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INTER-ORGANELLE METABOLISM AND SIGNALLING - POSTER ABSTRACTS -

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1- Abdur: Identification of novel candidate genes for inherited retinal disorders by analyzing consanguineous families from Pakistan and Iran

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Background: Consanguinity offers a unique opportunity to unveil novel gene-phenotype correlations, as children of consanguineous parents exhibit significantly higher genomic homozygosity, providing an ideal setting for identifying high-impact genomic variants associated with autosomal recessive disorders. Pakistan and Iran, characterized by high rates of consanguineous marriages (up to 70% and 38.6%, respectively), serve as valuable populations for such investigations.

Methods: This study encompasses the genetic analysis of 244 consanguineous families (913 affected individuals) from Pakistan and Iran, the majority having multiple affected individuals. Whole exome sequencing (WES) was conducted on affected individuals, followed by genotyping of potential variants in all family members, including parents and siblings.

Results: Among the families with known Inherited Retinal Disorder (IRD) genes, the diagnostic yield was 71% (174 out of 244 families). In the 70 undiagnosed families, several novel candidate genes were identified including, MOB1A (NM_001317110:c.503C>G:p.Ser168Cys), CCDC180 (NM_020893:c.1969_1970del:p.Asn657ArgfsTer10), PLA2G3 (NM_015715:c.601C>T:p.Arg201Ter), ATP8A1 (NM_001105529:c.3043C>T:p.Pro1015Ser), and MPHOSPH6 (NM_005792:c.414T>G:p.Tyr138Ter). Notably, PLA2G3 and CCDC180 have been associated with ciliary dysfunction which has previously been linked to various ocular diseases. A Ccdc180^{-/-} mouse model was shown to exhibit pronounced ocular phenotypes. MOB1A, a regulator of YAP1 activity, has implications in IRDs. Lastly, while ATP8A1 and MPHOSPH6 are expressed in the eye, their roles in visual function remain unclear.

Conclusions: This study underscores the effectiveness of WES in unraveling the genetic basis of IRDs in consanguineous families. The identification of novel candidate genes enhances our understanding of molecular pathways involved in these disorders, paving the way for future therapeutic interventions.

2- Aguetaz: An elusive PLIN3 splicing variant reveals a conserved mitochondrial targeting of the perilipin protein family.

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Perilipin3 (PLIN3) is a ubiquitous member of the Perilipins lipid droplet (LD)-coating protein family. PLIN3 contributes to LD growth, lipophagy, phosphatidylcholine synthesis and to the cellular protection from lipid toxicity. PLIN3 is highly expressed in skeletal muscle, where its levels have been correlated with fatty acid oxidation and exercise training. Interested in the study of proteins at the crossroad between lipid metabolism and muscle function, we decided to focus on Perilipin3.

We report on a previously uncharacterized PLIN3 coding sequence originating by an alternative splicing event, here named PLIN3B. PLIN3B overexpression in cells highlighted a specific mitochondrial targeting. Electron micrographs revealed alterations of the mitochondrial suborganellar organization. In accordance with imaging and electron microscopy data, mass spectrometry analysis of PLIN3B interactors identified several mitochondrial partners, with enrichment of intermembrane space proteins.

In cells, we confirmed the alternative splicing event with splicing-modulating antisense oligonucleotide experiments. Despite being able to modulate PLIN3B mRNA and detect the alternative transcript in multiple human cell lines and tissues, we were not able to detect endogenous PLIN3B with biochemical approaches, proteomics and databases exploration.

In Zebrafish, PLIN3B mitochondrial targeting is conserved. Two ubiquitously expressed perilipins Plin2 and Plin3 share the mitochondrial localization of the human isoform.

Despite confirming PLIN3B splicing variant at the transcriptional level, its translation appears absent in human. This project highlights and opens new questions on the complexity of protein expression regulation, organelle targeting and gene subfunctionalization.

3- Blanco-Fernandez: Mitochondrial gene expression supports anti-inflammatory macrophage immunometabolism

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Macrophages are innate immune cells that can polarize into functionally distinct subsets throughout the initiation and resolution of inflammation. Each polarization state is accompanied by a specific metabolic program that sustains function. While mitochondrial remodeling occurs during pro- and anti-inflammatory polarization, how mitochondrial function supports macrophage immunometabolism remains unclear.

Here, we investigate mitochondrial remodeling during macrophage polarization by applying quantitative protein mass spectrometry to highly purified mitochondria isolated from pro- and anti-inflammatory mouse bone marrow-derived macrophages. We identify mitochondrial DNA (mtDNA) expression as the most upregulated mitochondrial pathway during anti-inflammatory polarization. We confirm increased mtDNA transcription and translation of mitochondrial-encoded proteins, all of which are involved in oxidative phosphorylation (OXPHOS).

To better characterize the functional consequences of mitochondrial remodeling, we next assess the effect of a panel of small molecule inhibitors of mtDNA transcription, translation, and OXPHOS. We found that while blocking mitochondrial translation prevents the increase in oxygen consumption seen in anti-inflammatory macrophages, inhibiting the ATP synthase, which contains two mtDNA-encoded subunits, exhibits the strongest inhibition on the polarization of these macrophages. Importantly, we report that the role of the ATP synthase in polarization can be bypassed by treatment with the mitochondrial protonophore BAM15, pointing to a key role for the mitochondrial membrane potential in anti-inflammatory macrophage polarization.

Our work reveals how mitochondrial remodeling supported by increased mtDNA expression promotes anti-inflammatory macrophage polarization and function, providing new insights into their immunometabolic regulation.

4- Boya: Mitophagy at the crossroads of neuroinflammation during aging and disease

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Loss of proteostasis and dysregulated mitochondrial function are recognized as core hallmarks of aging, with recent revisions also highlighting impaired macroautophagy and chronic inflammation. Mitophagy, a crucial process in mitochondrial quality control, lies at the nexus of these aging-related phenomena; however, its age-associated perturbations have been largely unexplored. In our recent study, we conducted a comprehensive analysis of mitolysosome levels in mice and discovered that, contrary to the established decline in non-selective macroautophagy, mitophagy either remains stable or increases with age across all examined tissues. This upregulation is mediated by the PINK1-PRKN-dependent pathway. Further investigation revealed a simultaneous increase in mitochondrial DNA (mtDNA) leakage into the cytosol and activation of the CGAS-STING1 inflammation axis, phenomena that were also observed in primary fibroblasts from older human donors. We propose that mitophagy may be selectively upregulated during aging to enhance mitochondrial function and mitigate mtDNA-induced inflammation. Notably, treatment with the mitophagy inducer urolithin A alleviates age-related neurological decline, evidenced by improved synaptic connectivity, cognitive memory, and visual function. In alignment with our hypothesis, urolithin A decreases cytosolic mtDNA levels, reduces CGAS-STING1 activation, and diminishes neuroinflammation. Additionally, using an in vitro model of mitochondrial membrane permeabilization, we confirmed that PINK1-PRKN-mediated mitophagy is vital for resolving cytosolic mtDNA-triggered inflammation. These findings suggest a novel integrative approach to addressing aging and enhancing healthspan through the induction of mitophagy.

5- Castro-Sepulveda: Zebrafish as a Model for Human Skeletal Muscle Aging: Identifying Critical Windows for Intervention

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Aging leads to skeletal muscle (SkM) deterioration, marked by loss of mass, reduced aerobic capacity, mitochondrial dysfunction and increased lipid droplets (LD). Zebrafish presents a gradual SkM aging process like humans. This project aims to analyze organelles and SkM quality in aging zebrafish to identify ages in which preventive actions can be undertaken.

Zebrafish were divided into five age groups: 7-12[Y], 19-24[M], 30-36[MO], 42-48[O] and 54-55[OO] months-old; 11 females /11 males per group. Morphometric and physiological assessments included spontaneous locomotion, *in vivo* subcutaneous adipocytes imaging and maximal oxygen consumption (MO_{2max}). After euthanasia, SkM samples were analyzed via immunofluorescence to evaluate fibers cross-sectional area (CSA), electron microscopy to evaluate mitochondria and LD content, and Western blotting to evaluate mitochondrial proteins involved in oxidative phosphorylation (OXPHOS) and mitochondrial dynamics. Two-way ANOVA was performed to evaluate age and sex effects.

OO fish develop scoliosis and have larger adipocytes. Spontaneous swimming velocity and MO_{2max} /body-area declines with age, although males maintain higher velocity swimming and MO_{2max} . Fast fiber CSA decreases in OO fish with sex-specific patterns. Mitochondrial density and morphology vary by fiber type and age LD accumulate specifically in O fish slow fibers. Despite age-related declines in MO_{2max} , key mitochondrial proteins (e.g., OXPHOS complexes, dynamics regulators) remain stable.

Zebrafish present SKM aging patterns similar to observations made in human studies with loss of type II fibers CSA, reduction of mitochondrial content, increase in LD numbers and declines in exercise capacity. We now have a clear view when to study effects of intervention and mechanistic queries.

6- Crivelli: Astrocytes transfer mitochondria to neurons for metabolic support

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Mitochondria are secreted by astrocytes via large vesicles during stressful events, such as hypoxia, to support neurons. However, the mechanisms governing this process and the range of stressors triggering mitochondrial release remain unclear. Additionally, it is debated whether mitochondria are transferred directionally from astrocytes to neurons under physiological conditions.

Using a co-culture system of astrocytes with fluorescently labeled mitochondria and fluorescently labeled neurons, we tracked mitochondrial transfer under various conditions. To investigate physiological transfer in vivo, we developed an adeno-associated virus (AAV) for astrocyte-specific expression of green fluorescent protein (GFP) in mitochondria, co-injected with an AAV labeling neurons, into the hippocampus of wild-type mice. This enabled visualization of donor (astrocytes) and recipient (neurons) cells via fluorescence microscopy.

We observed directional mitochondrial transfer from astrocytes to neurons under physiological conditions, with the transfer rate doubling within 20 minutes upon exposure to a stressor like glutamate. In vivo data corroborated mitochondrial movement from astrocytes to neurons.

In conclusion, our preliminary findings suggest that directional mitochondrial transfer from astrocytes to neurons occurs in vivo under physiological conditions and intensifies under stressors like glutamate, potentially to provide metabolic support.

Key words: mitochondria transfer, astrocytes, neurons, energetic metabolism

7- Da Graça: ER-endosomes contacts generate a local environment promoting phagophore formation

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Autophagy is a cellular process initiated by the de novo formation of a double membrane vesicle called autophagosome, which emanate from a transient structure termed phagophore. Phagophore biogenesis requires phase transition and occurs at the immediate vicinity of endoplasmic reticulum (ER) subdomains which can be engaged in membrane tethering with other organelles such as mitochondria and plasma membrane. Recently, we demonstrated a dynamic recruitment of early endosomal membranes toward sites of phagophore biogenesis. Our present work shows that the number of endosomes-ER contact sites (EERCS) increase during acute response of starvation and are mobilized in early steps of autophagy pathway.

To relevantly tackle the implication of EERCS during phagophore biogenesis, we optimized different cutting-edge technologies specific for contact sites studies (i.e. expansion microscopy, machine learning-based analysis, split-turboID, biochemical purification). Deeper analyzes on the nature and the dynamics of EERCS unveiled their implication in the creation of a local environment with specific biochemical properties. These include phase transition and Ca²⁺ confined release, events that could support de novo membrane generation. Here, we identify a novel role for ER-endosomes membrane contact sites during early response to nutrients deprivation. We propose that dynamic mobilization of EERCS enable transient confinement of cytoplasm which in turns favors the local enrichment of proteins, lipids and ions required for phagophore biogenesis initiation.

8- Domeniconi: Novel photocage for the targeted delivery of native lipid species

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The metabolic fate and function of lipids are dependent on their subcellular localisation or acyl chain composition. However, tools to investigate lipid metabolism at such levels of specificity are scarce. We develop a chemical biology approach by delivering specific native lipid species to live cells in a time-controlled manner using a new type of photocleavable fluorescent 'cages' masking the lipid's function prior to release. Click-chemistry based modifications on the photocage allow sub-cellular targeting of the caged lipid probe to specific organelles² or membrane proteins. By using this technique to pulse-liberate deuterated fatty acid species in live cells, coupled with lipidomic analysis, we are addressing the organelle- and species- specific metabolic bias of the fatty metabolism flux. More broadly, with this innovative chemical biology technique, we intend to gain new insights in lipid signalling and metabolism at an unprecedented level of specificity.

9- Estrada-Meza: Local translation as a molecular mechanism underlying tanycyte-neuron communication for energy balance regulation

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Energy balance requires a fine-tuned crosstalk between the periphery and the central nervous system. In the hypothalamus, tanycytes line the walls and floor of the third ventricle and extend their processes into brain parenchyma. This strategic location allows tanycytes to integrate peripheral metabolic cues and modulate neuronal function in consequence. Here, we investigate local translation as a molecular mechanism underlying tanycyte-neuron communication for energy balance regulation.

We use a wide variety of technologies including electron microscopy, puromycin, live imaging, In Situ Hybridization and immunofluorescence to study translation in tanycytes. We show the presence of endoplasmic reticulum, mitochondria, the mRNAs of multiple genes that regulate energy balance and translating ribosomes throughout tanycyte processes, strongly suggesting that local translation takes place in tanycytes. We also show that the nutritional context modifies the tanycytic ultrastructure for local translation. Interestingly, using electron microscopy 3D models, we highlight that at the interface of tanycyte-neuron contacts, both cells are enriched in endoplasmic reticulum-plasma membrane and endoplasmic reticulum-mitochondria contacts. These types of contact sites participate in intracellular calcium homeostasis, and tanycyte calcium transients have been shown to trigger tanycyte-neuron communication. Using live calcium imaging, we show that the nutritional context also modulates tanycyte calcium transients. Finally, preliminary results suggest that the molecular mechanism linking tanycytic nutritional status and local translation is calcium.

Our results suggest that tanycyte processes contain ultrastructure that could participate in tanycyte-neuron communication via calcium homeostasis and the distal localization of protein synthesis.

Keywords: tanycyte; hypothalamus; local translation; ribosomes; calcium, energy balance

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10- Faitg: Age-related muscle function decline and Urolithin A

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Age-related muscle function decline is a major contributor to frailty and reduced quality of life, with mitochondrial dysfunctions emerging as key drivers. However, the effects of aging on mitochondria and their inter-organelle communication remain poorly understood. In this study, we present a comprehensive 3D reconstruction of mitochondria to explore how aging influences mitochondrial morphology, volume, complexity, and organelle interactions across slow- and fast-twitch fibers in anatomically distinct muscles.

Our findings reveal striking muscle- and fiber-specific adaptations to aging, including divergent changes in mitochondrial volume and complexity in oxidative versus glycolytic fibers, and a broad decline in mitophagy and nanotunnel formation.

Remarkably, Urolithin A (UA)—a naturally occurring gut metabolite known to enhance muscle health by activating mitophagy and improving mitochondrial quality—reversed most of these age-related disruptions. UA treatment restored mitochondrial complexity in oxidative fibers, rescued type IIA fibers in aged tibialis anterior muscles, and revived critical mitochondrial structures such as mitophagosomes and nanotunnels. Additionally, UA increased the proximity of complex mitochondria to one another and to cytosolic lipid droplets, fostering inter-organelle communication that may contribute to UA's beneficial effects on muscle health.

These findings reveal the heterogeneous impact of aging on muscle mitochondria and organelle communications, and deepen our understanding of mechanisms that establish UA as a natural intervention to preserve mitochondrial and muscle function during aging.

11- Gaifullina: Overexpression of UCP4 in mitochondria of astrocytes corrects Alzheimer's disease associated dysfunction of Ca²⁺ and K⁺ conductances in neurons.

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Neuron-based therapies for Alzheimer's disease (AD) repeatedly face setbacks. As a consequence, attention is now shifting toward non-neuronal cells such as astrocytes, microglia, and oligodendrocytes. We recently demonstrated that targeting astrocyte mitochondria can avert AD-associated neurodegeneration in the 3xTG AD mouse model. Viral vector-mediated overexpression of mitochondrial uncoupling protein 4 (UCP4) in astrocytes of AD mice administered at 2 months and recorded at 7-10 months prevented degeneration and aberrant electrical activity of subicular neurons (SN), the major output of the hippocampus (HIP). Our next steps are to test whether treatment in older mice with overt AD symptoms is effective and to establish the underlying mechanisms of this neuroprotection.

Whole-cell patch-clamp recordings from 10-12 months old 3xTG mice injected with the UCP4 virus at the age of 6-7 months, revealed rescue of intrinsic electrical properties of SN. In line, UCP4-mediated improvement in the local HIP and SN circuits but not in the HIP-SN interregional communication was also observed. Our findings suggest that astrocytic mitochondrial uncoupling enhances neuronal resilience in symptomatic AD mice possibly through observed changes in the neuronal firing-dependent Ca²⁺ influx and A-type Kv4, potentially underlying the detected neuroprotective effects. We are currently studying the impact of UCP4 on astrocytic Ca²⁺ signaling to better understand its role in shaping the function of single-cells and neuronal populations.

12- Gorsek: Role of Lipid Heterogeneity in Early Embryonic Development

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Lipids play crucial roles in a plethora of biological processes, ranging from cellular organization to signalling. It has recently been shown that they are involved in regulation of transcriptional programs, uncovering a previously unknown function proposing their involvement in establishing cell identity, tissue patterning and developmental processes. Early embryonic development is an essential phase in an organism's life, during which the organism establishes body axes and initiates the formation of the basic structure of organs. While most of our knowledge revolves around proteins and their roles in differentiation and specialisation for distinct functions, the role of lipids in these processes remains unclear.

This project aims to understand the nature and significance of cell-to-cell lipid state heterogeneity on the single-cell level. Investigating the crosstalk between transcriptome and lipid states will provide an insight into how lipids regulate early embryonic development, establishment of body axes and tissue patterning. Our preliminary data shows that mouse embryonic stem cells in culture exhibit a high degree of lipid heterogeneity, which subsequently becomes spatially confined to a specific area in the *in vitro* mouse embryo model, possibly representing specific regions of the embryo. Given that a lipid state can influence the transcriptional program and cell state, we aim to understand how induced changes in lipid metabolism impact mammalian development. To this end, we plan to utilize the *in vitro* model of early development and combine it with lipid analyses to reveal the role of lipids and the consequence of abnormal lipid metabolism on the shaping of an organism.

13- Harhai: An updated inventory of genes essential for oxidative phosphorylation identifies a mitochondrial origin in familial Ménière's disease.

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Mitochondrial disorders (MDs) are among the most common inborn errors of metabolism and dysfunction in oxidative phosphorylation (OXPHOS) is a hallmark. Their complex mode of inheritance and diverse clinical presentations render the diagnosis of MDs challenging and, to date, most lack a cure. Here, we build on previous efforts to discover genes necessary for OXPHOS and report a highly complementary galactose-sensitized CRISPR-Cas9 "growth" screen, presenting an updated inventory now with 481 OXPHOS genes, including 157 linked to MDs. We further focus on FAM136A, a gene associated with Ménière's disease, and show that it supports intermembrane space protein homeostasis and OXPHOS in cell lines, mice, and patients. Our study identifies a mitochondrial basis in a familial form of Ménière's disease (fMD), provides a comprehensive resource of OXPHOS-related genes, and sheds light on the pathways involved in mitochondrial disorders, with the potential to guide future diagnostics and treatments for MDs.

14- Köck: MYCT1-IFITM2/3 interaction balances energy storage and efficient antiviral defense of the endothelium

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Starvation and infection are major threats to all living organisms. Vertebrates rely on a complex network of blood vessels to meet tissue demands for oxygen and nutrients, though this system also facilitates pathogen spread. Here, we find that the interaction of the pan-endothelial transmembrane adaptor protein MYCT1 with the viral restriction factor IFITM2/3 is crucial for balancing nutrient transport and antiviral defense. In endothelial cells, constitutively expressed IFITM2/3 are sequestered by MYCT1 restricting their endosomal accumulation. In the absence of MYCT1 or upon interferon exposure, IFITM2/3 accumulate in early endosomes, promote endolysosomal cargo degradation and hyper-activation of mTORC1 signaling in endothelial cells, limiting energy storage in white adipose tissue. Our results reveal a novel endothelial-specific mechanism balancing energy storage and antiviral defense in the vascular system.

15- Lagarrigue: LACTB, a new player in mitochondrial function and lipid modulation

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Mitochondria evolved from alpha-proteobacteria through endosymbiosis. Several mitochondrial proteins are evolutionarily related to bacterial proteins although not always keeping similar functional properties. A good example of this is Lactamase B (LACTB). LACTB, derived from the penicillin-binding/beta-lactamase protein family involved in peptidoglycan synthesis of bacteria, is in eukaryotic cells localized in the mitochondrial intermembrane space. Since mitochondria do not synthesize peptidoglycan, our working hypothesis is that LACTB may have gained one or more novel function(s).

To elucidate the physiological role of LACTB, we created a zebrafish LACTB KO using CRISPR-Cas9 technology. We did not observe change in body weight, length but a significant increase in surface area in KO fish. Spontaneous locomotion shows that LACTB KO spend less time over a slow distance, with an increase in pause time. We performed an incremental swimming test by gradually increasing water flow rate in a hermetic chamber. LACTB KO fish had a significant higher oxygen consumption than CTRL for the same speed, suggesting that KO may have a defect in mitochondrial efficiency or substrate utilization. Skeletal muscle of LACTB KO fish show an increase in mitochondrial circularity. Interestingly, muscle from LACTB KO fish show an increase in lipid droplet and gene expression related to lipid synthesis. In parallel, LACTB KO fibroblasts show an increase in lipid droplets with a defect in fatty acid oxidation. Taken together, our results reveal that LACTB plays a potential role in mitochondrial efficiency, shape and lipid homeostasis. We pursue the effort to understand the physiological and pathophysiological functions of this endosymbiotic protein.

16- Landaluce-Iturriria: Macronutrition and signal-induced regulation of RNA splicing in adipose tissue depots

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Obesity and insulin resistance (IR) are currently recognized as major public health concerns due to their high global prevalence. An increased consumption of energy dense diets, high in fats and sugar triggering obesity, and a complex remodeling of white adipose tissue (WAT) which in fine leads to tissue dysfunction. Interestingly, RNA-seq analysis from WAT samples from C57BL/6 mice fed with a high fat diet (HFD), replicating conditions of obesity and IR, has shown an enrichment of the spliceosome pathway. RNA binding proteins (RBPs), as part of the spliceosome or by binding cis-regulatory elements in the pre-RNA transcripts, modulate the recruitment of the spliceosome, splicing efficiency and alternative splicing (AS).

To investigate if WAT splicing alterations contribute to adipocyte dysfunction in response to macronutrient and signaling cues remains unknown. To study RBPs-AS networks in adipocytes at a molecular level, we established in vitro models using 3T3L1 adipocytes treated with different stimuli replicating the conditions found in vivo in IR and obesity patients (inflammation, high levels of FFAs, hyperglycemia, hyperinsulinemia...). These in vitro models serve as tools to investigate whether RBPs or specific AS events can be targeted pharmacologically to treat obesity RNA-seq was performed to measure the expression of RBPs [IL3] and AS alterations in WAT in vivo, using samples from the C57BL/6 model and in vitro using samples from the established 3T3L1 models. The comparison of HFD induced changes in WAT samples and inflammation and/or hyperinsulinemia induced changes in vitro highlighted alterations in the expression of hundreds of RBPs, including Rbfox2, Mbnl1, Srsf3 and Fmr1; as well hundreds of AS alterations. In vitro, most splicing changes were triggered by inflammation (TNFa treatment), while hyperinsulinemia had a surprisingly modest effect. Changes in AS identified in vivo and in vitro occur in genes involved in cell metabolism, growth and organization; including Slc22a17, Insr, Spag9, Mink1 and Apoe. These sequencing results will be used to describe RBPs-AS networks and better understand how macronutrient and signaling-regulated AS coordinate cellular and tissue function in the context of metabolism.

17- Landoni: Mitochondrial pearling mediates mtDNA nucleoid and microdomain distribution

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The mitochondrial network carries an essential multi-copy genome packaged in nucleoids. These are regularly spaced along mitochondria and confined by cristae invaginations, enabling the regionalized distribution of mitochondrial function along the network and the peripheral ejection of damaged material.

Mechanistically, however, it is unclear how such a constrained system with seemingly static nucleoids can dynamically manage its contents, segregate and distribute nucleoids, and sense and isolate damaged regions.

The mitochondrial tubule can spontaneously and reversibly collapse into highly regular compartments, a process known as “pearling”. Using adaptive super-resolution microscopy and pharmacological and genetic modulation of its occurrence, we identified pearling as a major driver of nucleoid splitting and regular distribution along the tubule, regulated by calcium and fission-adjacent molecular mechanisms. Additionally, we further investigate its putative role in aiding the acute local biochemical changes required for subsequent mitochondrial fission and quality control.

18- Lisci: FBXW7 alleviates c-MYC repression of pyruvate carboxylase to support metabolic flexibility

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Metabolic flexibility, or the ability to adapt to environmental fluctuations, is key to the survival and growth of all living organisms. In mammals, the pathways supporting cell proliferation in nutrient-limiting conditions have not been fully elucidated, although cancers are known to display metabolic dependencies that can be targeted for therapy. Here, we combine systematic nutrient and genome-wide CRISPR/Cas9 screening to provide a comprehensive map of the signaling and metabolic pathways that support cell proliferation in glutamine-limited conditions. We focus on pyruvate anaplerosis and discover a mechanism by which the tumor suppressor FBXW7 controls a MYC-dependent cluster of epigenetic repressors that bind the pyruvate carboxylase (PC) promoter, leading to histone deacetylation, reduced PC expression and glutamine addiction. Our work sheds light on the molecular mechanisms that support metabolic flexibility, and on the nutrients and pathways involved in glutamine dependency, a hallmark of several cancers.

19- Lopez-Alcala: CDK4 modulates the integrated stress response in triple-negative breast cancer cells

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During triple-negative breast cancer (TNBC) the epithelial cells that line the ducts or lobules of the breast increase their ability to resist death, sometimes even after chemotherapy treatment with inhibitors of cyclin dependent kinases (CDKs). The integrated stress response (ISR) is one of several conserved reprogramming mechanisms that allows cells to adapt to hostile conditions, through eIF2 α phosphorylation, stress granules (SGs) assembly, and activating transcription factor 4 (ATF4) increase, which could restore homeostasis. However, if the stress cannot be alleviated, the ISR triggers apoptosis. An RNA-Seq analysis of MDA-MB-231 CDK4-knockout (KO) cells showed a strong decrease in the ISR pathway compared to wild type (WT) cells, manifested by suppression of ATF4 and its targets, as confirmed by RT-qPCR. Intriguingly, the amount or activation of eIF2 α protein, the cornerstone of the ISR, was not altered. Cells were then exposed to sodium arsenite, one of the main inducers of ISR and SGs, or amino acid deprivation. Cells exposed to the treatments formed more SGs. Furthermore, KO cells exposed to stressors showed a lower amount of SGs than WT cells exposed to such stimuli, which could be related to a higher tolerance to stress, as KO cells demonstrated higher survival under these conditions. Interestingly, it has been reported that the specific reduction of ATF4 also achieves a positive increase in survival associated with long-term amino acid deprivation. In addition, phosphoproteomics data unveil that eukaryotic translation initiation factor 2B subunit 5 (eIF2B5) of the eIF2B complex (guanine nucleotide exchange factor for eIF2 α), might be a substrate of CDK4 kinase, which is devoid of phosphorylation at S610 when CDK4 is missing. Altogether, our results suggest that the ISR pathway flux of TNBC CDK4-ablated cells enhances their resistance to cell death induced by stress insults, and that this harmful phenotypic advantage deserves to be unraveled.

20- McKay: The role of the acid-sensing ion channel Asic1a in thermoregulation

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Acid-sensing ion channel 1a (Asic1a) is a proton-sensing ion channel that is expressed in the central and peripheral nervous system of both humans and mice. Our group previously reported lower core body temperature (T_c) in male Asic1a knock-out (KO) mice when compared to wild-type (WT) littermate controls during the dark phase. To investigate the mechanism behind this reduced T_c, we performed indirect calorimetry simultaneously with T_c telemetry at a range of environmental temperatures including thermoneutrality (30°C) and room temperature (22°C). Our results recapitulated our previous findings of reduced T_c in male Asic1a KO mice at room temperature. However contrary to prior findings, this difference was more prominent in the light-phase than the dark-phase. The decrease in T_c during the light-phase of Asic1a KO mice at room temperature was seen alongside a decrease in respiratory exchange ratio (RER), indicating increased use of fat as a fuel source, consistent with what typically occurs with mice at a lower temperature. These T_c changes were observed despite no change in body mass, nose-anus length, fat depot mass, or locomotor activity, indicating that insulation and physical activity changes are not the cause of reduced T_c in Asic1a KO males. Interestingly when male Asic1a KO mice were housed at thermoneutrality (30°C), no change in T_c was observed when compared to wild-type mice. Characterisation of adrenergic receptor and Ucp1 expression in brown and white adipose tissue depots is now underway. Differences in these parameters may explain the reduced T_c in Asic1a KO mice.

21- Pajot: Role of Ephrinb3 in POMC neurons in the control of energy balance

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Today, one in eight people in the world is obese, which can result in comorbidities such as diabetes or pre-diabetes, characterized by insulin resistance and glucose intolerance. However, the mechanisms leading to this onset remain to be elucidated. In the brain, proopiomelanocortin (POMC) neurons have been primarily described as regulating feeding behaviour. Nevertheless, mounting accumulating evidence also highlights the pivotal role of these neurons in the control of glucose homeostasis.

The present project aims to elucidate novel mechanisms underlying the control of energy and glucose homeostasis through the synaptic plasticity of POMC neurons, and subsequently their activity. A recent study conducted within our research group demonstrated that modulation of EphrinBs (EphrinB1, B2, B3) proteins, which are well-known actors in the formation and plasticity of glutamatergic synapses, resulted in a reduced number of glutamatergic inputs into POMC neurons. This, in turn, led to impaired glutamatergic-dependent activity and insulin secretion in response to hyperglycemia.

In order to ascertain the role of EphrinB3 in the control of synaptic plasticity of POMC neurons, we silenced (Pomc-Efnb3-KD) Efnb3 (gene encoding EphrinB3) in POMC neurons of adult male mice by means of stereotactic viral infusion and exposed the animals to a high-fat diet (HFD), a potent modulator of synaptic plasticity of POMC neurons.

The results obtained demonstrated that Pomc-Efnb3-KD male mice exhibited a greater body weight gain, accompanied an increased fat mass, compared to the control group.

22- Panfilova: Lipid droplets in neural stem cells show cell-state specific differences in their composition, potentially influencing cellular identity

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Lipid droplets (LDs) are protein-coated organelles that regulate storage and hydrolysis of neutral lipids, and they have been increasingly recognized for their versatility beyond energy storage. LDs have gained interest in the field of neuroscience because they accumulate in various cell types in neurodegenerative diseases. Our recent studies have shown that LDs are also present in healthy young brains in various cell types to a much greater extent than previously thought (Madsen et al., Nature Communications 2024). Furthermore, we have demonstrated that LDs play an important role in neural stem/progenitor cell (NSPC) metabolism and proliferation (Ramosaj, Madsen et al., Nature Communications 2021). However, it remains unknown whether LDs differ in their composition between cell types and how this may influence their behavior. To investigate a potential role of LDs in cellular identity, we established an LD isolation protocol using primary mouse-derived NSPCs. In this study, we generated a comprehensive LD proteome and lipidome dataset, comparing proliferative NSPCs, quiescent NSPCs (qNSPCs) and NSPC-derived astrocytes. Our data revealed clear cell-type specific differences in both the LD coat proteins and the stored lipids. Interestingly, the qNSPCs showed many LD coat proteins unique to this cellular state. CIDE (cell death-inducing DNA fragmentation factor alpha-like effector) proteins, which are LD-coat proteins known to regulate LD dynamics, were specifically and highly upregulated on LDs of qNSPCs. These proteins appeared to regulate LD size of qNSPCs and may have the potential to influence NSPC behavior. In summary, we have created the first proteome and lipidome of endogenous LDs isolated from NSPCs and their progeny and demonstrated that these datasets can be used to further understand the role of LDs in NSPCs behavior.

23- Parashar: Cyclin Dependent Kinase 4 (CDK4) acts as a brake for Triple Negative Cancer (TNBC) cell migration

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Cyclin-dependent kinase 4 (CDK4) is a cell cycle regulator promoting cell cycle progression through the phosphorylation of the retinoblastoma (Rb) during G1 phase. In the clinical context, CDK4/6 inhibitors, in combination with anti-hormonal drugs, effectively arrest ER/PR/HER2-positive breast cancer cells at G1, reducing cell proliferation. In contrast its effect on triple negative breast cancer cell type (ER/PR/HER2- negative; TNBC) remains limited. However, it has been proposed that a reduction in cell proliferation may inversely enhance cell migration, as both processes are energy-intensive. In addition to the established role of CDK4 in cell cycle regulation, emerging evidences points to additional functions of CDK4 in the control of other cellular pathways, including cell metabolism or cytoskeletal dynamics and cellular morphology that remains poorly understood. This study aims to elucidate the role of CDK4 in the organization and dynamics of cytoskeletal filaments. Using genetic, and phospho-proteomic analyses in CRISPR-mediated CDK4 knockout cells we revealed significant alterations in cellular morphology and differential phosphorylation of mostly actin cytoskeleton proteins. We further demonstrated that CDK4 restrains TNBC cell migration by maintaining cell front back polarity and limiting filopodia number. Mechanistically, phospho-proteomics analysis unraveled that CDK4-mediated Myo9b phosphorylation may modulate its RhoGAP function, thereby increasing RhoA activity. These findings uncover a previously unrecognized role for CDK4 beyond proliferation, identifying the Myo9b-RhoA axis as a potential therapeutic target in TNBC metastasis.

24- Poddar- Role of small intronic RNAs in the crosstalk between immune cells and β -cells during type 1 diabetes development

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High-throughput sequencing technologies have revealed a vast repertoire of small non-coding RNAs in mammalian cells. While the roles of microRNAs and other small RNA classes have been extensively studied, the functions of small intronic RNAs (sinRNAs) remain largely unexplored. In this study, we identified two sinRNAs, designated sinR-D and sinR-T, whose levels are elevated in pancreatic β -cells of prediabetic NOD mice, a well-established model of type 1 diabetes. In vivo RNA-tagging experiments demonstrated that these sinRNAs are packaged into extracellular vesicles released by CD4⁺ T lymphocytes infiltrating the islets of Langerhans and are subsequently transferred to β -cells during the early stages of type 1 diabetes. Functional analyses revealed that while overexpression of sinR-T had no significant impact on β -cell survival, elevated levels of sinR-D were associated with increased β -cell apoptosis. This suggests that the transfer of sinR-D contributes to the destruction of insulin-secreting cells and the onset of diabetes. Further investigation using pull-down assays with biotinylated sinRNAs showed that sinR-D interacts with Ago2, a core component of the RNA-induced silencing complex (RISC), indicating that this sinRNA may function through mechanisms similar to those of microRNAs. Collectively, these findings expand the known repertoire of small non-coding RNAs delivered to insulin-secreting cells via extracellular vesicles under diabetic conditions. They also highlight a potential role for sinRNAs in cellular regulation and disease progression.

**25- Popadin: Decoding the Puzzle of Human Somatic mtDNA Mutagenesis:
Bridging Species Life-History Traits and Organ-Specific Cellular Properties**

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Mitochondrial DNA (mtDNA) mutagenesis in human cancers is still enigmatic: external mutagens like UV light or tobacco smoke, known to impact nuclear DNA, don't seem to affect mtDNA. This discrepancy suggests a dominant, mitochondria-specific internal mutagen. To understand this, we analyzed mtDNA mutational spectra across a wide range of vertebrates, uncovering that some mutations (A>G on the heavy chain) are linked to internal, age-related chemical damage, likely oxidative (DOI: 10.1093/nar/gkac779). Others (C>T on the heavy chain) stem from internal replication processes (DOI: 10.1101/2023.12.08.570826). Leveraging our insights, we hypothesized that in somatic mtDNA mutagenesis, slow-dividing cells would exhibit more damage-related mutations, while fast-dividing cells would show a predominance of replication-associated mutations, i.e. we posited a variable damage-to-replication ratio (A>G/C>T) across tissues with different replication rates. To test this, we reanalyzed the two most comprehensive human mtDNA datasets: the TCGA, with 7,611 mutations across 37 cancer types and 21 tissues, and the GTEx, with 2,762 mutations in 25 healthy tissues. In both datasets, a higher damage-to-replication ratio was found in organs with slow-dividing cells (neurons, ovary, heart, skeletal muscles) and a lower ratio in those with rapid cell division (colon, intestine, stomach, vagina). Furthermore, a decrease in this ratio in late-stage somatic mutations across all cancer types (TCGA data) aligns with the anticipated increase in cell turnover during carcinogenesis. Our research shows that mtDNA mutations can reveal cell traits, unveiling their potential importance in biomedical applications.

26- Raja: Nicotinamide and Pyridoxine boost myonuclear accretion and enhance muscle hypertrophy in response to voluntary exercise

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Skeletal muscle growth, repair, and maintenance are critically dependent on the function of tissue-resident muscle stem cells (MuSCs). While their role in regeneration and tissue repair is well established, their contribution to muscle adaptations in response to exercise training and physical activity is less understood. We previously identified nicotinamide and pyridoxine (NAM/PN) as food derived bioactive nutrients that synergistically stimulate MuSCs and demonstrated that oral NAM/PN supplementation enhances muscle regeneration following acute muscle injury in mice and in humans. In this study, we used an in vivo Pax7mTmG/+ lineage-tracing model to evaluate that impact of oral NAM/PN supplementation on myonuclear accretion and muscle hypertrophy in an in vivo model of chronic exercise using 2 weeks of voluntary running in wheels with increasing resistance. As previously reported, exercise-induced muscle hypertrophy was observed exclusively in the soleus muscle and not in the tibialis anterior (TA) and associated with mild but specific transcriptional signatures by RNAseq in freshly isolated MuSCs. Using GFP tracing of Pax7-MuSC progenies and PCM1 staining of newly fused nuclei, we demonstrate that NAM/PN supplementation selectively increased myonuclear accretion in the exercise-responsive soleus, but not in the exercise-resistant TA muscle. Pax7mTmG/+ tracing after exercise also revealed two distinct populations of GFP+ fibers where a rare subset of GFP^{High} myofibers had more centralized nuclei, suggesting they may arise from contraction-induced myofiber damage. In contrast, a more abundant population of GFP^{Low} fibers lacked centralized nuclei and were increased and enlarged after NAM/PN supplementation. Our results demonstrate that oral administration of NAM/PN synergizes with chronic exercise to enhance myonuclear accretion and muscle hypertrophy. Our work also suggests an inter-fiber heterogeneity in the amount of myonuclear accretion after exercise and that NAM/PN is a new tool to uncover the mechanistic bases of heterogeneity of exercise-induced myonuclear accretion. Altogether, these findings establish NAM/PN as a promising nutritional solution to optimize muscle adaptations during exercise training.

27- Rey: Mitochondrial fatty acid β -oxidation in astrocytes is important for postnatal brain development and brain lipid homeostasis.

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The synthesis, degradation, storage and recycling of lipids within cells directly affect energy production, cell membrane turnover, signaling pathways, and lipotoxicity. Therefore, lipid homeostasis plays a crucial role in optimal cellular function across diverse tissues. In the brain, dysregulated lipid metabolism is linked to several neurodegenerative disorders, highlighting the significance of lipid homeostasis for normal brain function. Recent studies have emphasized the role of astrocytes in neutralizing toxic lipids from stressed neurons through lipid transfer and mitochondrial fatty acid beta-oxidation (FAO)^{1,2,3}. However, the contribution of astrocytic FAO to lipid homeostasis and postnatal brain development remains poorly understood. To investigate this, we characterized the expression pattern of one of the key enzymes of FAO, carnitine palmitoyl-transferase 1a (Cpt1a), and evaluated the functional role of FAO in astrocytes by selectively deleting Cpt1a at both early and late stages of postnatal development. Our findings demonstrate that Cpt1a is predominantly expressed in glial progenitors and astrocytes throughout the entirety of postnatal development and into adulthood. Single-nucleus RNA sequencing, lipidomics, behavioral tests, and imaging revealed that the deletion of Cpt1a in astrocytes disrupts lipid homeostasis, adversely affecting both astrocyte maturation and normal brain development.

28- Tabasso: Organellar DAG fatty acid composition is mediated by diet and alters mitochondrial function

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Obesity is related to muscle insulin resistance (IR). The lipotoxicity theory hypothesized that excess intramyocellular lipids overwhelm mitochondria, generating toxic intermediates, which specific lipid classes are still debated. DAG effects depend on subcellular localization, fatty acids (FA) stereoisomeric position and composition. DAG can be precursors of phospholipids (PL), which are parts of cellular membranes and mediate organelle function. In this work, we tested two hypotheses: 1) that modulating dietary FA intake in mice would modify muscle mitochondria and lipid droplet (LD) lipid composition. 2) that we could replicate the phenotype by modifying organellar DAG composition *in vitro*.

Mice fed with a 12-week saturated FA rich western diet (WD) became obese and IR. Stereoisomeric proportions of mitochondrial and LD DAG were unaffected, while DAG FA composition was directly linked to dietary FA supply. Among PL, PE and PG were modified by diet in LD. All PL classes FA composition were altered, with changes being class-specific and not correlated to diet or DAG composition.

To study the mechanisms behind organelle specific lipids, we used an organelle-targeted lipid delivery system. When delivering those DAG leading to IR in mice to plasma membrane, we replicated the *in vivo* phenotype in muscle cells. When targeted to mitochondria, DAG increase mediated mitochondrial function.

This study, combining *in vivo* phenotyping and *in vitro* mechanistic queries, points to the causal mechanism linking dietary FA composition to IR. This work will be repeated in humans to unveil organelle specific lipids roles.

29- Ziegler: CDK4 inactivation inhibits apoptosis via mitochondria-ER contact remodeling in triple-negative breast cancer

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The shift on energetic demands of proliferating cells during tumorigenesis requires an intense crosstalk between cell cycle and metabolism. Beyond their role in cell proliferation, cell cycle regulators also modulate intracellular metabolism of normal tissues. Nevertheless, in the context of cancer, where CDK4 is upregulated or stabilized, the metabolic role of CDK4 is barely understood. Accounting for 15-20% of breast cancer worldwide, triple-negative breast cancer (TNBC) is characterized by its aggressiveness and remains a challenging disease due to the lack of selective therapies. Using both genetic and pharmacological approaches, we aimed to determine the metabolic role of CDK4 in TNBC cells. Unexpectedly, CRISPR-Cas9-mediated deletion of CDK4 only slightly reduces cell proliferation of TNBC cell line and allows tumour formation. Furthermore, CDK4 deletion deeply affects mitochondrial morphology, leading to hyperfused mitochondrial network. Surprisingly, multiple pro-apoptotic stimuli including several chemotherapeutic agents, fail to induce proper cell death in CDK4-depleted or long-term CDK4/6 inhibitors-treated TNBC cells. Mechanistically, CDK4 depletion impairs mitochondrial-ER contacts thus reducing calcium fluxes upon pro-apoptotic stimuli. While CDK4/6 inhibitors constitute future valuable anti-tumoral therapeutic approaches, altered mitochondrial pro-apoptotic function upon CDK4 inhibition could generate cell-death resistant cells, further increasing future risk of cancer relapse in treated patients. In this work, we highlight CDK4's role in mitochondrial apoptosis and suggest that targeting MERCs-associated metabolic shifts could enhance TNBC therapy with CDK4/6 inhibitors.