changes in disease burden and support evaluation of therapeutic interventions in future mitochondrial disease clinical trials and further research.

24

Investigating a role for the transcriptional pausing factor SPT5 in one-carbon metabolism and tRNA methylation in hematopoiesis

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Emerging evidence suggests that the poor prognosis of acute myeloid leukemia (AML) may be linked to metabolic reprogramming during disease progression, but the transcriptional programs regulating this remain unknown. Transcriptional pause-release is a critical regulatory step of RNA polymerase II-dependent transcription, particularly for genes involved in metabolism. SPT5 stabilizes RNA polymerase II at promoter-proximal pause sites and promotes its release into productive elongation, suggesting a link between transcriptional and metabolic regulation. Analysis of public patient data associated high SPT5 expression with reduced long-term survival. Furthermore, SPT5 co-dependencies from DepMap were enriched for genes involved in mitochondrial gene expression. To investigate how SPT5 may regulate cellular metabolism, we used zebrafish as a vertebrate model of hematopoiesis. We found that zebrafish spt5 mutant embryos have a pronounced defect in erythroid differentiation accompanied by elevated levels of S-adenosyl-L-methionine (SAM), the major methyl donor in cells. Because increased mTORC1 signaling can elevate SAM levels, we investigated nprl3 expression, which encodes a key inhibitor of mTORC1 signaling, and found it to be downregulated in spt5 mutants. Additionally, we found that pharmacologically inhibiting mTORC1 rescues the erythroid differentiation defect in spt5 mutants. Next, we examined the downstream consequences of elevated SAM levels on RNA, which revealed increased levels of methylation at specific guanosine residues (2-methyl- and 2,2-dimethyl-guanosine) on tRNAs in spt5 mutants. Knocking down the writer of both these RNA modifications, trmt1, synergizes with the loss of spt5. Together, these findings provide mechanistic insight into how altered Spt5 function may couple transcriptional regulation to metabolic programs.

25

Exploring the Connection Between Mitochondria Membrane Potential and Tau Pathology in an Alzheimer's Disease Mouse Model

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Tau pathology is a defining hallmark of Alzheimer's disease (AD), yet the mechanisms that drive its progression are not well understood. Mitochondrial dysfunction emerges early in AD, including loss of the membrane potential and declining bioenergetic function, but whether these defects directly contribute to tau pathology remains unclear. We previously used a novel optogenetic tool called mitochondria-ON (mtON) that increases the mitochondrial membrane potential independent of the electron transport chain and is activated upon light activation at 561nm. Following ischemic stress, the activation of mtON improved neuronal health. Here, we use mtON to directly test whether boosting the mitochondrial membrane potential will impact tau pathology in vivo. PS19 mice, a tauopathy mouse model, were transduced with neuronally targeted mtON packaged in adeno-associated virus in the hippocampus and subsequently exposed the mice to light stimulation through a cordless fiber optic implant. Two weeks after delivery, mtON showed robust neuronal expression and mitochondrial localization. 561nm light was applied at varying intensities, and immunoblotting was used to determine how exposure to various light intensities along with mtON activation affects phosphorylated tau levels. These studies could help establish the impact of mitochondrial dysfunction on tauopathy in AD and potentially open a new pathway of treatments for the disease.

26

The IDH2 inhibitor AGI-6780 limits viral and mitochondrial DNA synthesis in HCMV-infected fibroblasts

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Human cytomegalovirus (HCMV) is a ubiquitous beta-herpes virus and a major cause of congenital disease and morbidity in transplant recipients. HCMV drives widespread metabolic reprogramming of infected cells, relying on cellular metabolic resources to support virion production. Pathways known to be modulated by HCMV during infection include glycolytic flux, glutaminolysis, and fatty acid biosynthesis. We find that AGI-6780, an allosteric inhibitor developed against mutant IDH2 (R140Q), potently blocks productive HCMV infection in human fibroblasts with infectious progeny decreasing more than 10,000-fold at a dose of 5 µM. AGI-6780 treatment reduced transcript abundances of the IE1, UL26, and UL99 viral genes measured at 48 hours post infection (hpi), representing a decrease in immediate-early, early, and late HCMV gene expression. Viral genome replication fell in AGI-6780-treated cells at 48 hpi, and mitochondrial DNA abundance decreased from its elevated level during infection. We examined the effectiveness of AGI-6780 in inhibiting viral infection when added at different time points following infection. AGI-6780 was most effective at inhibiting productive viral infection when added to infected cells prior to 48 hpi, with decreased efficacy when added at later time points following infection. We then performed LC-MS metabolomic analyses to identify differences in metabolite abundances caused by AGI-6780 treatment during HCMV infection. We determined that AGI-6780 resulted in intracellular nucleotide pools shifting towards dephosphorylated species, possibly indicating a defect in viral genome replication through reducing dNTP supply. This work expands our knowledge about viral metabolic reprogramming and may offer therapeutic targets for the attenuation of viral infection.

27

Uncovering a novel role for the ESCRT pathway in protecting against mitochondrial protein import stress

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Introduction: Mitochondrial damage can trigger a cytosolic response known as mitochondrial precursor overaccumulation stress (mPOS) which results in cell death. We have previously shown that the vacuole/lysosome plays a critical role in handling these unimported mitochondrial precursor proteins. In this study, we used an unbiased genome-wide genetic screen to identify specific genetic pathways that are required for the survival of actively respiring yeast cells upon partial depletion of mitochondrial DNA (mtDNA).
Methods: We performed a genome-wide deletion screen in Saccharomyces cerevisiae to detect genes that protect cells against ethidium bromide (EB)-induced mtDNA depletion under respiring conditions. EB-sensitive strains were then crossed with our genetic model of mPOS overexpressing the AAC2 gene (AAC2OE), a homologue of human Ant1. Phenotypes were analyzed under fluorescence microscopy to assess vacuolar morphology and cytosolic responses.
Results: We identified 80 genes grouped into ten functional pathways that protect against mPOS. Five of these pathways are involved in vacuolar function: lipid homeostasis, amino acid/nucleotide metabolism, vesicular trafficking, and pH regulation. We determined that mutant strains lacking endosomal sorting complex required for trafficking (ESCRT) are particularly sensitive to mPOS and exhibit morphological changes to the vacuole. Additionally, these ESCRT mutants demonstrated temperature sensitivity under respiring growth conditions.
Conclusion: Our study revealed key genetic pathways that protect cells from mitochondrial protein import stress. Furthermore, we highlight a previously unknown role for the ESCRT pathway in handling unimported mitochondrial precursor proteins. These data suggest a novel functional link between mitochondrial and vacuolar pathways that may underlay mitochondrial disease pathology.

28

Mitochondrial Function in Skeletal Muscle in Myotonic Dystrophy Type 1

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Myotonic Dystrophy Type 1 (DM1) is a multisystemic disease that affects nearly every organ system. DM1 is primarily attributed to the global dysregulation of alternative splicing across thousands of transcripts. Approximately 60% of patient mortality is caused by respiratory failure due to muscle weakness and wasting. However, the exact molecular mechanisms underlying this respiratory dysfunction remain unclear. Skeletal muscle function depends on mitochondria to generate ATP through oxidative phosphorylation, suggesting that mitochondrial function may play a critical role in the development of respiratory muscle dysfunction in DM1. The diaphragm, the primary muscle responsible for respiration, is unique among other skeletal muscles due to its higher mitochondrial volume density, which supports its high metabolic demands. Here, we aim to investigate mitochondrial function in permeabilized diaphragm tissue isolated from DM1 mouse models. To achieve this, we are using high-resolution respirometry tools, such as the Hansatech and the Oroboros O2k, to measure real-time oxygen consumption. Our studies utilize a SUIT protocol and use control wild-type and DM1 mouse models that include Cav1.1Δe29 (exon 29 removed from the CACNA1S gene), Mbnl1 knockouts (Mbnl1-/-), and Cav1.1Δe29/Mbnl1-/-. We hypothesize that diaphragm isolated from DM1 mouse models will have significantly impaired state 3 and state 4 respiratory function, alongside an overall reduction in electron transport chain activity.

29

Effects of large-scale mitochondrial deletions on lymphoblast growth and metabolism

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Large-scale deletions occur in human mitochondrial DNA (mtDNA) due to misannealing during asymmetric replication. We are studying how lymphoblast cultures are affected by increased levels of mtDNA bearing these deletions, examining growth, metabolism, and response to ionizing radiation.

30

Altered mitochondrial reactive oxygen species metabolism: implications in cadmium and PFAs toxicities

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Cadmium (Cd) and per- and polyfluoroalkyl substances (PFAS) are environmental contaminants of increasing ecotoxicological concern. Mitochondria play a central role in cellular stress responses and organismal adaptation; and are a potential common target of these toxicants. However, the effects of Cd and PFAS co-exposure on mitochondrial metabolism remain poorly characterized. Here, we used genetic and pharmacological approaches in Caenorhabditis elegans to investigate the effects of Cd and PFAS co-exposure on mitochondrial function and organismal physiology. Our findings indicate that dysregulation of mitochondrial metabolism and reactive oxygen species (ROS) emission is associated with altered worm behavior, lifespan and stress response. Our results show different organismal outcomes in response to different mitochondrial ROS production sites. These results highlight the importance of site-specific mitochondrial ROS production and signaling in mediating toxicological responses to Cd and PFAS co-exposure, providing insights into the mitochondrial mechanisms underlying toxicant-induced physiological dysfunction.

31

Empirical evaluation of computational tools that predict the thermodynamic consequences of single amino acid substitutions on the SOD1 protein

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease associated with, in part, mutations in the superoxide dismutase 1 (SOD1) gene. The consequences of these mutations vary, and they may alter SOD1 protein stability, structure, and function. With the proliferation of an ever-increasing number of computational tools that can predict the effects of individual point mutations, different models may produce conflicting results. This study compares multiple computational models to assess their consistency in predicting changes in SOD1 protein stability, evaluate their strengths and limitations, and better understand the ultimate consequences of the actual mutations.

In this work, we performed saturation mutagenesis across every possible point mutation at every possible site within the SOD1 protein. Our analysis utilized the canine SOD1 protein, as it has a high-quality crystal structure dataset that allowed for this comprehensive thermodynamic characterization using an assortment of computational tools and the protein is highly conserved functionally across diverse eukaryotic organisms.

Following analysis with these tools we compiled our results and compared the predictions across different algorithms. We will then select several mutations that we will create and empirically test using site directed mutagenesis via PCR on a series of plasmid vectors. These plasmids will then be transformed into yeast that lack a functional SOD1 gene, and the consequences of the mutation will be assessed to investigate their functional effects. By unifying computational predictions with experimental analysis in the yeast model, this study aims to determine how reliably these tools predict changes in protein stability and how those predictions relate to the functional effects of SOD1 mutations.

32

Exploring the role of MIX23 in mitochondrial protein import

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MIX23 resides in the mitochondrial intermembrane space and is expressed broadly across tissues and cell types. Proteomic studies have shown that it interacts with several components of the PAM motor complex (DNAJC19, DNAJC15, and PAM16), which mediate protein import across the mitochondrial inner membrane through the TIM23/17 channel. In yeast, MIX23 is upregulated by mitochondrial protein import stress and genetically interacts with a temperature sensitive TIM17 import channel to support protein import. However, the precise molecular function of MIX23 in this context and in mammalian cells is still unknown. We investigated the interaction of MIX23 with several proteins involved in protein import across the inner mitochondrial membrane. We also tested whether their steady-state protein levels were altered in WT vs MIX23 KO HeLa cells or WT vs MIX23 mutant mouse brains. Immunoprecipitation of MIX23 co-purified DNAJC19, but not TIM23/17 channel subunits. Additionally, DNAJC19 co-precipitates at higher levels with MIX23 than with the TIM12/17 subunits and shows weaker binding to TIM23 in the absence of MIX23. We found loss of MIX23 in HeLa cells resulted in lower levels of PAM16, no change in DNAJC19, and sometimes increased DNAJC15. This suggests that loss of MIX23 is disrupting the PAM motor complex in HeLa cells. However, these steady state protein changes were also not found in the brains of MIX23 mutant mice. Future studies will seek to more directly test whether loss of MIX23 impairs mitochondrial protein import.

33

Cardiac over-expression of SOD2 reduces pulmonary vascular disease in adult mice exposed to neonatal hyperoxia

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