Abstract Program

2025 Cell Biology and Physiology Research Day

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111 Mason Farm Rd Chapel Hill, NC 27599

UNC SCHOOL OF MEDICINE
Cell Biology and Physiology

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Proximity proteomics of RAMP3 identifies MYO6 as a regulator of adrenomedullin signaling in lymphatic endothelial cells

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The receptor activity-modifying protein 3 (RAMP3) heterodimerizes with the G-protein coupled receptor (GPCR), calcitonin receptor-like receptor (CLR), to form an adrenomedullin (AM)-receptor. RAMP3 allosterically regulates CLR to coordinate signaling, internalization, and endosomal recycling after stimulation by the pro-lymphangiogenic peptide, AM. Importantly, the host of cellular machinery that RAMP3 engages to regulate CLR remains unknown. Here, we utilize proximity proteomics to identify the RAMP3 interactome and characterize how it changes in response to AM ligand. The unconventional myosin VI (MYO6) protein, an actin-based molecular motor, was identified as a proximal RAMP3-CLR interactor. We then interrogate within primary lymphatic endothelial cells the role of MYO6 on AM-mediated signaling and functional cellular outcomes. We found that loss of MYO6 inhibits AM-mediated GαS, ERK, and AKT activation while simultaneously increasing PKA-activity. We further show that loss of MYO6 has broad cellular outcomes on AM-mediated adherens junction remodeling and migration. This RAMP3-CLR interactome shines light on a novel spatial-temporal regulator of AM-signaling within lymphatic endothelial cells.

Lymphatic activation of ACKR3 signaling regulates lymphatic response after ischemic heart injury.

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Ischemic heart disease is a prevalent cause of death and disability worldwide. Recent studies reported a rapid expansion of the cardiac lymphatic network upon ischemic heart injury and proposed that cardiac lymphatics may attenuate tissue edema and inflammatory mechanisms after ischemic heart injury. Nevertheless, the mechanisms through which hypoxic conditions affect cardiac lymphangiogenesis and function remain unclear. Here, we aimed to characterize the role of the adrenomedullin decoy receptor atypical chemokine receptor 3 (ACKR3) in the lymphatic response following ischemic heart injury.

Using the novel ACKR3-Tango-GFP reporter mice, we detected activation of ACKR3 signaling in cardiac lymphatics adjacent to the site of ischemic injury of left anterior descending artery (LAD) ligation. *Ackr3*$^{\Delta Lyve1}$ mice exhibited better survival and were protected from the formation of acute tissue edema after ischemic cardiac injury. *Ackr3*$^{\Delta Lyve1}$ mice exhibited a denser cardiac lymphatic network after LAD ligation, especially in the injured tissues. Transcriptomic analysis revealed changes in cardiac lymphatic gene expression patterns that have been associated with extracellular matrix remodeling and immune activation. We also found that ACKR3 plays a critical role in regulating continuous cell-cell junction dynamics in LECs under hypoxic conditions.

Lymphatic expression of ACKR3 governs numerous processes following ischemic heart injury, including the lymphangiogenic response, edema protection and overall survival. These results expand our understanding of how the heart failure biomarker adrenomedullin, regulated by lymphatic ACKR3, may exert its roles after ischemic cardiac injury.

The Serine Protease Htra2 is Required for Myoblast Differentiation

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Proteases are enzymes which permanently alter the structure of peptides by cleaving the covalent bonds between amino acids. In some cases, these alterations to the intracellular protein environment can act as a differentiation signal and regulate cell fate decisions. Using a biotin-conjugated probe, we measured the global serine protease activity within differentiating C2C12 mouse myoblast cells to identify potential protease drivers of muscle differentiation. Among other mitochondrial proteases, we identified the serine protease High Temperature Requirement Protein A2 (Htra2) as a highly active protease in myotubes which is required for myoblast differentiation. This effect on myogenesis is independent of Htra2's reported role in the mitochondrial unfolded protein response. We hypothesize that Htra2 monitors mitochondrial protein import in differentiating myoblasts and is required for proper mitochondrial biogenesis during myotube maturation.

Alternative splicing of a clathrin microexon is essential for proper skeletal muscle development

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Most mammalian genes are alternatively spliced into two or more distinct messenger RNAs. Skeletal muscle exhibits one of the highest levels of tissue-specific alternative splicing in mammalian tissues; the functional consequences of these tissue-specific splicing events, however, are not well understood. Clathrin heavy chain (Cltc), a main component of the clathrin triskelion, is alternatively spliced almost exclusively in skeletal muscle, heart, and brain. Alternative splicing generates two distinct Cltc transcripts: a long mRNA that includes a 21-nucleotide microexon (microexon 31) and a short mRNA that skips this microexon. In healthy skeletal muscles, the short Cltc transcript is predominant in fetal stages, whereas the long transcript is the primary form in adulthood. However, it was not known at which time during development the Cltc splicing transition occurred in skeletal muscles, or whether its mis-regulation would impact skeletal muscle development. We found that the splicing switch was mostly concentrated to the first four postnatal weeks of skeletal muscle development in mice. To determine the consequences of Cltc mis-splicing during development, we forced the skipping of Cltc microexon 31 in mice using CRISPR/Cas9 gene editing. We found that adult homozygous (HO) mice, which express only the short CLTC isoform throughout development, displayed increased skeletal muscle mass yet decreased skeletal muscle performance compared to wild-type (WT) mice. Mass spectrometry revealed extensive proteomic differences between genotypes. Skeletal muscles undergo dramatic structural remodeling during the first postnatal month; this is when transverse tubules (T-tubules) and neuromuscular junctions (NMJs) mature. Our transmission electron micrographs revealed that T-tubules and NMJs were strikingly abnormal in muscles of HO mice. Our data indicate that the inclusion of a 21-nucleotide microexon in Cltc is essential for the proper development of skeletal muscles.

A Novel G13-PKCθ-RAPGEF2-RAP1 Signaling Pathway Mediating Platelet Adhesion Under High Shear Rates

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Platelet plug formation is mediated by the activation of the integrin αIIbbβ3 on the platelet surface. Activation of αIIbbβ3 allows for binding of fibrinogen, which facilitates platelet aggregation by binding to other platelets. Activation of αIIbbβ3 is mediated by the small GTPase, RAP1, the activation of which strongly relies on strongly relies on signaling by G protein-coupled receptors (GCPRs). The predominant activator of RAP1 is the guanine nucleotide exchange factor (GEF) CalDAG-GEFI downstream of Gq-coupled GPCRs. However, our studies and existing literature show that loss of CalDAG-GEFI or Gq signaling does not completely abolish platelet aggregation, suggesting that other G protein signaling pathways may also be facilitating RAP1 activation. Recently, our lab has identified RapGEF2 as an additional RAP1 activator in platelets which is activated by GPCRs coupled to Gq and G13. We found that in response to low doses of the thromboxane A2 (TXA2) analogue, U46619, platelets lacking PKC θ displayed defective platelet aggregation, comparable to platelets lacking RapGEF2 or G13. Additionally, following treatment with aspirin and apyrase, we found that PKC θ -/- platelets exhibited reduced and reversible aggregation in response to high doses of U46619, also comparable to RapGEF2mKO (megakaryocyte lineage knockout) and G13 mKO platelets. Finally, we found that PKC θ phosphorylation decreases in G13 mKO platelets when compared to controls. Together, these findings (1) establish a mechanism of action of RapGEF2 in platelets, (2) provide a novel mechanistic explanation for the antithrombotic actions of aspirin, and (3) suggest PKC θ as a potential target for antiplatelet therapy.

ER Dynamics Control T Cell Immunity in Solid Cancer

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T cells play a vital role in the attenuation of tumor growth; which serves as the driving principle behind many cancer immunotherapies. It has been demonstrated that the tumor microenvironment (TME) stimulates organelle alterations in T cells detrimental to their antitumor function. While mitochondrial morphology in tumor infiltrating lymphocytes (TIL) have been well-characterized, limited investigation has been conducted into the TME's effects on the endoplasmic reticulum (ER) architecture. Here, we show that T cells entering the TME experience expansion of ER volume, and this expansion is associated with a dysfunctional T cell state. Furthermore, we demonstrate that this expansion is specifically induced by the hypoxic nature of the TME. Alteration of the ER morphology to prevent ER distention results in improved tumor control, highlighting, for the first time, the central role of ER morphology in T cell antitumor immunity.

Mechanism of Intracellular ANGPTL3 and ANGPTL8 Trafficking

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Lipids circulate in the blood in lipoproteins including chylomicrons and very low-density lipoproteins (VLDLs). Lipoprotein Lipase (LPL) is the main enzyme that hydrolyzes the triglycerides from circulating lipoproteins into free fatty acids that can be taken up by cells. Without LPL, dangerously high levels of lipoproteins circulate in the blood, which can lead to cardiovascular disease. LPL inhibitors, known as angiopoietin-like (ANGPTL) proteins, have key roles in the regulation of lipid metabolism. ANGPTL3 is a potent inhibitor of LPL. ANGPTL8 can form a complex with ANGPTL3 which results in more efficient secretion of ANGPTL8 and greater inhibition of LPL than ANGPTL3 alone. Based on immunofluorescence staining of LAMP1 (lysosomal-associated membrane protein 1), I have found that ANGPTL8 when expressed alone, gets trafficked toward lysosomes. However, when ANGPTL8 is co-expressed with ANGPTL3, both ANGPTL3 and ANGPTL8 are effectively trafficked out of the cell. Using the Retention Using Selective Hooks (RUSH) assay, I confirmed that a population of ANGPTL8, when expressed alone, gets trafficked to lysosomes. I have also found that ANGPTL3 and ANGPTL8 are synthesized and form a complex in the endoplasmic reticulum (ER) and get trafficked out of the cell together. Thus, ANGPTL3 diverts a pool of ANGPTL8 from lysosomal degradation. Future goals include understanding why ANGPTL8 is unstable when it is not in complex with ANGPTL3, as well as visualizing the trafficking dynamics of the ANGPTL3/8 complex.

The development of white matter astrocyte morphology and tiling in the *corpus collosum*

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Astrocytes are morphologically and functionally complex glial cells that play varied and critical roles in brain homeostasis, synapse development, and neurovascular coupling. via their extensive interactions with their tissue microenvironment. The morphological complexity of astrocytes is a prerequisite for their functional complexity, and disruptions to astrocyte morphology are observed in numerous neurological disorders. Our current understanding of the relationship between astrocyte form and function rests almost entirely on studies of protoplasmic gray matter (GM) astrocytes, which interact extensively with neuronal synapses. GM astrocytes have a well-defined set of morphological features which describe how they develop and interact with their microenvironment. For example, they tile with their neighboring astrocytes, form end feet which encapsulate the vasculature, and shape themselves around interactions with thousands of synapses. In contrast, we know shockingly little about the development and morphology of fibrous astrocytes in the white matter (WM). While WM constitutes only ten percent of the rodent brain, it is fifty percent of the human brain by volume and contains the highest glia-to-neuron ratio of any brain region. GM contains neuronal synapses that drive the development of GM astrocytes, yet WM is largely devoid of synapses. WM astrocytes support myelination and regulate signal propagation down the axon and these specialized functions are accompanied by their striking differences in morphology. A detailed characterization of WM astrocyte development and morphology does not exist, impeding our ability to understand the functional complexity of these cells in both health and disease. To address this knowledge gap, I designed a multi-viral labeling strategy to perform an in-depth comparison of GM and WM astrocyte morphology and tiling at multiple timepoints during postnatal mouse development. I established new imaging and analysis workflows to quantify

Investigating the roles of FXR1 in cardiac biology

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RNA-binding proteins (RBPs) are regulators of RNA biology, including splicing and translation. Alternative splicing is an RNA processing mechanism that allows single genes to produce multiple mRNA transcripts. The heart and skeletal muscle exhibit high levels of tissue specific alternative splicing, which contributes to the specialized contractile function of sarcomeres. The RBP Fragile X messenger ribonucleoprotein 1 autosomal homolog 1 (FXR1) is enriched in skeletal muscle and heart, where it is known to regulate the translation of multiple sarcomere mRNAs. Previously, our group demonstrated that Fxr1 exon 15 is alternatively spliced in a developmentally regulated and tissue specific manner. Recessive mutations in Fxr1 exon 15 are linked to congenital multi-minicore myopathy, an inherited neuromuscular condition where affected individuals often experience heart failure. Our work is concerned with (1) characterizing the impact of FXR1 translation regulation on the cardiomyocyte proteome, and (2) defining the functional consequences of Fxr1 exon 15 expression in the adult heart. We hypothesize that FXR1 controls cardiomyocyte contractility via translation regulation and exon 15 inclusion. To address the translational function of FXR1 in cardiomyocytes, we performed mass spectrometry on HL-1 cells with and without si-RNA induced Fxr1 depletion. Fxr1 depleted cells exhibited more downregulated proteins than upregulated proteins (versus control cells), suggesting that FXR1 primarily promotes translation. In agreement with previously proposed FXR1 functions, several of these downregulated proteins are involved in translation, RNA processing, actin dynamics, muscle contraction, and calcium ion homeostasis. We have also identified many novel candidates for mRNAs potentially regulated by FXR1. To dissect the role of Fxr1 exon 15 in heart function, we have developed a mouse model where exon 15 was deleted via CRISPR/Cas9 editing. We are beginning to assess the functional co

Understanding how actomyosin dynamics drive apical constriction

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Apical constriction is a critical cell shape change that drives cell internalization and tissue bending. This process is driven by the contraction of the actomyosin network and its attachment to adherens junctions on the plasma membrane. Dynamic remodeling of the actomyosin network is also fundamental to processes such as cell migration and division. However, how the actin cytoskeleton is regulated and functions during apical constriction remains poorly understood. Actin-binding proteins are thought to play critical roles in the remodeling process.

We therefore hypothesize that different actin-binding proteins act in distinct subcellular regions to dynamically regulate the actomyosin network during apical constriction. *C. elegans* gastrulation provides a valuable model to test this hypothesis. To investigate the function and regulation of actin-binding proteins in this context, we used CRISPR/Cas9 genome editing to endogenously tag all actin-binding proteins expressed in early embryos. We are now applying quantitative fluorescence imaging, RNAi, and targeted protein degradation to systematically map the spatial organization and dynamic remodeling of the actin network. Our goal is to determine how different actin-binding proteins coordinate in space and time to assemble, stabilize, and remodel the actomyosin network that drives apical constriction.

In utero fetal denervation impacts nephron progenitor's dynamics during kidney development

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Proper kidney function is intricately regulated by intrinsic mechanisms and extrinsic signals from peripheral nerves. Our lab has recently established that peripheral neurons composed of both sympathetic and sensory fibers innervate the kidney concomitantly during arterial differentiation, following guidance cues released by renal stromal cells. Moreover, renal nerves establish neuroeffector junctions with renal structures during development. Yet, it remains unknown whether renal nerves actively direct organogenesis. Preliminary studies from our lab showed that whole animal *Ntrk1* (TrkA) knockout leads to total renal denervation which results in a reduced number of glomeruli, and thereby nephrons, with an increase in glomerular diameter at postnatal day 0.5 (P0.5). To determine which type of nerve was responsible, we began by selectively ablating sympathetic nerves using a floxed-STOP Diphtheria Toxin A (DTA) allele crossed with a *DBHCre/+* driver. Using 3D light-sheet imaging and IMARIS analysis on control and DTA-ablated kidneys, we determined that sympathetic nerve ablation resulted in a decrease in total glomerular/nephron number and an increase in glomerular diameter at P0.5 and P3.5. Thus, we hypothesize that renal nerves, likely via sympathetic signaling, support proper nephrogenesis through modulation of nephron progenitor proliferation or differentiation. To test this hypothesis, we used 3D light-sheet imaging to quantitatively assess nephron progenitor dynamics between wild-type and Ntrk1 knockout kidneys at P0.5. Our results revealed a decrease in the nephron progenitor niche number and an increase in the number of nephron progenitor cells (NPCs) per niche in denervated kidneys. Taken together, these results suggest that signals from renal nerves could be essential for maintaining the balance between the self-renewal and differentiation of nephron progenitors.

Measurement of cellular traction forces during confined migration

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Cells migrate by exerting physical forces against the surrounding extracellular matrix. These forces are generated by actomyosin contractility and are responsive to both biochemical and physical stimuli. Traction force microscopy (TFM), which quantitatively measures these forces, is an indispensable tool to study cell migration. However, the majority of TFM applications have utilized flat planar geometries or homogeneous 3D hydrogels. To better understand how cells migrate through complex three- dimensional geometries, similar to those they might encounter invivo, we have developed a versatile traction force microscopy platform that enables the generation of compliant substrates in a variety of micron-scale, user-defined 3D geometries. Using this system, we have measured cell migration parameters, cellular traction forces, and nuclear deformation as cells navigate through complex 3D structures, including narrow confinements. Our initial analysis demonstrates cells migrate slower through compliant than rigid confinements and with a greater degree of nuclear deformation. While treatment with the myosin contractility inhibitor blebbistatin reduced nuclear deformation during transit, it did not significantly increase transit speed. High resolution imaging and TFM shows that fibroblasts exert significantly higher traction forces while migrating through constrictions as compared to channels without constrictions. Lastly, TFM revealed that fibroblasts use internal cytoskeletal forces to deform the nucleus rather than extruding the nucleus through constrictions during transit, at least under the rigidities and geometries studied here. Future work will probe the interplay between cytoskeletal and adhesive structures, biochemical signaling pathways, and the effects of substrate rigidity and geometry on cell migration.

Investigating the Role of Enteroendocrine Cells in Intestinal Epithelial Barrier Function

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Enteroendocrine cells (EECs) are rare nutrient-sensing cells in the intestinal epithelium that synthesize and secrete hormones, metabolites, and small molecules in response to nutrient and microbial cues. EECs and the hormones they produce are often dysregulated in gastrointestinal (GI) diseases such as inflammatory bowel disease (IBD). However, little is known about how EECs regulate their local intestinal environment, and how they may act in important roles in the development and pathophysiology of GI disease. Studies in our lab have previously indicated that EECs are essential regulators of intestinal cell biology and function. Through single-cell RNA sequencing and lipidomic analysis on intestinal tissues lacking EECs, our lab found that lipid metabolic processes and inflammatory pathways were altered in the absence of EECs. Furthermore, we discovered that several species of ceramides and glucosylceramides were altered without EECs. Ceramides are sphingolipids important for maintaining barrier integrity and function in epithelial tissues, including the intestine.

To investigate the mechanisms between EECs and ceramides, we analyzed intestinal tissue permeability and barrier function in EEC-deficient mice and in EEC-deficient human epithelial enteroids plated on transwells. We observed a significant decrease in barrier integrity and permeability compared to control enteroid cultures. The barrier function decrease in EEC-deficient enteroids was significantly exacerbated by the addition of Tnf-alpha. We rescued this phenotype by administering EEC hormones somatostatin and PYY on the EEC-deficient monolayers. We show that junctional proteins promoting a tight epithelial barrier are decreased and Claudin-2, a pore-forming junctional protein, is increased in human EEC-deficient models. Additionally, ceramide synthases that synthesizes long-chain ceramides, are altered in enteroid cultures, supporting our lipidomic analysis.

Misregulation of ceramides has been implicated

Investigating dynamic protein palmitoylation during cardiac and skeletal muscle development

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Emerging evidence suggests that protein lipidation, a post-translational modification (PTM) involving the covalent attachment of lipids, plays a critical role in tissue physiology and development. Among these lipid modifications, protein S-acylation—a reversible PTM characterized by the attachment of the fatty acid palmitate (palmitoylation) to cysteine residues—regulates protein function, signaling, and membrane tethering. This modification is catalyzed by a family of zinc finger and DHHC domain-containing acyltransferases (ZDHHC enzymes) and reversed by select acyl-thioesterases. Our recent quantitative proteomic analysis of murine embryonic heart development revealed that ZDHHC enzymes are enriched in early cardiogenesis, whereas acyl-thioesterases are more enriched in later stages, suggesting that the temporal regulation of palmitoylation dynamics contributes to distinct stages of cardiogenesis. Additionally, we found that ZDHHC enzymes and acyl-thioesterases are differentially expressed during myoblast differentiation, indicating palmitoylation is tightly regulated during specific time points of skeletal muscle development. However, the precise mechanisms by which palmitoylation influences muscle cell differentiation remain unclear. To quantify palmitoylation dynamics during muscle cell differentiation, we utilize acyl resin-assisted capture (Acyl-RAC) to selectively capture S-acylated proteins via a thiol-reactive resin in murine embryonic hearts and skeletal muscle myoblasts. Our results show that S-acylated proteins are enriched following thioester bond cleavage, providing a direct snapshot of the “S-acylome” during cardiogenesis and skeletal muscle cell differentiation. We will couple this technique with mass spectrometry-based proteomics to identify endogenous S-acylated proteins and elucidate key signaling pathways and cellular processes that are regulated by palmitoylation dynamics in cardiac and skeletal muscle development.

CD73 restrains mutant β-catenin oncogenic activity in endometrial carcinomas

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β-catenin is an important oncogene commonly (20-30%) activated by somatic missense exon 3 *CTNNB1* mutations in low grade, early stage endometrial cancer (EC). Although exon 3 *CTNNB1* mutation associates with increased risk of recurrence in 30% of patients with low grade, early stage EC, many patients with β-catenin mutant tumors will not recur. It remains unclear why patient outcomes are so variable. The variability suggests there are unknown determinants of β-catenin mutant tumor aggressiveness. The purpose of this study was to evaluate CD73, a cell surface 5'-nucleotidase, as a critical factor controlling mutant β-catenin oncogenic activity in EC. We previously reported that CD73 localizes wild-type β-catenin to the membrane, and we hypothesized that mutant β-catenin could be similarly localized. Accordingly, oncogenic activity was evaluated by assessing β-catenin localization, β-catenin transcriptional activity, and recurrence in *CTNNB1*-mutant tumors. We developed 7 patient-specific β-catenin mutant constructs (D32N, S33F, S33Y, G34R, S37C, S37F, S45F) for expression in EC cells with genetic deletion of CD73 and alternate EC cells with re-expression of CD73. We found a positive correlation between the loss of CD73 and nuclear β-catenin localization in EC tumors. Cellular fractionations showed that loss of CD73 in EC cells resulted in increased nuclear and/or chromatin mutant β-catenin expression. Loss of CD73 also increased transcriptional activity for all 7 β-catenin mutants. With re-expression of CD73 in alternate EC cells (normally $CD73^{-/-}$), transcriptional activity of some but not all β-catenin mutants decreased, which provides evidence for the first time that β-catenin mutants are differentially controlled in endometrial cancer. RNA-seq uncovered β-catenin mutant-specific transcriptomic changes with CD73 loss, such as dysregulation of oncogenic ZNF transcription factors and lncRNAs. Additionally, we found that CD73 downregulation in exon 3 *CTNNB1* mutant EC pred

Sex-Specific Regulation of Cardiac Excitation-Contraction Coupling by the RNA Helicase DDX3X.

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Sex disparities exist in cardiac anatomy and physiology and the prevalence of types of heart disease. These differences are often attributed to sex hormones. Through a series of proteomics- based approaches, we demonstrated that in addition to sex hormones, genes on the X- chromosome are both necessary and sufficient to regulate cardiac sex chromosome-derived protein differences. Genetic screens in mice identified RNA-binding protein DEAD-Box Helicase 3 X- Linked (Ddx3x) as a candidate for regulating these sex chromosome-dependent differences. Consistently, point mutations in DDX3X have been shown to cause cardiac defects in females predominantly, in both heart structure and proper action potential propagation. To date, no investigation into the cardiac function of DDX3X has been performed. We have generated a Ddx3x cardiomyocyte (CM) conditional allele in mice. My data demonstrate that Ddx3x is essential for embryonic cardiac viability, with females but not males dying prior to E12.5. I have shown that loss of Ddx3x in female CMs also causes broad dysregulation of protein expression at E10.5, including those associated with cardiac electrical signaling and cardiac muscle development. DDX3X has multiple proposed functions in RNA biology, including in translation initiation, splicing, and nuclear export, having been shown to bind and resolve secondary structures in mRNA. Consequently, we have immunopurified the endogenous cardiac interactome under physiological conditions and demonstrated that DDX3X interacts with ribosomal proteins in the developing heart. To identify the E10.5 DDX3X cardiac target mRNAs, we performed Enhanced Crosslinking and Immunoprecipitation (eCLIP) in parallel with ribosome profiling of female Ddx3x null hearts. Our findings show that DDX3X interacts with proteins and regions of mRNAs implicated in translation initiation, and with targets and proteomic differences characterizing dysfunction in excitation-contraction coupling (ECC). Based on th

The ARP2/3 Complex Regulation of Clathrin Dynamics is Induced through ACK

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Clathrin-mediated endocytosis (CME) is an important cellular process that plays a role in cell-cell communication, signaling, and migration. In addition to the canonical clathrin coated pits, clathrin can form large flat lattice structures, alternatively known as clathrin plaques. Clathrin plaques have been proposed to serve as a receptor hub, increasing signaling by increasing the lifetime of cargo and provide a structural role in straited muscle cells and osteoclasts. Recently, Arp2/3 branched actin has been shown to be involved in the breakdown of plaques. Consistent with this finding, we can induce accumulation of clathrin plaques in mouse fibroblasts (JR20 cells) via conditional knockout of Arpc2. This plaque accumulation is accompanied by a decrease in endocytosis of fluorescently tagged transferrin, a CME-dependent cargo. Identifying factors involved in the regulation of clathrin plaques is an area of ongoing research. Our JR20 cells present an interesting model to study this pathway because parental JR20 cells do not readily form plaques under typical experimental conditions, but form large, stable plaques upon Arpc2 deletion. Phospho- proteomic data comparing Arpc2 KO and control JR20 cells revealed that many CME- related proteins have increased levels of tyrosine-phosphorylation upon Arpc2 deletion. Immunofluorescence shows that plaques co-stain with a pan-phospho-tyrosine antibody. Alternatively, plaques which form naturally in ITGB1 null GD25 cells do not costain. This suggests the tyrosine phosphorylation drives ARP2/3 branched actin plaque resolution. Based on the phospho-proteomic data, we have identified activated-Cdc42 kinase (ACK), a non-receptor tyrosine kinase, as a potential plaque sensor upstream of Arp2/3 activation. Exogenously expressed mScarlet-tagged WT ACK shows preferential binding to large clathrin plaques over pits when imaged via TIRF microscopy. Additionally, ACK KO, small molecule inhibition, or overexpression of a kinase dead mutan

Elucidating the mechanism of ENaC activity reduction by CC-90009

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Epithelial sodium channel (ENaC) activity reduction is a potentially promising, mutation-agnostic therapeutic strategy for cystic fibrosis (CF) and other muco-obstructive lung diseases [1]. However, numerous ENaC-inhibiting drugs have failed in clinical trials due to efficacy, pharmacodynamics, and safety issues [1]. We have reported that the small-molecule cereblon E3 ubiquitin ligase modulating drugs CC-90009 and SJ6986 reduce ENaC activity in air-liquid interface (ALI) cultures of human airway epithelial cells (HAECs) [2]. Here, we interrogate the unknown mechanism by which this class of drugs reduces ENaC activity by knocking down cereblon (CRBN) and eRF3a (GSPT1), two proteins that interact with CC-90009 in its known mechanism of action (Figure 1A), in five HAEC lines using CRISPR-Cas9 [3]. The cells were grown into well-differentiated ALI cultures before treating with drug or vehicle control for 24h and measuring ENaC activity with a 24-channel transepithelial current clamp (TECC-24) amplifier [2]. For comparative mass spectrometry, ALI cultures of primary HAECs from three non-CF donors were treated for 18h with either drug or vehicle control. LC-MS/MS was performed using a Thermo Vanquish Neo/Orbitrap Astral.

CC-90009 reduced ENaC activity in cells that underwent no electroporation or electroporation with negative-control or GSPT1 sgRNAs. However, CC-90009 did not reduce ENaC activity in CRBN knockdown cultures (Figure 1B-F). The abundance of 56 proteins decreased (log2 fold change[treatment/control]≤-1) and 9 increased (log2 fold change[treatment/control]≥1) after CC-90009 treatment.

These data demonstrate that CC-90009 reduces ENaC activity in HAECs in a cereblon-dependent, eRF3a-independent fashion. This suggests that CC-90009 leverages the CRL4CRBN E3 ubiquitin ligase to cause the ubiquitination and subsequent proteasomal degradation of an unknown neosubstrate involved in regulating ENaC activity. While additional experimentation is required to identify t

Unraveling the Role of Protein Myristoylation in Embryonic Heart Development

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Congenital heart diseases (CHDs) affect nearly 1% of all live births. However, only 15–20% of CHD cases are attributed to known genetic causes, highlighting the need to uncover additional molecular mechanisms. Disruptions in sarcomere formation contribute to CHDs, yet the regulatory pathways governing this process remain elusive. Myristoylation is a lipid modification in which an N-myristoyltransferase (NMT1) attaches a 14-carbon myristic acid to the N-terminal glycine of proteins, regulating protein localization, interactions, and stability. Recent findings in *Caenorhabditis elegans* show that protein myristoylation is required for sarcomere formation and muscle function.
Further, previous studies show the loss of NMT1 activity impairs the differentiation and function of ESC-derived cardiomyocytes. Therefore, we hypothesize that protein myristoylation is critical for sarcomere formation and function during cardiogenesis. We first assessed the temporal and cell-type specific expression of NMT1 in the developing heart. Quantitative proteomic analysis revealed that NMT1 is abundant and dynamically expressed during cardiogenesis. Further, immunohistochemical analysis of heart tissue at embryonic day 9.5 (E9.5), E12.5, and E16.5 show that NMT1 is abundantly expressed in the heart chambers and is enriched in cardiomyocytes. To determine if protein myristoylation regulates sarcomere formation and function, we treated embryonic cardiomyocytes with an NMT1 inhibitor. We found that impaired
myristoylation leads to disrupted sarcomere organization and reduced sarcomere length. To further dissect the molecular basis of this phenotype, we are optimizing two approaches -- a metabolic labeling strategy and proximity proteomic methods (TurboID)-- to identify the myristoylated cardiac proteins involved in sarcomere assembly. These findings will provide insight into how myristoylation regulates sarcomere formation and may reveal molecular mechanisms and therapeutic targets for CHDs.

Studying the role of brain enriched E3 ubiquitin ligase TRIM9 in Alzheimer's Disease

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In the last decade, we have demonstrated that the brain-enriched E3 ubiquitin ligase TRIM9 regulates cytoskeletal dynamics, membrane remodeling, and netrin-dependent signaling pathways in all stages of neuron development, including the maturation of dendritic spines and electrophysiological activity. Moreover, TRIM9 protein levels increase in the adult brain and are maintained throughout adulthood. In the adult mouse, TRIM9 is enriched within the postsynaptic density (PSD), a proteinaceous rich region in the post synapse, containing neurotransmitter receptors, scaffolding proteins, and cytoskeletal elements. Our published proximity labelling experiments to characterize the TRIM9 interactome identified several proteins implicated in synaptic function and Alzheimer's disease (AD), including Tau. Here we investigate the role of TRIM9 in tau-mediated neurodegeneration, using the P301S (PS19) mouse model, primary neuronal cultures, and HEK cells, in order to understand the mechanistic role that TRIM9 plays in the adult brain. We found that Iba1 positive microglia are enriched in the hippocampus, entorhinal cortex, and amygdala of six month old *Trim9*$^{-/-}$*:PS19* mice compared to *PS19* only mice. Pathological tau (p-Tau stained with AT8) accumulation was significantly higher in the hippocampus of *Trim9*$^{-/-}$*:PS19* mice compared to PS19 only mice. Ongoing experiments are exploring if pathological tau accumulation is different in neuronal cultures from WT or *Trim9*$^{-/-}$ mice. Unbiased quantitative proteomics of the PSD fractions from six month old mice from each genotype showed that mitochondrial proteins were decreased in *Trim9*$^{-/-}$*:PS19* mice compared to *PS19* only mice. Isolated mitochondria from *Trim9*$^{-/-}$*:PS19* cortical tissues also show the lowest oxygen consumption rate through Seahorse Assay. Future experiments will determine the how TRIM9 affects mitochondria function in disease context, and explore the role of TRIM9-dependent tau ubiquitination in cultured neurons with tau pathology

TRIM9 Regulates Melanoma Adhesion, Actin Dynamics, and Motility

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Cell shape change and motility involve remodeling of the actin cytoskeleton, cell adhesions, and plasma membrane. How these cytoskeletal and membrane remodeling are altered in pathological states remains largely unknown. Netrin is an extracellular morphogen which promotes neuronal morphogenesis and cancer progression. Here we examine the role of a brain enriched E3 ubiquitin ligase TRIM9 implicated in netrin dependent neuronal morphogenesis in the context of melanoma. We previously identified that TRIM9 regulates netrin dependent actin dynamics and exocytosis in developing neurons. Deletion of murine Trim9 impairs neuronal migration, netrin induced axon turning, and axonal and dendritic branching, and increases exocytosis and filopodial stability.

TRIM9 alters the dynamics of the actin polymerase VASP at filopodia tips via non degrative ubiquitination. TRIM9 is expressed in other motile cells, but the non neuronal role of TRIM9 is unknown. TRIM9 was identified as a potential prognostic biomarker in melanoma and high TRIM9 expression correlates with low patient survival. Melanomas undergo phenotype switching, where three distinct phenotypes exist that are associated with differential gene expression. Single cell RNAseq data from patient-derived melanoma indicate TRIM9 is highly expressed in phenotypes that correlate with poor prognosis. Broadly our findings support the hypothesis that TRIM9 coordinates actin dynamics, adhesion, and exocytosis in melanoma to regulate cell motility and potentially invasion. We show that in several human melanoma lines TRIM9 protein is enriched and netrin is secreted. Here we examine the role of TRIM9 in regulation of focal adhesions, exocytosis, migration, and invasion.

Genetic loss of TRIM9 increased random migration velocity, but reduced directional persistence. We find that TRIM9 plays a role in promoting bleb like morphology and inhibits the ability of cells to durotax on soft shallow. Fluorescence recovery after photobleaching, Total inte

Twist1 promotes endothelial phenotypic transition during atherosclerosis

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Endothelial cell (EC) dysfunction during atherosclerosis has been traditionally assessed using a small number of markers, leading to disparate views of this process. More recently, limited investigation with single-cell 'omics' has resulted in the proposal that ECs undergo multiple alternative cell fate decisions during disease, but lack of lineage tracing and/or spatial localization of ECs complicates interpretation of these data. *TWIST1*, a causal gene for multiple atherosclerotic vascular diseases, is activated with disturbed flow in endothelial cells (ECs), and EC-*Twist1* knockout results in reduced atherosclerosis. However, it remains unclear how *Twist1* affects EC phenotype and plaque biology. We performed a combination of EC lineage tracing, *in situ* analysis and scRNA-Seq in *ApoE-/-* mice, both prior to disease and after 16 weeks of high-fat diet-induced atherosclerosis. We also performed these studies with EC-specific *Twist1* deletion. We found that EC phenotypic modulation during atherosclerosis is characterized by an upregulation of pro-inflammatory and partial EndMT gene programs, occurring simultaneously along a single cell fate transition. EC-*Twist1* deletion led to a significant reduction in lesion area, reduced EC migration into the lesion, and reduced EC phenotypic modulation. *TWIST1*-overexpression in HCAECs led to changes in atherosclerosis-relevant pathways, and integration with the mouse data identified *TWIST*-specific effects during EC phenotypic modulation *in vivo*. Our combined transcriptomic and *in situ* assessment of EC phenotypic modulation, coupled with *TWIST1* perturbation, has identified important cellular and molecular mechanisms by which ECs and *TWIST1* influence risk for human vascular disease.

Investigating neuroimmune and structural signatures of peripheral neurodegeneration

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While defects in the endo-lysosomal pathway are implicated in many neurodegenerative disorders such as Parkinson's and ALS, the underlying mechanisms remain elusive. From a forward genetic screen for microglia mutants in zebrafish, we uncovered a loss of function mutation in *atp6ap2*, a vacuolar ATPase lysosomal accessory protein gene. We previously showed *atp6ap2* deficient microglia have irregular shape, lack lysosomal acidification, and exhibit disrupted endo-lysosomal dynamics. This was accompanied by severely disrupted peripheral axonal projections. To more fully investigate neuronal *atp6ap2*, we are studying this gene in the mechanosensory posterior lateral line (pLL) system. Using dynamic in vivo imaging, we found progressive physical breakdown of axons, cell bodies, and terminals, accompanied by accumulation of macrophages around the pLL ganglia. The onset of macrophage infiltration appears prior to overt structural defects, suggesting possible early neuroimmune crosstalk. Using both live pH-sensitive-dye LysoTracker and genetically encoded neuronal pHLARE, we found pLL cargos lack proper acidification in *atp6ap2* mutants, and lysosomal transport appears disrupted and stalled. Loss of neuromast hair cells, the innervation targets of pLL, provide a means to assess functional implications of degenerating neurons. Next we will dissect cell- specific *atp6ap2* functions and cell-cell interactions in neurodegeneration using a Gal4-UAS-CRISPR system. We are developing quantitative label-free imaging and models to perform large-scale phenotyping after these genetic perturbations. We will examine early molecular signals that may mediate neuron-macrophage communication during degeneration. This work will shed light on endo-lysosomal dynamics in neuronal homeostasis and how macrophages may interact with dysfunctional neurons. Uncovering distinct macrophage interactions may serve as signatures of peripheral neurodegeneration, providing means for early detection and treatment.

Dimerization-dependent fluorescent proteins as a tool to study ER-LD contact sites

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Eukaryotic cells compartmentalize cellular processes through membrane-bound organelles that can interact with one another at membrane contact sites (MCSs) to coordinate functions. These interaction sites, often spanning less than 30 nm between organelles, are difficult to visualize in live cells. Lipid droplets (LDs) are dynamic organelles that store neutral lipids, originate from the ER, and are enclosed in a lipid monolayer. Proteins key to LD function, such as triglyceride synthesis and lipid metabolism, target to LDs from either the cytosol or through the ER at MCS. To better understand the function of these MCSs and how they affect LD morphology and lipid distribution within cells, we have applied novel biosensors using dimerization-dependent fluorescent proteins (ddFPs) to label MCS in a live-cell context. This system pairs a weakly fluorescent A monomer with a non-fluorescent B monomer to produce a bright fluorescent signal when the two monomers are in close proximity, and can be detected using confocal microscopy. Using ddFPs targeted to ER and LDs, we can effectively and specifically label ER-LD MCSs in a live-cell context. We have shown that our tool effectively identifies changes to ER-LD contacts under conditions of LD biogenesis. Upon supplementation of the common dietary fatty acid oleic acid to induce LD biogenesis, we identified increased ER-LD contacts, detected through higher total ddFP intensity around LDs. These contacts also appear to be enriched on larger LDs. This tool can also be used to induce MCSs through transfection of high amounts of ddFP. Inducing such contacts increases LD size that is specific to ER-LD MCSs and is not present with other ddFP monomer pairings, such as mitochondria-LD MCSs, which also participate in lipid metabolism. Overall, ddFPs are a useful tool to aid our understanding of ER-LD MCS.

Elucidating the Role of Centrosome Positioning in Oriented Cell Divisions

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During development, the orientation of cell division is crucial for balancing differentiation, proliferation, and stem cell maintenance. In the epidermis, basal progenitor cells divide either parallel to or perpendicular to the epithelial surface. Division orientation dictates fate outcomes; for example, planar divisions are symmetric and self-renewing, while perpendicular divisions are asymmetric and differentiative. The scaffolding protein LGN/Gpsm2 is an evolutionarily conserved key player in regulating this choice. Previous work in our lab has shown that LGN localizes to the apical cell cortex during prophase, and that LGN is required for perpendicular divisions. While this role for LGN follows the classical dogma that it and its family members (e.g. Pins in *Drosophila* and GPR-1/2 in *C. elegans*) play in regulating asymmetric divisions, other studies suggest that oriented cell divisions in epidermal progenitors are more complex. As one example, our lab found that basal progenitors remain uncommitted to their ultimate plane of division until well beyond metaphase, and that LGN promotes perpendicular “telophase correction” in this context. This led us to explore whether LGN may also play roles in centrosome migration and positioning prior to the establishment of the mitotic spindle. Because centrosomes are localized apically in interphase basal cells, and in many other systems mother and daughter centrioles show differential microtubule organizing center (MTOC) activity, a simple model to explain establishment of the spindle plane would be migration of one centriole pair basally to establish a perpendicular axis. In this scenario, perpendicular divisions would be the “default” state, and planar divisions would occur following spindle rotation by 90°. To our surprise, we find that in the absence of LGN, both centrosomes appear to migrate to lateral positions prior to spindle formation, suggesting a novel role for LGN in regulating centrosome positioning and MTOC act

Calcium regulated plasma membrane expansion and remodeling during neuronal morphogenesis

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The plasma membrane (PM) of a developing neuron undergoes dramatic expansion and remodeling to establish the morphology necessary for neural network connectivity and function. The insertion of new membrane material is facilitated by Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated exocytosis, which is required for neuronal morphogenesis. Two SNARE proteins, Vesicle-associated membrane protein (VAMP) 2 and VAMP7, are enriched in the embryonic brain and are associated with distinct vesicle populations even prior to synapse formation. While knockout studies in mice have shown that both VAMP2 and VAMP7-mediated vesicle fusion are required for proper neuronal morphogenesis, the specific mechanisms regulating the trafficking and fusion of these vesicles during development is not known. Here I show that VAMP2 and VAMP7 mediate non-synaptic exocytic events that cluster in different areas of the developing neuron, suggesting the existence of distinct regulatory pathways governing their distribution and fusion. Additionally, my preliminary data indicate that VAMP2-mediated exocytosis is sensitive to Ca^{2+} chelation, whereas VAMP7-mediated exocytosis is not, mirroring the differential Ca^{2+} sensitivity of VAMP2 and VAMP7 vesicle pools observed at the synapse of mature neurons. This suggests a potential role for Ca^{2+} signaling in the regulation of VAMP2 and VAMP7-mediated exocytosis during neuronal morphogenesis. Endoplasmic reticulum (ER)-PM membrane contact sites, key regulators of Ca^{2+} signaling, may also be involved in this regulatory process. Here I investigate the relationship between Ca^{2+} signaling, SNARE-mediated exocytosis, and ER-PM contact sites in stage 2 and stage 3 neurons and how they are contributing to neuronal shape change during development.

Coro1A and TRIM67 collaborate in netrin-dependent neuronal morphogenesis

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Neuronal morphogenesis depends on extracellular guidance cues accurately instructing intracellular cytoskeletal remodeling. Here, we describe a novel role for the actin binding protein Coronin 1A (Coro1A) in neuronal morphogenesis, where it mediates responses to the axon guidance cue netrin-1. We found that Coro1A localizes to growth cones and filopodial structures and is required for netrin-dependent axon turning, branching, and corpus callosum development. We previously discovered that Coro1A interacts with TRIM67, a brain enriched E3 ubiquitin ligase that interacts with a netrin receptor and is also required for netrin-mediated neuronal morphogenesis. Loss of Coro1A and loss of TRIM67 shared similar phenotypes, suggesting that they may function together in the same netrin pathway. A Coro1A mutant deficient in binding TRIM67 was not able to rescue loss of Coro1A phenotypes, indicating that the interaction between Coro1A and TRIM67 is required for netrin responses. Together, our findings reveal that Coro1A is required for proper neuronal morphogenesis, where it collaborates with TRIM67 downstream of netrin.

Regulation of delamination behavior of basal keratinocytes in the murine epidermis

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Development of the embryonic skin is dependent on the cell fate choices of basal keratinocytes. These progenitor cells contribute to epidermal stratification and differentiation through two primary mechanisms: 1. Delamination and 2. Oriented Cell Divisions (OCDs). Delamination is a non-mitotic differentiative event wherein a basal cell loses contact with the underlying basement membrane (BM) and adopts the molecular identity of a spinous keratinocyte. In contrast, OCDs produce differentiated daughter cells through perpendicular, asymmetric divisions. Our lab has previously described several key pathways that regulate OCDs, including both spindle orienting machinery and cell-cell adhesions. Whether integrin-based adhesions regulate these two processes and if delamination or OCD dysregulation may contribute to post-natal skin fragility disorders is unknown. To address this knowledge gap, we used an ultrasound-guided lentiviral gene inactivation method developed in the lab (LUGGIGE) to knock down expression of Integrin-β subunits—core components of basal receptors responsible for basement membrane attachment in the developing mouse epidermis—and the Integrin-β4 ligand, Laminin-α3. Through novel approaches to identify actively delaminating cells, we demonstrate that integrin adhesions, likely through Laminin-α3 binding, is required for resisting delamination in the developing skin. Additionally, we show using immunofluorescence microscopy and transmission electron microscopy (TEM), that loss of basement membrane attachment sites, and not basal keratinocyte polarity, correlates with delamination in Integrin-β knockdown cells. We are also analyzing telophase correction, a division orientation refinement program, and delamination in real time through *ex vivo* live imaging of Integrin-β1 and 4 knockdown tissue. This work is the first to identify a direct regulator of delamination *in vivo*, and thereby bolsters our knowledge of the mechanisms guiding development of the epiderm

Characterizing the Role of the Vinculin Isoform, Metavinculin in Force Transmission

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Vinculin (Vcn) and its larger splice isoform, Metavinculin (MVcn), are core adhesion proteins that link the actin cytoskeleton to the cell periphery to modulate adhesion formation, strength, and motility. Vcn, a well-characterized mechanosensory protein, tightly links filamentous actin (F-actin) into bundles or stress fibers, which are key to maintaining adhesion strength and force transmission. MVcn is limited to smooth muscle and cardiac tissue and is expressed sub- stoichiometrically compared to Vcn. Yet MVcn's role in force transmission, cell morphology, and adhesion formation is understudied. Notably, patients with MVcn-specific mutations show defects in actin organization, cell morphology, and development of the often-fatal group of cardiac diseases, cardiomyopathies (CM). While both Vcn and MVcn bind F-actin, MVcn lacks F-actin bundling capabilities. When increasing amounts of the actin-binding tail domain of MVcn are mixed with Vcn *in vitro,* actin-bundling progressively diminishes. These findings suggest that MVcn may negatively regulate F-actin bundling when co-expressed with Vcn. Moreover, MVcn CM mutations promote higher-order yet more disordered F-actin assemblies. **Thus, I hypothesize that MVcn acts as a negative regulator of Vcn to fine-tune cell morphology and force transmission**. To understand how the stoichiometric relationship between the isoforms affects actin-associated functions, I will utilize murine embryonic fibroblasts that conditionally express mScarlet-tagged MVcn. Under differing doxycycline concentrations, I can mimic the endogenous sub-stoichiometric expression of MVcn to test differences in actin organization, motility, and focal adhesion characteristics by subjecting cells to fluorescent confocal microscopy, random motility assays, and 2D traction force experiments. Additionally, I will repeat these experiments in fibroblasts expressing the R975W MVcn CM mutant that has been previously shown to perturb higher-order actin structures.

Installation of Poly(A) Signals in the Human UBE3A-ATS Using Cytosine Base Editors.

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Angelman Syndrome (AS) is a rare genetic neurodevelopmental disorder resulting from loss of function of the maternal *UBE3A* ubiquitin ligase gene, for which there exists no cure. In neurons, the paternal *UBE3A* allele is silenced through genomic imprinting by an antisense transcript called the *UBE3A-ATS*. As such, loss of the maternal allele results in a functional lack of neuronal *UBE3A* expression. Previous research has shown unsilencing of paternal *UBE3A* and behavioral rescue in AS mice following treatment with an adeno-associated viral vector with Cas9 targeting the *ATS.* However, it has become clear that AAV genomes integrate at the site of Cas9-induced double stranded breaks (DSBs). These integrations contribute to unsilencing but are random, uncontrollable, and unacceptable for human therapies. Cas9 DSBs may also result in increased genotoxicity *in vivo*. To avoid AAV integration, we are investigating cytosine base editors, which consist of a cytosine deaminase fused to a nickase Cas9, allowing for precise, stable C→T nucleotide conversion without generating DSBs. Preliminary data in mouse cortical neurons indicates that use of a cytosine base editor to install a poly(A) signal results in early termination of the *ATS* and unsilencing of paternal *UBE3A*. Using computational tools, we have identified sites in the human *ATS* where a polyadenylation signal could be installed, which has allowed us to design human guide RNAs. Preliminary studies have revealed a human SaCas9 guide, cg146, which installed the expected edit in a reporter experiment and appears to generate a functional Poly(A) signal. We plan to screen this guide RNA as well as others *in vitro* using human AS model iNeurons. Furthermore, we will test lead candidates *in vivo* by administering AAV containing our base editor and human gRNAs to AS model mice with a human *ATS* knockin to confirm phenotypic rescue.

Regulation of gastric epithelial proteins in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest adult cancers with a 5-year survival rate of only 13% and is one of the top five leading causes of cancer-related deaths for men and women in the United States. These dire statistics underscore the need for a better understanding of the mechanisms that promote pancreatic cancer initiation and progression. Recently, the gastric epithelial genes Gastrokine (Gkn) 1 and 2, as well as trefoil factor 1 (Tff1), have been shown to be *de novo* upregulated in metaplastic epithelial cells in pancreatic tumorigenesis. These gastric proteins are abundantly expressed by normal stomach epithelial cells and are considered tumor suppressor proteins in gastric cancer. However, the triggering event(s) and functional relevance of these proteins in pancreatic neoplasia and cancer remain unclear. Here, we investigate the etiology of the gastric program expression and identify signaling pathways that are required for oncogenic Kras-mediated modulation of this gastric program in pancreatic cancer. Expanding our knowledge of the upstream regulatory networks will help illuminate how a gastric identity gene program emerges early in transformed pancreatic epithelium and acts as a potential impediment to the progression of this aggressive cancer.

Intracellular pH Dynamics as a regulator of Cell Cycle Progression and Genome Stability

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Intracellular pH (pHi) is dynamic within the cell cycle, but how pHi mechanistically directs cell cycle progression leading to division and impacts genome stability is unknown. Here, we utilize selective ion transporters inhibitors to manipulate intracellular pH within metastatic-derived lung cancer cells, H1299s. We demonstrate by flow cytometry how intracellular pH decreases as a result of the inhibition of ion transporters on the plasma membrane. We then investigate the cell cycle effect from alterations in pHi. Utilizing the PIP-FUCCI system that allows delineation of cell cycle phases, we found that decreasing the pHi in H1299s leads to a G1 arrest by live cell imaging. Following this finding, we discuss future directions and anticipated results involving origin licensing, genome instability, and CDK activity.

APOE4 Disrupts Lipid Droplet Metabolism in Astrocytes

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Alzheimer's disease (AD) is the most common neurodegenerative disease and the global leading cause of dementia. The strongest genetic risk factor for the development of late-onset AD is the *APOE4* allele, which encodes for a variant of the secreted protein apolipoprotein E (APOE). APOE is highly expressed in astrocytes, glial cells that play important roles in brain lipid metabolism. Astrocytes store lipids in spherical organelles called lipid droplets (LDs). Neutral lipids such as triglycerides and sterol esters are stockpiled in the LD core, which is enveloped in a monolayer of phospholipids decorated by an extensive network of proteins. When astrocytes were treated with the common dietary fatty acid oleic acid (OA) to promote lipogenesis, we observed a marked shift of APOE from the secretory pathway to the surface of LDs. We also found that astrocytes expressing APOE4 exhibited larger and fewer LDs than APOE3-expressing astrocytes following oleic acid pulse-chase, and that the LDs in *APOE4* cells were enriched in unsaturated triglycerides. These observations suggest that APOE plays a role in LD turnover. LDs can be turned over by two distinct pathways: lipolysis via cytoplasmic lipases, or macroautophagy (called lipophagy). Following pharmacological inhibition of these two pathways of LD turnover, we observed that the effect of inhibition of lipolysis but not lipophagy was occluded in *APOE4* astrocytes. This suggests that APOE regulates LD size and composition by modulating lipolysis, and that APOE4 expression disrupts this function. However, co-immunoprecipitations failed to show any physical interaction between APOE and adipose triglyceride lipase (ATGL), the most upstream lipase in the lipolysis pathway. Therefore, we performed affinity purification mass spectrometry to identify novel protein-protein interactions of APOE on the lipid droplet interface. This work will elucidate a novel function of APOE and potentially provide a new avenue of drug development in AD

Elucidating the role of VSMCs in guiding kidney innervation

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Effective alternative therapeutic strategies for kidney disease, such as *ex vivo* tissue engineering, will rely on sufficient vascularization and innervation. This necessitates a thorough understanding of the developing vasculature and how it interacts with both the nerves and the kidney. Despite the importance of innervation and vascularization for proper kidney development, there is still little known about how signaling between the nerves and the vasculature influences kidney organogenesis. To interrogate this, we use iDISCO+ tissue clearing with light-sheet microscopy to elucidate the mechanisms of neurovascular development in the kidney. During the onset of kidney innervation at embryonic day 13.5 (E13.5) in mice, nerves are tightly associated with vascular smooth muscle cells (VSMCs) in wild-type mice as well as in a model of aberrant vascular patterning. This association is tightly maintained throughout development. Using a mouse model of denervation, we found that loss of nerves decreases kidney glomerular number and increases tubule diameter, indicative of impaired nephron development. Taken together, we hypothesize that VSMCs play a critical role in guiding nerves to their appropriate location which is required for sufficient nephrogenesis. We have used a SMC Cre mouse model paired with a diphtheria toxin (DTA) allele to assess the impact of ablating VSMCs on kidney development. Our preliminary results suggest that VSMC ablation after the establishment of innervation alters nerve patterning in the kidney. This suggests an important role for VSMCs in guiding proper innervation of the kidney. Future studies will continue using this VSMC ablation model to assess the role of these cells at different stages of organogenesis, uncovering their contribution to normal kidney development and innervation.

Role of branched actin assembly and disassembly at sites of actin-endomembrane Interaction

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Branched actin localizes to a wide variety of intracellular membrane surfaces, also called endomembranes, where it operates as a cellular force generator and membrane insulator. Cellular membrane trafficking is critical for cell function: regulating processes such as cargo sorting and molecular transport, degradation, secretion, response to pathogens. *We hypothesize that both polymerization and depolymerization of branched actin regulate endomembrane trafficking and maturation.*

I am developing targeted approaches to disrupt endomembrane-localized branched actin in a cultured mammalian cell system. I will disrupt actin branch assembly by depleting proteins critical to actin branch formation, promote actin branch disassembly by overexpressing branch disrupting proteins, and promote actin branch stability by depleting actin debranching proteins. Aim 1 establishes a baseline of branched actin involvement in endomembrane compartment homeostasis and trafficking. Aim 2 and Aim 3 complement each other by focusing on two different aspects of branched actin dynamics: branch formation (Aim 2) and branch disassembly (Aim 3).

With deeper understanding of the role of branched actin at endosomes and lysosomes, further questions can be asked about the role of actin at other organelles or how actin structures transition between different intracellular compartments. Understanding the roles and regulation of actin-dependent membrane trafficking within and between organelles is valuable for understanding homeostatic cell function and disease states with underlying alterations in membrane trafficking, such as tumor metastasis, viral infection, and protein processing disorders. Actin has been demonstrated to localize to endosomes, but there is much to discover about actin's role in endomembrane maturation, trafficking, and fusion.

Interrogating the Cell Death Effects of IL-22 on Intestinal Stem Cells

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Interleukin (IL)-22 is a cytokine upregulated in inflammatory bowel disease[1], though its role on intestinal stem cells (ISCs) is not fully elucidated. IL-22 is primarily thought to induce intestinal epithelial regeneration, with IL-22 knockout mice showing impaired healing and more severe disease[2]. However, previous work from our lab shows decreased ISC expansion after IL-22 treatment, suggesting this epithelial repair is not stem cell-driven[3]. Thus, I hypothesize that rather than solely promoting proliferation, IL-22 also induces death of intestinal stem cells. To interrogate these cell death effects of IL-22 on ISCs, a 2D crypt system developed in the lab called a planar crypt microarray (PCM) was used to allow for compartmentalization of ISCs *in vitro.* PCMs were treated with PBS or IL-22 and single cell RNA sequencing was ran to probe for cell death gene expression changes. Cell death was also measured on these PCMs through immunofluorescence staining of the apoptotic effector caspase, cleaved caspase 3. PCMs have a decreased percentage of stem cells after IL-22 treatment, as well as a lower "stem cell score" based on our lab's published differentially expressed colon stem cell genes[4]. IL-22-treated PCMs also show increased expression of apoptotic initiator caspase (CASP) genes *CASP8* and *CASP10*. In addition, at the protein level, there is a significant increase in cleaved caspase 3 staining over PCM microholes after IL-22 treatment. Together these data define a novel role of IL-22 in inducing apoptosis of intestinal stem cells. This mechanism will be further studied to determine whether IL-22 is acting through CASP8 or CASP10 or an alternative mechanism to promote apoptosis. As phase 2 clinical trials are ongoing using IL-22 as an inflammatory bowel disease therapy to promote mucosal healing[5], understanding a potential role of IL-22 in inducing death of intestinal stem cells is vital for patient outcome.

Elucidating the role of lymphatic vessel integrity in CGRP induced migrainePathophysiology

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Migraine is one of the most common disorders worldwide, affecting females three times more than men1. During migraine attack calcitonin gene related peptide (CGRP) is elevated in the dural and cerebral blood supply and cerebrospinal fluid (CSF) causing pain, vasodilation of dural vessels, and extravasation of plasma proteins resulting in immune activation2,3. CGRP acts through its cell membrane associated g protein-coupled receptor calcitonin receptor-like receptor (CLR) and its associated protein, receptor activity modifying protein 1 (RAMP1). CLR-RAMP1 are abundant in and crucial for maintenance of lymphatic vessel integrity in development and adulthood4,5. Our lab previously demonstrated that lymphatic endothelial cells (LECs) treated with CGRP results in tightened linear cell-cell junctions *in vitro* and reduced CSF drainage by meningeal lymphatic vessels *in vivo*6. These data suggest that under conditions of high CGRP, as in the dura during migraine, the permeability and drainage functions of meningeal lymphatics are significantly attenuated. To test the hypothesis that impaired meningeal lymphatic vessel function leads to increased intensity or duration of CGRP mediated migraine pain, we are utilizing a genetically engineered mouse model wherein cell-cell junctional protein, vascular endothelium cadherin (VE-Cadherin), is knocked out in lymphatic endothelial cells. Preliminary grimace assay data indicates that at one day of nitroglycerine (NTG) induced migraine, mice deficient in VE-Cadherin do not differ in pain behavior from wildtype controls. Subsequent days of migraine induction are being analyzed. Interestingly, female animals exhibit greater pain at baseline, prior to migraine induction, than males regardless of genotype.

Molecular drivers of CD8 T cell exhaustion across cancer and chronic infection

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Although CD8 T cells are crucial to the antitumor immune response, persistent antigen stimulation drives T cell differentiation into a dysfunctional or exhausted cell state. To resolve the factors involved in the development and maintenance of exhaustion our lab performed paired scRNAseq and scATACseq on antigen specific CD8 T cells across 11 conditions, including acute and chronic infection, and 4 orthotopic mouse tumor models. Upon integration, we identified numerous transcription factors (TFs) predicted to influence gene expression and regulate specification. Various members of the NFkB TF family were enriched across CD8 T cell states. Although NFkB is of known importance to the immune response, the exact role in CD8 T cells responding to chronic antigen remains unclear. We employed genetic mouse models to deplete *Ikbkg* (encoding IKKγ), the master regulator of canonical NFkB signaling, in the tumor microenvironment and in chronic infection. We found that IKKγ maintains the long-term persistence of exhausted CD8 T cells in cancer and chronic infection. IKKγ may also enforce distinct gene expression programs as we detected discrete phenotypes in each disease setting in *Ikbkg* deficient CD8 T cells. Therapeutic modulating of the canonical NFkB pathway may present a promising path for effectively manipulating T cell differentiation and durability in chronic infection or cancer.

Characterization of Pancreatic Ductal Adenocarcinoma Fibroblasts

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a 5-year survival rate of just 12%. Tumors are composed of tumor cells and a dense fibrotic stroma made up of cancer associated fibroblasts (CAFs), immune cells, and extracellular matrix (ECM). CAFs have been shown to promote tumors through mechanisms like secretion of soluble factors and ECM deposition and remodeling, however, ablation or broad targeting of CAFs can have a similar effect – suggesting that these cells may play both positive and negative roles in the tumor microenvironment. Through transcriptomic analysis of patient tumors, we have identified two prognostic molecular subtypes of CAFs: tumor restrictive (restCAFs) and tumor promoting (proCAFs), the latter of which shows worst clinical outcomes. We hypothesized that patient derived proCAFs would promote more aggressive tumor phenotypes in vitro compared to restCAFs. Interestingly, tumor cells stimulated with restCAF conditioned media displayed an increased invasive phenotype compared to proCAF conditioned media. As CAFs are major contributors to ECM deposition, we derived cell free matrix from both CAF subtypes. We found that proCAF matrix promoted tumor cell migration compared to restCAF matrix. Overall, both proCAFs and restCAFs can promote an aggressive tumor phenotype in vitro, but through different mechanisms.

Comprehensive mathematical modeling of the planar cell polarity complex to understand its role in regulating the human intestinal epithelium

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The core planar cell polarity (PCP) complex regulates cellular phenotypes in a diverse range of tissues throughout life. While recent literature has elucidated several mechanisms that regulate polarity formation and maintenance, a comprehensive model that connects these mechanisms to tissue-scale polarity and mutant phenotypes is currently lacking. Here, we develop a comprehensive computational model centered around the phosphorylation of DVL and VANGL that captures the key mechanisms believed to underlie PCP. Our computational model reproduces key experimental phenotypes, including polarity alignment in the absence of a biasing signal, robust sensitivity to signaling gradients, resistance to biological noise, and the ability to reproduce known PCP mutant phenotypes. Interestingly, it predicts that PCP is highly regulated by 'nodes' – individual cells or tissue niches with different PCP component expression compared to the surrounding tissue, providing a potential mechanistic explanation for diverse phenotypes ranging the establishment of Drosophila wing polarity to leader-cell driven tumor metastasis. Importantly, our model also predicts that VANGL-dependent DVL phosphorylation may be a key underappreciated mechanism in polarity regulation. To test this prediction, we culture primary human intestinal epithelium (hIE) on planar-crypt microarrays (PCMs) as a new biological model for studying PCP, using single-cell RNA sequencing to confirm PCM biological significance and investigate PCP gene expression. We utilize a doxycycline- inducible VANGL2-iOE primary hIE stem cell line to confirm model predictions and show that alterations to PCP impairs collective migration of neighboring WT cells during both homeostasis and wound healing. Together, our model provides a novel framework for understanding the mechanisms that regulate PCP in a plethora of biological contexts, while our results highlight an unappreciated role for planar cell polarity in the intestine.

Sublethal DNA Damage in Neurons: Is the Apoptotic Pathway Even Activated?

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Throughout life cells will experience a variety of stressors, both extrinsic and intrinsic, which result in the accumulation of DNA damage. Once DNA damage has been detected, most cell types will employ mechanisms to repair the damage. However, if the DNA damage is deemed unrepairable, the cell will undergo a form of programmed cell death known as apoptosis. The death and removal of these irreparably damaged cells is critical as accumulation and replication of cells with DNA damage may lead to cancer, cellular dysfunction, or a loss of homeostasis.
Neurons however, are a resilient and unique post mitotic cell type whose long-term survival is vital for organism survival. Therefore, neurons have developed strategies to restrict cell death, even in the presence of apoptotic stimuli. Interestingly, it has been reported that ‘healthy’ neurons accumulate DNA damage throughout their lifetime and that this DNA damage largely does not kill neurons. This suggests that neurons may uniquely tolerate DNA damage or neurons activate the apoptotic pathway at sublethal levels. Excitingly, our work shows that neurons indeed activate the apoptotic pathway in response to sublethal DNA damage. In this work, we are examining to what extent the neuronal apoptotic pathway is activated in response to sublethal DNA damage and how neurons maintain resilience with sublethal DNA damage.

The role of tubulin code in organelle contacts during neuronal differentiation

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A functional nervous system depends on the correct neuronal morphology established during differentiation. Microtubule (MT) cytoskeleton is required for successfully completing all the morphological transitions of differentiation, providing structural support, positioning organelles, and enabling their transport. MT dynamics and properties are regulated by a variety of post-translational modifications (PTMs), which have been shown to interact with membrane-bound organelles in non- neuronal cells. However, how neurons rearrange organelles through modifying tubulin PTMs to meet their transition from stem cells remains to be fully delineated. We recently found that organelle contact increases during axon formation, coinciding with enriched tubulin PTMs. We hypothesize that neurons spatiotemporally coordinate the distribution of MT PTMs throughout neuronal differentiation to drive organelle reprogramming during differentiation.
We derived cortical neurons (iNeurons) from human induced pluripotent stem cells and found that α tubulin K40 acetylation and polyglutamylation display distinct changes in abundance and subcellular localization during differentiation. We downregulated acetyltransferase, ATAT1, or polyglutamylase, TTLL1, then applied 3D multispectral imaging analysis to study how tubulin PTMs affect eight membrane-bound organelles simultaneously in day 7 iNeurons. We found that reducing MT acetylation alters lysosomal morphology, inter-organelle contacts, lysosomal function, and autophagy. In contrast, reduced tubulin polyglutamylation leads to Golgi fragmentation and disrupts Golgi-related organelle contacts. Moreover, reduction of tubulin acetylation or polyglutamylation differently affects iNeuron morphology. Together, our results suggest that changes in the amount and localization of MT PTMs are a mechanism of organelle remodeling throughout neuronaldifferentiation.

Phenotypic Modulation of Vascular Smooth Muscle Cells: a case for redox regulation

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Vascular smooth muscle cells (VSMCs) are essential for maintaining vascular integrity and function. Under pathological conditions involving oxidative stress, inflammation, and mechanical stress, VSMCs undergo phenotypic modulation, shifting from a contractile state to a range of synthetic phenotypes. This transition is characterized by increased proliferation, migration, and changes in the composition and remodeling of the extracellular matrix, which contributes to the progression of various vascular diseases. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical regulator of defense mechanisms against oxidative stress, and its activation has been implicated in mitigating VSMC phenotypic modulation.

This study investigates the potential of triterpenoid Nrf2 activators, specifically Bardoxolone and Omaveloxolone, to inhibit phenotypic modulation in VSMCs. We hypothesize that the activation of Nrf2 by these compounds prevents the shift towards synthetic phenotypes and supports vascular health. Our approach involves both in vitro and in vivo models. *In vitro*, VSMCs are treated with Bardoxolone and Omaveloxolone, followed by assessments of cell proliferation, migration, and phenotypic modulation markers. *In vivo*, we utilize a mouse arteriovenous fistula (AVF) model, which is critical for hemodialysis patients but has a failure rate exceeding 50%, to assess the effects of Nrf2 activation on AVF functionality, employing Light Sheet Fluorescent Microscopy (LSFM) to visualize and analyze vascular remodeling within the AVF.

Preliminary data indicate that Nrf2 activation reduces VSMC proliferation, migration, and adverse ECM remodeling, suggesting a potential therapeutic benefit in vascular diseases associated with oxidative stress. This research provides new insights into phenotypic modulation and proposes that local delivery of Nrf2 activators to the mouse AVF model could be a novel therapeutic strategy for improving vascular health, particularly in the context

Dissecting the Role of Folate-Driven One Carbon Metabolism in Tumor-Associated Macrophages

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The emergence of immune-checkpoint blockade cancer immunotherapies, such as anti-PD-1 (aPD-1), that activate a patient's adaptive immune response against their malignancy have revolutionized the cancer treatment landscape. Despite this success, a majority of cancer patients remain refractory to immunotherapy. Resistance to aPD-1 is associated with the expansion of a subset of suppressive innate immune cells called tumor-associated macrophages (TAMs) that dampen the anti-tumor immune response. The development of novel strategies to therapeutically reprogram TAMs to an anti-tumor cell state is a clinically relevant approach to overcome cancer immunotherapy resistance and broadly improve patient outcomes. Our lab has identified a population of folate receptor β (FRβ)-expressing immunosuppressive TAMs enriched in aPD-1 resistant tumors. FRβ is a surface protein that mediates the high-affinity binding and uptake of folate from the extracellular environment. Folate is a requisite cofactor for the one-carbon (1C) metabolism pathway, which maintains cellular redox balance, nucleotide synthesis, and global methylation reactions. The regulatory role of folate-driven 1C metabolism on TAM biology in the tumor is not known. Based on our preliminary findings, we hypothesize that immunosuppressive TAMs require FRβ for folate uptake and downstream 1C metabolism to sustain survival and functional suppression of the anti-tumor immune response.

The Role of TNIK and tPA in Tumor-Stroma Crosstalk, ECM Degradation and Metastasis in Pancreatic Cancer

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5-year survival in Pancreatic Ductal Adenocarcinoma (PDAC) is 11-13%. Our lab has identified two molecular subtypes of PDAC, classical and basal, based on RNA expression that hold clinical relevance. Classical PDAC has improved response to chemotherapy, and longer overall survival, though more work is needed to develop therapeutics leveraging knowledge of subtype differences. Through a comprehensive unbiased proteomic approach that enriches for kinases called multiplex inhibitor bead mass spectrometry, Traf2 and Nck interacting kinase (TNIK) was identified as the top differentially expressed classical kinase.

TNIK is a germinal center kinase, a known regulator of the beta-catenin/TCF4 – Wnt signaling pathway, and its inhibition has been studied in colorectal cancer. In contrast to TNIK's role in colorectal cancer, our lab has observed that TNIK may be required to maintain PDAC differentiation, organization and ***restrain*** invasion. We first observed an increase in PDX cell line invasion across a transwell with shRNA mediated knockdown (KD) of TNIK. Next TNIK was knocked out in a classical PDAC organoid where we observed an increase in invasive and migratory potential when plated on collagen. Transcriptomic analysis of control and TNIK knockout organoids showed an increase of basal-associated genes as well as a significant increase in tissue plasminogen activator (tPA) expression.

tPA is a secreted serine protease responsible for the activation of plasminogen to plasmin which breaks down fibrin, an abundant component of the PDAC tumor microenvironment. Media from TNIK KO organoids was collected and found to have increased plasminogen activity by SDS- PAGE zymography. A fibrin degradation assay showed increased fibrin matrix degradation in TNIK KO organoids compared to control. These findings may provide a potential mechanism for observed increase invasive phenotype in TNIK KO models. More work is needed to elucidate the molecular link between TNIK and tPA in PDAC.

Noise-Induced Hearing Loss Alters Structure and Function of Inhibitory Cells in the Dorsal Cochlear Nucleus

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Noise-induced hearing loss (NIHL) permanently impairs ~40 million US adults. While it is known that NIHL can damage peripheral auditory structures, its effects on the central auditory system are still being investigated. The first central auditory structure to receive input from the cochlea via the auditory nerve is the cochlear nucleus (CN). The CN has two main divisions: the ventral and the dorsal cochlear nucleus (DCN). DCN neurons integrate auditory information with somatosensory, vestibular, and proprioceptive signals for external sound localization. Key inhibitory neuronal types of the DCN circuit are tuberculoventral cells (TV), and cartwheel cells (CWC). Structural changes in the axon initial segment (AIS)—a macrodomain responsible for action potential initiation and propagation—leading to increased excitability have been described in a subset of avian CN neurons after hearing loss. While these and other studies provide insight into the structural and functional changes of auditory neurons in the context of sensorineural hearing loss, there is limited information about the effects of NIHL on DCN-interneurons.

Experiments were performed in VGAT-ChR2-EYFP, GlyT2-EGFP, and GlyT2-EGFPxNF107 mice for CWC and TV identification. Mice 40 to 45 days old were exposed to 8-16 kHz octave broadband noise at 106 or 115 dB SPL for 2 hours. Two weeks later, mice were subjected to immunofluorescence or brain slice electrophysiology. AIS lengths were measured through IMARIS. A subset of cochleae were evaluated via light-sheet fluorescence microscopy.

We observed a lengthening in CWC AIS, and a shortening in TV AIS post noise exposure. Electrophysiologically, CWCs exhibited slower action potential kinectics (depolarizing and repolarizing phases) after noise exposure. Both interneurons have wider action potentials and lower action potential thresholds post noise exposure. TVs exhibit higher input resistances and resting membrane potentials after noise exposure. Although not sign

Photometric measurement of bodily physiology in freely behaving mice

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Recently, renewed interest in the biological mechanisms of brain-body communication has driven great strides in understanding the links between body, brain, and behavior. This research is providing convincing evidence that interoceptive feedback is an important regulator of higher-order neural activity, emotion, and behavioral outflow. However, this research remains limited by the available technologies allowing bodily physiology to be easily and non-invasively monitored alongside widespread techniques in neuroscience. To address this need, we are developing a minimally invasive and easy-to-use tool to simultaneously measure pupil size and heart rate in freely-moving mice. This tool uses infrared light from an implanted source that travels through tissue and out of the eye, where it is transduced to electrical signal by a photodiode. Modulation of light propagation occurs as a function of certain physiological changes such as pupil diameter and blood volume/oxygenation, which allows frequency filtering of the resulting signal to track these changes over time. We have successfully validated pupil size measurements from our device in both head-fixed and freely-moving animals using camera-based ground-truth recordings. Currently, we are in the process of validating this approach in measuring heart rate in freely-moving animals. This approach offers great potential in providing an easy-to-use method of recording multiple physiological signals in behaving animals. Furthermore, this approach can be used alongside techniques like optogenetics and calcium imaging to inform research into the neural mechanisms of physiological change. Combining existing approaches to perturb and record neural activity with convenient methods to measure bodily physiology during behavioral testing may yield added insight into the connection between internal state, neural activity, and outward behaviors.

Characterizing the cellular context of RAMP1 signaling in migraine

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Migraines are a debilitating neurological condition afflicting approximately 10% of the global population. While the causes of migraine remain unclear, the most well-established mediator is calcitonin gene related peptide (CGRP), a neuropeptide elevated in the plasma and cerebral spinal fluid during migraine attacks. CGRP primarily acts upon its canonical receptor, which is a heterodimer composed of the G-protein coupled receptor calcitonin-receptor like receptor (CLR), and its allosteric regulator, receptor activity-modifying protein 1 (RAMP1). The CGRP receptor (RAMP1-CLR) is expressed in various cell types involved in migraine pathophysiology, including those within the central and peripheral nervous systems as well as the blood vasculature. Recently, our laboratory was the first to show that CGRP-mediated signaling in meningeal lymphatic vessels significantly contributes to migraine pain. CGRP tightens the cell-cell junctions of the lymphatic endothelial cells (LECs) that line these vessels, which attenuates permeability and impairs their drainage function. Currently, it is unknown whether RAMP1 modulates CLR activity uniformly across all cell types or whether its regulation is shaped by both internal cellular mechanisms and external cues. To investigate whether RAMP1 regulates CLR signaling in a cell-dependent manner, we leveraged proximity labeling technology to compare the proximal protein network of RAMP1 in LECs to Schwann cells, a glial cell in the peripheral nervous system implicated in migraine pain. Preliminary results suggest that while LECs and Schwann cells both recruit endosomal proteins upon CGRP treatment, they also interact with various unique proteins. Ongoing experiments aim to delineate the impact of the differentially enriched proteins on receptor activation and downstream signaling profiles in each cell type.

Protein Geranylgeranylation Regulates Cardiac Morphology and Contractility During Embryonic Development

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Protein prenylation is a post-translational modification involving the covalent addition of isoprenoid lipids to proteins. Our previous studies implicate geranylgeranylation, a type of prenylation, as a critical regulator of heart development, but the precise role that geranylgeranylation plays in this process remains unknown. In this project, we aimed to determine how loss of geranylgeranylation activity affects the development and morphology of the mouse heart during embryonic development. We generated a genetic knockout mouse model of *Pggt1b,* a critical enzyme that mediates protein geranylgeranylation. We found that loss of *Pggt1b* expression in cardiomyocytes results in reduced compact myocardium thickness and altered interventricular septum morphology. To investigate the mechanisms by which geranylgeranylation regulates cardiomyocyte development and function, we treated cardiomyocytes with a geranylgeranyltransferase inhibitor and performed a quantitative proteomics analysis. We found that several sarcomere proteins were significantly downregulated in geranylgeranylation-inhibited cardiomyocytes. In addition, we assessed contractility and sarcomere organization following inhibition of geranylgeranylation in cardiomyocytes using a combined approach of a calcium dye-based contractility assay and immunohistochemistry. We show that inhibiting geranylgeranylation results in significantly reduced cardiomyocyte contractility and impaired sarcomere structure. Finally, to identify the subset of prenylated proteins that regulate cardiomyocyte sarcomere formation and function, we metabolically labeled prenylated proteins in cardiomyocytes using an isoprenoid-analogue probe and performed quantitative proteomic analysis. Using this approach, we have identified candidate prenylated proteins that may regulate cardiomyocyte development and function. Overall, our findings indicate that geranylgeranylation may contribute to cardiac morphogenesis by regulating sarcomere formation and function in developing cardiomyocytes.

Social threat recognition induces unique behavioral syllables and reshapes functional brain connectivity

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Social cues guide a significant portion of mammalian behavior, informing motivated action in response to positive or negative valence social stimuli to optimize an individual's chance of survival. This can be seen through recognition and subsequent approach of positive valence social stimuli, such as potential mates, or by recognition and subsequent avoidance of negative valence social stimuli, such as aggressive or threatening individuals. While the neuronal representations of positive valence social cues are relatively well characterized, there is a substantial lack of research on the recognition and response to threatening social stimuli. Using a modified social fear conditioning (SFC) task that automates the pairing of interaction with a same-sex, novel mouse with a mild foot-shock, we successfully drove defensive behavioral responses toward a social stimulus on the day of training. Furthermore, stimulus-specific aversion persisted over time, as shown by place avoidance in the three-chamber sociability test on the day following SFC. We then examined cFos protein expression across hundreds of brain regions to identify changes in brain-wide functional connectivity evoked by social threat recognition. We observed notable changes in correlations in cFos expression following SFC, particularly in the infralimbic region of the medial prefrontal cortex (IL) and the nucleus accumbens (NAc). We then employed an optogenetic approach to examine the role of excitatory projections from the IL to NAc in the processing of negative valence social experiences. We saw that optogenetic stimulation of this circuit rescued deficits in social preference induced by SFC. The results described here expand our understanding of the neural circuitry underlying recognition and subsequent behavioral responses toward social threats, and may open doors to future treatment of fear-based anxious behaviors.

Genetic Analysis of Allostery within the Multi-Subunit Exocyst Complex

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The exocyst is a highly conserved multi-subunit tethering complex responsible for the delivery and tethering of post-Golgi vesicles to specific sites on the plasma membrane. It is regulated by a multitude of interactors, but the mechanisms behind its regulation remain poorly understood. In budding yeast, it is known that the exocyst can be released from its basal autoinhibited state through one of two distinct pathways that drive its allosteric activation: the Rho/Exo70 pathway or the Sro7/Exo84 pathway. In each pathway, binding of an activator (Rho3/Cdc42 or Sro7) results in specific structural changes that release the exocyst from an autoinhibited state, activating its tethering activity. These pathways exhibit similarities to insulin-stimulated GLUT4 translocation in fat and muscle cells where binding of the small GTPases TC10 and RalA to the exocyst causes an increase in tethering activity, paralleling Rho and Sro7 in yeast. To generate a comprehensive map of exocyst allosteric regulation sites, we used both structure-guided and random mutagenesis to identify gain-of- function (GOF) mutants in yeast that mimic activation by Rho or Sro7. Most of these GOF mutants were found in subunit:subunit interfaces, suggesting that precise disruption of interactions within these interfaces causes structural changes that result in specific activated states of the exocyst. To investigate whether this allele- specific response is conserved in mammals, we also generated mammalian GOF homologues to test in a mouse adipocyte model of GLUT4 translocation. While there is a cryoEM structure of yeast exocyst, the same has not yet been achieved for mammalian exocyst. We therefore utilized AlphaFold Multimer to model subunit:subunit interfaces and designed our mammalian GOF alleles using these models as reference. This work demonstrates the functional relevance of the yeast exocyst cryoEM structure and aims to investigate conservation of allosteric activation mechanisms in mammals.

See More with the Hooker Imaging Core

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The Hooker Imaging Core (HIC) provides light microscopy and electron microscopy instrumentation, training and expertise. The HIC is an open core housed within CBP that serves the UNC biomedical research community and outside users. Come by the poster to learn about all our instruments and services, including the Leica Stellaris 8 FALCON STED confocal—flexible excitation wavelength selection for 7+ fluorophores, super-resolution imaging down to 50nm, and lifetime imaging—, slide scanner, high content imaging, live imaging, montage imaging for EM, and more!

Anatomic and genetic profile of prepronociceptin-expressing neurons projecting from the bed nucleus of the stria terminalis to the medial amygdala

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Social anxiety disorder (SAD) is characterized by persistent fear of social interactions, worsened by adverse social experiences. Despite being the third most prevalent psychiatric disorder worldwide, treatments for SAD have limited effectiveness, necessitating the need to understand its neural circuitry. The bed nucleus of the stria terminalis (BNST) is recognized for regulating behavioral responses related to anxiety. We previously identified BNST neurons that express the prepronociceptin (*Pnoc*) gene, the precursor for nociceptin, regulate anxiety responses. Additionally, *Pnoc*BNST neurons project to the medial amygdala (MeA), which regulates social behaviors; however, their role in social anxiety is unknown. To better understand *Pnoc*BNST-MeA neurons, we used fluorescent in situ hybridization (FISH) to label genetic markers co-expressed in *Pnoc*BNST neurons, including somatostatin (*som*), cholecystokinin (*Cck*), and protein kinase C delta (PKCδ), along with retrograde tracing and transgenic mouse line strategies to anatomically and genetically characterize this circuitry. By quantifying subregions of the BNST, we found that *Pnoc*BNST neurons are densely expressed dorso-laterally, particularly in the oval nucleus. Given the BNST's sexual dimorphism, we assessed sex differences in *Pnoc* expression and found none. Next, we tested our FISH and retrograde tracing approaches. We successfully labelled secondary genetic markers *som* and *Cck* in the BNST, and are currently testing PKCδ expression and tracing BNST→MeA neuron projections using Cholera Toxin Subunit B. We will combine these strategies to test whether *Pnoc*BNST-MeA neurons can be distinguished by their anatomical or genetic profiles. Collectively, these findings will inform future studies on *Pnoc*BNST-MeA circuitry in social behavior and anxiety.

Regulation of visual signaling and adaptation in photoreceptors via cAMP-dependent phosphorylation of GRK1 and GRK7

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In the vertebrate retina, phosphorylation of photoactivated visual pigments in rods and cones by G protein- coupled receptor kinases (GRKs) is essential for sustained visual function. Previous *in vitro* analysis demonstrated that GRK1 and GRK7 are phosphorylated by PKA, resulting in a reduced capacity to phosphorylate rhodopsin. *In vivo* observations revealed that GRK phosphorylation occurs in the dark and is dependent on cAMP and PKA. GRK1 appears to be universally expressed in vertebrate rods, whereas GRK7 has been identified as a cone-opsin kinase in all vertebrates that have been examined to date - with the exception of mice and rats, which have lost the gene for GRK7. Interestingly, only human, non-human primates, and some fish express both GRK1 and GRK7 in cones, while other vertebrates only express GRK7. In zebrafish under dark-adapted conditions, Grk1a and Grk7a are phosphorylated in rods and cones, respectively, in response to elevated cAMP which reduces the catalytic activity of these kinases based on our prior *in vitro* studies. However, GRK1 is not phosphorylated in either zebrafish or mouse cones, suggesting differences in the role of GRK1 in dark adaptation between rods and cones that are shared across vertebrates. Consistent with these results, we found that the phospho-null GRK1 mutant (GRK1-S21A) expressed in mouse rods is a more active kinase that slows rod dark adaptation, while cone dark adaptation is not affected. Additionally, zebrafish larvae lacking Grk7a are insenstive to a forskolin-induced decrease in cone photoresponse recovery to successive stimuli observed in wild type and *grk1b-/-* larvae. These and future studies will expand our knowledge of important aspects of the photoresponse in the vertebrate retina that are relevant to human visual physiology.

Initial Purification and Proteomic Analysis of Filopodia

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Filopodia are dynamic, finger-like cellular protrusions with important roles including synapse formation, angiogenesis, and cancer. Although purifications have been devised for most other major cellular organelles such as nuclei, mitochondria, and cilia, filopodia have not yet been purified with the full set of their components remaining unknown. We report here an initial purification of filopodia and their characterization by microscopy, cryoEM, and proteomics.

Filopodia can be sheared from HeLa cells by passage through a 22G needle and then low-speed centrifugation can be used to pellet the cells. Imaging the supernatant revealed large numbers of cylindrical structures averaging 3-4 um in length. These structures stain positive for F-actin, membrane, and plasma membrane marker wheat germ agglutinin. Isolating filopodia from HeLa cells transiently transfected with GFP-Myo10 or from HeLa Knock-In cells expressing Halo-tagged Myo10 revealed that many filopodia contain puncta of Myo10 at their tips and/or along their length, confirming their identity as filopodia. Isolated filopodia are surprisingly stable and retain their morphology for a day or more when stored on ice in the presence of EGTA and the F-actin stabilizing agent SiR Actin. Sheared filopodia can be concentrated and purified using an Optiprep step gradient, and further purified using density gradient centrifugation.

A trial proteomics analysis showed that actin was the most abundant protein present, with other actin- binding proteins such as radixin, plastin-3, fascin, and myosin-1c particularly abundant. Numerous other proteins were also detected. Future directions include improving the purification, identifying the full set of proteins in filopodia, developing a molecular model of the filopodium with the copy number of each protein present, and defining the molecular relationships between filopodia and other cellular protrusions such as microvilli, retraction fibers, and cancer cell invadopodia.

Utilizing a reversible chemical crosslinker to uncover protein interactomes in cultured cells and zebrafish embryos.

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Identifying the interactome of a given protein is crucial for determining its function(s) during development or within a specific biological process. However, current techniques for identifying protein-protein interactions (PPIs) (1) entail extensive experimental optimization, (2) result in unacceptable levels of background for downstream applications, and/or (3) require genetic manipulation, which can be experimentally impractical in some cases. One potential remedy for this problem is to covalently stabilize PPIs using a reversible chemical crosslinker *in vivo* prior to cell or tissue lysis. Here, we show that reversible covalent bonds formed between interacting proteins not only enable the use of strong lysis/wash conditions but also significantly reduce unwanted bait protein and IgG elution into samples. In addition, we demonstrate that crosslinked cells can still be fractionated into nuclear and cytoplasmic lysates. We combined our crosslinker-assisted co-immunoprecipitation workflow with mass spectrometry (co-IP/MS) and successfully determined the interactomes of Hic-5 and Lamin B1 in C2C12 mouse myoblast cells. We then sought to expand the scope of the technique and uncover the proteins required for myotendinous junction (MTJ) formation in *D. rerio* embryos. Utilizing a similar co-IP/MS workflow, we identified proteins that interact with kindlin-2, a focal adhesion protein enriched at the MTJ, in 24-hpf, wild-type zebrafish with minimal optimization and significantly reduced background. In short, our approach allows one to readily examine the role of highly dynamic proteins in different subcellular compartments and whole animals without genetic manipulation.

Increased Ciliation of CA3 Hippocampal Neurons in Alzheimer's Disease

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Essential for memory encoding and retrieval, the CA3 subregion of the hippocampus produces brain derived neurotrophic factor in support of the dentate gyrus (DG), entorhinal cortex and the basal forebrain. In Alzheimer's disease (AD), hippocampal BDNF production is significantly reduced, however the mechanism underlying BDNF reduction remains elusive. We hypothesize that primary cilia, signaling organelles found on neurons, regulate BDNF production. To determine whether primary cilia dysfunction in AD is causing BDNF reduction by CA3 neurons, we first scored neuronal primary cilia in the CA3 of human postmortem AD brains compared to non-AD controls. We noted a significant increase in percent ciliation of MAP2+ neurons in the CA3 in post-mortem human AD brains compared to controls. Our preliminary data also suggest longer primary cilia in the CA3 neurons of AD brains compared to controls. Future studies will examine BDNF transcription in CA3 neurons as a function of ciliation. Because the remaining CA3 neurons are increasingly ciliated, our current data indicate that neuronal primary cilia may confer resilience to AD pathology.

Motor assisted commutator to harness electronics in tethered experiments

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Research that combines advanced technological devices with complex behavioral tasks has enabled investigations into the neural mechanisms underlying brain and behavioral states. Freely moving rodent experiments often require a tether - a wired connection between an implanted device and an external power supply or data acquisition system. Traditionally, these experiments have used passive commutators to manage tethers, but such setups are often inadequate for reducing twisting and mechanical strain during behavioral tasks. Existing motorized commutators have extended the range of motion for these experiments but generally rely on stepper motors that produce auditory noise, potentially interfering with behavior. To address these limitations, we developed the Motor Assisted Commutator to Harness Electronics in Tethered Experiments (MACHETE), a motor-assisted commutator featuring a low-noise brushless motor. MACHETE dynamically adjusts tethers based on mouse movement, reducing torque and mechanical strain, and minimizing the animal's physical exertion during behavioral assays. Its onboard microcontroller provides customizable controls and seamless integration with custom electronic devices. The design includes a central through-hole to accommodate wires or fibers from external devices such as head-mounted miniature microscopes, electrophysiology probes, and optogenetic systems. We validated MACHETE across standard behavioral assays, including the open field test, the splash test, and the three-chamber social test. Our results showed no significant changes in mobility or behavior compared to untethered controls. By combining precise motor control, low auditory noise equipment, and accessibility, MACHETE can be used to support research that aims to adapt tools for use in freely moving behavior experiments.

Genetic Factors Mediating Teratogenic Susceptibility to Alcohol

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Genetics are a major factor explaining the range of observed craniofacial birth defects caused by prenatal alcohol exposure. In a mouse model, different inbred strains can exhibit different rates of craniofacial defects after prenatal alcohol exposure. For example, the widely studied C57BL/6J (B6J) strain is highly susceptible to alcohol-induced defects. In this study we characterize a mouse strain resistant to alcohol-induced defects: the 129S1/SvImJ (129S1) strain. The 129S1 strain is resistant to birth defects after alcohol and cannabinoid exposure. Both drugs cause similar birth defects through inhibition of the Sonic hedgehog (Shh) pathway. 129S1 embryos have higher expression of several Shh pathway-related genes. Alcohol causes apoptosis in the embryo, so we compared the amount of apoptosis in alcohol-exposed embryos of B6J and 129S1 strains. 129S1 embryos had significantly fewer apoptotic cells as compared to B6J embryos. In contrast, 129S1 dams have much higher blood alcohol concentrations than B6J dams. Alcohol metabolism can possibly influence the development of alcohol-induced birth defects by competing with retinol (which is used to synthesize retinoic acid) for alcohol dehydrogenase. We compared maternal alcohol metabolism in 129S1 and B6J mice, specifically identifying differences in liver alcohol dehydrogenase activity. 129S1 mice had lower enzyme activity than B6J mice. Finally, to identify specific genes that mediate teratogenic susceptibility to alcohol, we analyzed transcriptomic differences in B6J versus 129S1 embryos using RNA-sequencing. 129S1 mice have greater resistance to alcohol-induced defects in spite of their slower alcohol metabolism. This is possibly because of a combination of higher basal Shh pathway activity and lower apoptosis levels following alcohol exposure. These results can be used to better understand how different genetic factors work together to alter susceptibility to prenatal alcohol exposure.