

16TH ANNUAL GDBBS DSAC STUDENT RESEARCH SYMPOSIUM

Monday, January 28th, 2019
Claudia Nance Rollins and Grace Crum Rollins

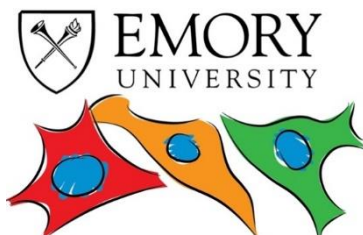


DIVISION STUDENT ADVISORY COUNCIL
GRADUATE DIVISION OF BIOLOGICAL AND BIOMEDICAL SCIENCES

Also Sponsored By:



Graduate Division of Biological
and Biomedical Sciences



Integrated Cellular
Imaging Core
Emory Integrated Core Facilities



Emory Antibiotic
Resistance Center

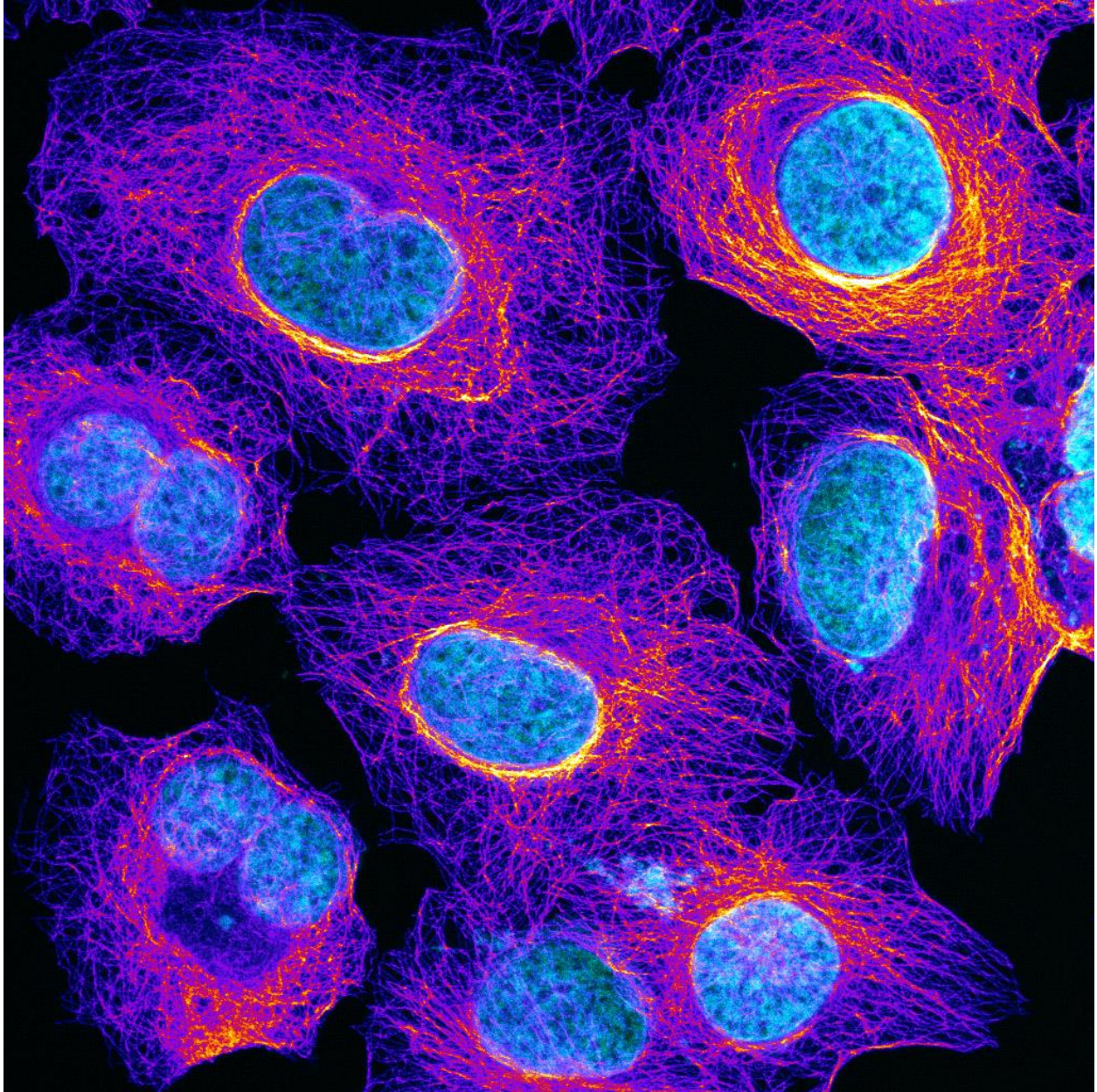
Table of Contents

Schedule of Events	4
Oral Presentation Abstracts	8
Session 1: Human Disease and Therapeutics	10
Session 2: Proteins: Dynamics and Signaling	16
Session 3: Neurological Disorders	22
Session 4: Epigenetics and Gene Expression	27
Session 5: Immunity and Pathogens	33
Poster Presentation Abstracts	39

ICI Image Contest Winners

1 st Place – Emily Summerbell, CB	3
2 nd Place – Brandon Ware, CB	9
Runner-Up – Joanna Perez, BCDB	39
Runner-Up – Brian Pedro, CB	39
Runner-Up – Alyssa Scott, GMB	40
Runner-Up – Cara Schiavon, CB	40

1st Place Image, ICI Image Contest



Emily Summerbell, Marcus Lab, CB
Tubulin (purple) and DNA (blue) in cancer cells

**The 16th Annual GDBBS DSAC Student
Research Symposium**
Monday, January 28th, 2019
Claudia Nance Rollins/Grace Crum Rollins

8:00-8:30AM – Breakfast, 8th floor, Rita Anne Rollins Room

Session 1: Human Disease and Therapeutics

8:30 - 9:45AM, GCR 8th floor, Rita Anne Rollins Room

8:30 – Sabrina Lynn (BCDB)

ASSOCIATION OF DYNAMIN-2 WITH CHANGES IN ALVEOLAR
EPITHELIAL TIGHT JUNCTION MORPHOLOGY

8:45 – James Ross (CB)

INVESTIGATION OF TUMOR ASSOCIATED MACROPHAGE DYNAMICS
IN PEDIATRIC HIGH-GRADE GLIOMA

9:00 – Akram Salam (MSP)

NOVEL COMPOUNDS ISOLATED FROM CASTANEA SATIVA LEAF
EXTRACT WHICH INHIBITS STAPHYLOCOCCUS AUREUS QUORUM
SENSING

9:15 – Brian Pedro (CB)

DISSECTING THE BIOLOGY OF LEADER AND FOLLOWER CELLS IN
COLLECTIVE CANCER INVASION

9:30 – Tyler Moser-Katz (BCDB)

A ROLE FOR SYNTENIN-1 IN MULTIPLE MYELOMA CELL SURVIVAL

9:30-9:45AM – Break

Session 2: Proteins: Dynamics and Signaling

9:55 - 11:10AM, GCR 8th floor, Rita Anne Rollins Room

9:55 – Matt Tillman (MSP)

FATTY ACIDS BIND TO THEM1, A NEGATIVE REGULATOR OF
THERMOGENESIS IN BROWN ADIPOSE TISSUE

10:10 – Alexandra Wolfarth (IMP)

PRAP1 IS A NOVEL HOST PROTEIN HIGHLY SECRETED IN THE PRE-OVULATORY UTERUS AND MODULATES THE MICROBIOTA OF THE FEMALE REPRODUCTIVE TRACT

10:25 – Brandon Ware (CB)

INTERLEUKIN-6 IN BILIARY TRACT CANCERS PROMOTES EXPANSION OF IMMUNE SUPPRESSIVE MYELOID CELLS AND IS ASSOCIATED WITH INCREASED MYELOID CELLS IN PATIENT TUMORS

10:40 – Emma D’Agostino (BCDB)

DEVELOPMENT OF A ROBUST DIRECT BINDING ASSAY FOR NR5A NUCLEAR RECEPTORS

10:55 – Dillon Patterson (GMB)

IRF4 REGULATES THE RATE OF CELL CYCLE DURING B CELL DIFFERENTIATION

11:10 - 11:20AM – Break

Session 3: Neurological Disorders

11:20AM – 12:20PM, GCR 8th floor, Rita Anne Rollins Room

11:20 – Erica Akhter (NS)

REGULATION OF THE POTASSIUM CHLORIDE COTRANSPORTER-2 (KCC2) ON SPINAL MOTONEURONS FOLLOWING PERIPHERAL NERVE INJURY

11:35 – George Inglis (GMB)

EXPLORING THE IMPACT OF SCN8A DIIS4 VOLTAGE-SENSOR DYSFUNCTION ON MOTOR FUNCTION AND NERVE CONDUCTION

11:50 – Mary Herrick (NS)

LRRK2: LURKING BETWEEN THE BRAIN AND GUT

12:05 – Sarah Suci (GMB)

THE CILIARY PROTEIN ARL13B REGULATES AXON GUIDANCE IN THE MOUSE HINDBRAIN

Poster Sessions & Lunch

12:30-2:30PM

CNR 1st Floor Lobby/Bridge

12:30-1:30 – Odd-Numbered Poster Presentations

1:30-2:30 – Even-Numbered Poster Presentations

Session 4: Epigenetics and Gene Expression

2:40 – 3:55PM, GCR 8th floor, Rita Anne Rollins Room

2:40 – Hannah Ratner (MMG)

CRISPR SYSTEMS DIVERSIFY FUNCTIONS WITH SUBTLE RNA VARIATIONS

2:55 – Sarah Curtis (GMB)

EXPOSURE TO POLYBROMINATED BIPHENYL (PBB) ASSOCIATES WITH DNA METHYLATION DIFFERENCES ACROSS THE GENOME

3:10 – Aimee Paulk Tierney (MMG)

A LysR-TYPE TRANSCRIPTIONAL REGULATOR IMPACTS MULTIPLE PHENOTYPES IN *ACINETOBACTER BAUMANNII*

3:25 – Kelsey Maher (BCDB)

CROSS-SPECIES GENOME-WIDE PROFILING REVEALS DEPLETION OF CHARACTERISTIC ENHANCER HISTONE MODIFICATIONS AT ACCESSIBLE CHROMATIN SITES IN PLANTS

3:40 – Christine Doronio (GMB)

A ROLE FOR EPIGENETIC MECHANISMS IN HOMOLOGOUS CHROMOSOME RECOGNITION DURING MEIOSIS

3:55 - 4:05PM – Break

Session 5: Immunity and Pathogens

4:05-5:20PM, GCR 8th floor, Rita Anne Rollins Room

4:05 – Kara Phipps (MMG)

VIRUS-HOST INTERACTIONS DETERMINE INFLUENZA A VIRUS CO-INFECTION DEPENDENCE

4:20 – Madeline Price (IMP)

EPIGENETIC PRIMING ACCELERATES MEMORY B CELL REACTIVATION

4:35 – Brindar Sandhu (GMB)

IDENTIFICATION OF A β -LACTAMASE THAT CONTRIBUTES TO INTRINSIC β -LACTAM RESISTANCE IN *CLOSTRIDIODES DIFFICILE*

4:50 – Signe White (PBEE)

THE EVOLUTION OF VIRULENCE IN A HETEROGENEOUS HOST POPULATION

5:05 – Dominika Swieboda (IMP)

ABUNDANT EXPRESSION OF CCR5 ON EARLY HOFBAUER CELLS MAY INCREASE HIV-1 SUSCEPTIBILITY

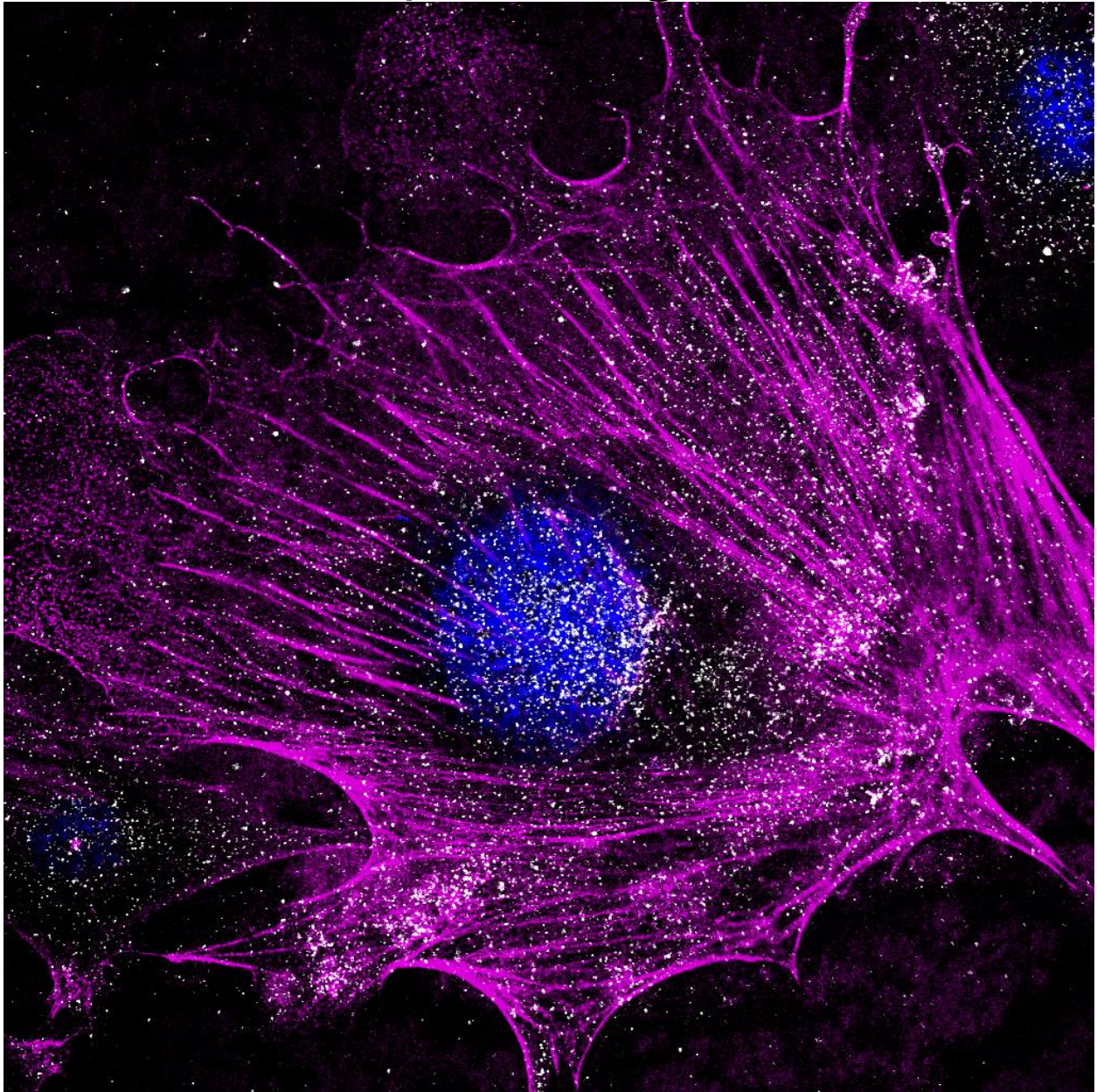
Reception and Awards

CNR 1st Floor Lobby/Bridge

5:30 - 7:00PM

Oral Presentation Abstracts

2nd Place, ICI Image Contest



Michael Brandon Ware, Lesinski Lab, CB

Fibroblast Activating Protein alpha (FAP) staining of a pancreatic stellate (star-like) cell isolated from a pancreatic tumor.

Session 1:

Human Disease and Therapeutics

8:30AM

ASSOCIATION OF DYNAMIN-2 WITH CHANGES IN ALVEOLAR EPITHELIAL TIGHT JUNCTION MORPHOLOGY

K. Sabrina Lynn,¹ Barbara Schlingmann,¹ Max Cornely,¹ Samuel A. Molina,¹ Michael Koval¹

¹Department of Medicine, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine and Department of Cell Biology

Previous work in the Koval lab determined that chronic alcohol ingestion increases expression of tight junction protein claudin-5 by the alveolar epithelium, which impairs alveolar epithelial barrier function and sensitizes the lung to acute respiratory distress syndrome. Claudin-5 interacts with claudin-18, causing a molecular rearrangement of tight junctions into spike-like structures perpendicular to the cell junction interface, with super-resolution microscopy revealing altered distribution of tight junction proteins. These “tight junction spikes” (TJ spikes) appear to be active areas of junction remodeling driven by increased endocytosis of tight junction proteins. Treatment with the endocytosis inhibitor Dynasore, which targets the actin-binding protein dynamin, significantly reduces the number of TJ spikes; upon removal of Dynasore, TJ spikes reform, indicating this phenotype recovers and persists after inhibition. This suggests a role for clathrin-mediated, dynamin-dependent endocytosis in TJ spike formation. My current work focuses on identifying the molecular machinery involved in TJ spike formation and further investigating the role of TJ spikes in barrier function. The long-term goal is to identify novel therapeutic targets to improve barrier function by redirecting spike-associated claudin-18 into barrier forming tight junctions.

INVESTIGATION OF TUMOR ASSOCIATED MACROPHAGE DYNAMICS IN PEDIATRIC HIGH-GRADE GLIOMA

James Ross^{1,2}, Zhihong Chen², Frank Szulzewsky³, Lenore Monterroza¹, Matthew Schniederjan⁴, Oren Becher⁵, Dolores Hambardzumyan²

¹ Emory University Graduate Division of Cancer Biology, Atlanta, GA

² Department of Pediatrics, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, GA

³ Department of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA

⁴ Department of Pathology and Laboratory Medicine, Emory University

⁵ Department of Pediatrics, Northwestern University, Chicago, IL

Pediatric high-grade gliomas (pHGG) account for the most cancer-related deaths in children under the age of 19 years old, as there are no effective therapies available. Tumor associated macrophages (TAMs) play important roles in immune suppression and tumor promotion in adult HGG, yet little is known about their role in the pediatric setting. To uncover the composition and behavior of TAMs in pHGG we utilize immunocompetent genetic mouse models that recapitulate the human disease. We ectopically overexpress PDGFA or PDGFB in *Cdkn2a* wild type or knockout mice. We observe PDGFB-driven tumors have a significantly lower median survival compared to PDGFA-driven tumors. PDGFB tumors also have increased infiltration of TAMs and pericytes, as demonstrated by immunohistochemistry. Flow cytometry indicates the increased number of TAMs is due to the inflammatory monocyte population. Gene expression analysis indicates several chemokines and their receptors are highly expressed in PDGFB tumors, suggesting chemokine mediated infiltration of TAMs is occurring. To establish correlations between PDGF signaling and TAM infiltration in humans, we stained 37 tumors and found a positive correlation between TAM infiltration and the presence of vasculature and stroma. Together, this data provides the necessary foundation for the development of immunotherapies in pHGG.

NOVEL COMPOUNDS ISOLATED FROM *CASTANEA SATIVA* LEAF EXTRACT WHICH INHIBITS *STAPHYLOCOCCUS AUREUS* QUORUM SENSING

Akram M. Salam,¹ Gina Porras-Brenes,² James T. Lyles,² Cassandra L. Quave^{2,3,4,5*}

¹ Graduate Program in Molecular and Systems Pharmacology, Laney Graduate School, Emory University, Atlanta, Georgia

² Center for the Study of Human Health, Emory University, Atlanta, GA

³ Department of Dermatology, Emory University School of Medicine, Atlanta, GA

⁴ Emory Antibiotic Resistance Center, Atlanta, GA

⁵ Emory University Herbarium, Atlanta, GA

*E-mail: cquave@emory.edu Lab Website: <http://etnobotanica.us/>

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents one of the most serious infectious disease concerns worldwide, with the WHO having labeled it as a “high priority” pathogen in 2017. The current arsenal of antibiotics works by inhibiting bacterial growth, which exerts great selective pressure for the development of resistance. The development of novel anti-infectives that attenuate quorum sensing (QS) in MRSA has been recurrently proposed as a promising therapeutic approach. QS refers to a population density-dependent system of stimuli and response between cells in a bacterial population that, in MRSA, serves as the key coordinator of virulence. Previously, our lab reported the non-bactericidal quorum quenching (QQ) activity of a fraction of a *Castanea sativa* leaf extract, 224C-F2, demonstrating high bioactivity against MRSA *in vitro* and in a mouse model of skin infection. Bioassay-guided fractionation of this extract led to the isolation of a number of novel compounds. Fractionation was guided by a reporter strain screen of inhibition of the QS system. Identities of the compounds were elucidated via x-ray crystallography, nuclear magnetic resonance, and mass spectrometry. These compounds, in isolation or in combination, represent promising leads for the development of a novel quorum quenching approach against MRSA.

DISSECTING THE BIOLOGY OF LEADER AND FOLLOWER CELLS IN COLLECTIVE CANCER INVASION

B. Pedro^{1,2}, J. Konen³, E. Summerbell^{1,2}, J. Mouw², M. Rupji⁴, B. Dwivedi⁴, J. Kowalski⁴, P. Vertino⁵, A.I. Marcus²

¹ Graduate Program in Cancer Biology, Emory University, Atlanta, GA

² Hematology and Medical Oncology, Emory University, Atlanta, GA

³ Thoracic Head and Neck Oncology, University of Texas MD Anderson Cancer Center, Houston, TX

⁴ Biostatistics and Bioinformatics, Emory University, Atlanta, GA

⁵ Radiation Oncology, Emory University, Atlanta, GA

Previous work has demonstrated heterogeneity within collectively invading packs of lung cancer cells, including leader and follower cells that cooperate to facilitate invasion into the microenvironment. We utilized RNA-seq of purified populations of leader and follower cells from the H1299 non-small cell lung cancer cell line to identify 17 point mutations found uniquely in leaders and 18 point mutations found uniquely in followers, thus representing the first known compilation of leader- and follower-enriched genetic variants. Notable leader-enriched mutated genes included *NAE1*, *NUP93* and *ACTR3*, while notable follower-enriched mutated genes included *NADK*, *NDUFS1* and *LEO1*. We then performed single-cell RNA-seq on H1299 cell spheroids in a 3-D matrix. Interestingly, we found that leader-enriched and follower-enriched mutations are mutually exclusive on the single cell level, and leader and follower mutational profiles correlate with expression of leader cell genes (e.g. *MYO10* and *JAG1*), and follower genes (e.g. *IL13RA2* and *HTATIP2*), respectively. Subgroups expressing higher and lower levels of proliferative markers were also found, indicating further levels of heterogeneity within the population. Taken together, these data suggest novel drivers of leader and follower cell biology in collective invasion, opening the door to new potential strategies for targeting and inhibiting metastasis in human lung cancer.

A ROLE FOR SYNTENIN-1 IN MULTIPLE MYELOMA CELL SURVIVAL

Tyler Moser-Katz¹, Catherine M. Gavile¹, Benjamin G. Barwick², Sagar Lonial¹ Lawrence H. Boise¹

¹ Department of Hematology and Medical Oncology, Emory University

² Department of Radiation Oncology, Winship Cancer Institute, Emory University

During multiple myeloma, malignant transformation of plasma cells imparts survival independently of the bone-marrow microenvironment. Our lab has previously shown that signaling of the receptor protein, CD86 through its cytoplasmic tail is important for myeloma cell survival. CD86 contains a PDZ-binding domain which may allow it to signal through PDZ-domain containing proteins. We identified syntenin-1 as a PDZ-domain containing protein expressed in myeloma cells. Syntenin-1 has been shown to mediate vesicular trafficking of numerous proteins including CD138, a plasma/myeloma cell marker. However, when we studied correlation of syntenin-1 gene expression with CD86 and CD138 in myeloma patients, we found a positive correlation with CD86 but not CD138. We used short hairpin RNA to knock down syntenin-1 in two different myeloma cell lines. We found increased cell death in syntenin-1-silenced cells compared to our empty vector controls in both cell lines suggesting that syntenin-1 is important for myeloma cell survival. Silencing syntenin-1 decreases CD86 surface expression but causes no change in CD86 transcript or total cellular CD86 protein levels. Moreover, knockdown of CD86 results in increased protein expression and transcript levels of syntenin-1. Taken together, these data suggest that syntenin-1 may regulate myeloma survival via CD86 expression on the cell surface.

Session 2:

**Proteins:
Dynamics and
Signaling**

9:55AM

FATTY ACIDS BIND TO THEM1, A NEGATIVE REGULATOR OF THERMOGENESIS IN BROWN ADIPOSE TISSUE

Matthew C. Tillman¹, C. Denise Okafor¹, Manoj Khadka¹, Puneet Juneja¹, Eric A. Ortlund¹

¹ Department of Biochemistry, Emory University School of Medicine, Atlanta, GA

Obesity is a global epidemic, greatly increasing in prevalence over the past 30 years. In mammals, brown adipose tissue counteracts obesity through thermogenesis: the burning of fat to produce heat. Thioesterase superfamily member 1 (Them1) is a multi-domain enzyme in brown adipose tissue that slows thermogenesis by converting acyl-CoA into fatty acids. Additionally, Them1 contains a lipid-binding StAR-related lipid transfer (START) domain with an unknown function. We used mass spectrometry, X-ray crystallography, and microscale thermophoresis to demonstrate that long-chained fatty acids, products of Them1's enzymatic reaction, bind to the START domain. Additionally, we determined a low-resolution structure of full-length Them1 using negative stain electron microscopy and found that Them1 forms a trimer, making contacts between the enzymatic domains. Using this structure, we ran molecular dynamic simulations to compare protein dynamics in the presence and absence of fatty acid, and found the largest differences were localized to the START domain. In conclusion, we demonstrated that fatty acids bind the START domain, making Them1 the first protein of the START domain family identified to bind fatty acids. Additionally, we provide the first structure of full-length Them1, which enhances our limited knowledge of multi-domain thioesterases.

PRAP1 IS A NOVEL HOST PROTEIN HIGHLY SECRETED IN THE PRE-OVULATORY UTERUS AND MODULATES THE MICROBIOTA OF THE FEMALE REPRODUCTIVE TRACT

Alexandra A. Wolfarth¹, Xu Liu¹, Krisztina Z. Hanley¹, Eric A. Ortlund¹, Andrew S. Neish¹

¹ Emory University, Atlanta, GA

Proline rich acidic protein-1 (PRAP1) is a 17 kDa intrinsically disordered secreted protein conserved in placental mammals with no known function or recognizable sequence homology. To define the physiological role of PRAP1, we have made polyclonal antibodies, PRAP1 recombinant protein and *Prap1* null mice. Measurement of PRAP1 expression by qPCR and western blot reveal PRAP1 expression is highest in the proximal small intestine, and interestingly, in a hormonally dependent manner in uterine endometrial cells of female mice. This expression pattern is consistent with immunofluorescence staining demonstrating high expression of PRAP1 in the pre-ovulatory uterine endometria, as well as in the extracellular space of the corresponding mucosal tissue. *In vivo* data shows *Prap1* null females have decreased litter sizes, increased uterine inflammation and an altered bacterial community in the uterine fluid 12 hours after mating. Given the localization of PRAP1, its secretion into the lumen of the pre-ovulatory uterus, and its modulation of the uterine bacterial community 12 hours post coitum, we hypothesize that PRAP1 is a novel intrinsically disordered protein whose secretion influences the normal microbiota of the female reproductive tract, consequently contributing to colonization resistance against ascending pathogenic infections.

INTERLEUKIN-6 IN BILIARY TRACT CANCERS PROMOTES EXPANSION OF IMMUNE SUPPRESSIVE MYELOID CELLS AND IS ASSOCIATED WITH INCREASED MYELOID CELLS IN PATIENT TUMORS

Michael B. Ware^{1*}, Jennifer Yang^{2*}, Mohammad Y. Zaidi³, Alyssa Krasinskas⁴, Thomas A. Mace², Matthew R. Farren¹, Yiman Li⁵, Wanqi Chen⁵, Zhengjia Chen⁵, Gregory S. Young², Omar Elnaggar¹, Zheng Che¹, Shishir K. Maithel³, Tanios Bekaii-Saab¹, Bassel El-Rayes¹, Gregory B. Lesinski¹

* Denotes equal contribution

¹ Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University;

² Department of Internal Medicine, Divisions of Medical Oncology and Gastroenterology, The Ohio State University, Columbus, OH;

³ Department of Surgery, Winship Cancer Institute of Emory University;

⁴ Department of Pathology, Winship Cancer Institute of Emory University;

⁵ Biostatistics and Bioinformatics, Winship Cancer Institute of Emory University;

⁶ Center for Biostatistics, The Ohio State University, Columbus, OH

Biliary tract cancers (BTC) are an aggressive malignancy frequently associated with elevated immunosuppressive cell types in patients. We hypothesized that BTC-derived cytokines act through distinct pathways to promote immune suppressive features of the disease. Cytokine and chemokine secretion, and activation of associated signaling pathways, were studied in a panel of BTC cell lines. We used flow cytometry and immunoblot to study the effects of BTC supernatants on human peripheral blood mononuclear cells (PBMCs). Based on results, a human BTC tissue microarray (TMA, n=33) was stained for IL-6 and CD33+S100a9+ myeloid cells. BTC cell lines demonstrated active STAT3 and STAT5 signaling, and secreted several immunomodulatory factors including IL-6, GM-CSF, and MCP-1. Antibody neutralization of IL-6 and GM-CSF in these supernatants limited STAT3 and STAT5 phosphorylation in BTC cell lines and in PBMCs exposed to these supernatants. The expansion of myeloid derived suppressor cells (MDSC) from PBMCs cultured with BTC supernatants was inhibited with antibody neutralization of IL-6 but not GM-CSF. TMA analysis revealed significant association between IL-6 expression and CD33+S100a9+ myeloid cell infiltration of tumor samples ($p < 0.001$). This study highlights IL-6 as a potential target in BTC for future clinical trials, as it promotes the expansion of immunosuppressive cell types.

DEVELOPMENT OF A ROBUST DIRECT BINDING ASSAY FOR NR5A NUCLEAR RECEPTORS

Emma H. D'Agostino¹, Jeffery Cornelison², Suzanne G. Mays¹, Autumn Flynn², Nathan T. Jui², Eric A. Ortlund¹

¹ Department of Biochemistry, Emory University

² Department of Chemistry, Emory University

The human NR5A nuclear receptors, steroidogenic factor-1 (SF-1) and liver receptor homolog-1 (LRH-1), are phospholipid-sensing regulators of steroidogenesis, development, and metabolism. Their control of such diverse biological processes renders them attractive pharmacological targets for the treatment of several cancers and metabolic diseases. However, evaluation of candidate endogenous ligands and development of synthetic modulators have been hindered by the lack of a robust direct binding assay. Using a structure-guided approach, we recently developed a potent NR5A agonist (EC₅₀: 15 nM) targeting the ligand-binding pocket. We have leveraged this molecule to create a fluorescent probe that binds SF-1 and LRH-1 with 12 nM and 1 nM K_d, respectively. This probe enabled the creation of a fluorescence polarization (FP)-based competition assay suitable for both synthetic small molecules and mammalian phospholipids. We have found that for 25 LRH-1 agonists derived from the previously-reported RJW100 scaffold, affinities determined by FP correlate with potencies in a luciferase reporter assay, suggesting that *in vitro* binding affinity may predict in-cell activity for this molecule class. Moreover, affinities reported for NR5A modulators harboring different chemical scaffolds agree with our FP-based measurements. Finally, we show that the previously-reported dilauroylphosphatidylcholine agonist binds LRH-1 with 80 nM K_i, suggesting that this assay may be used to evaluate candidate endogenous NR5A ligands.

IRF4 REGULATES THE RATE OF CELL CYCLE DURING B CELL DIFFERENTIATION

Dillon G. Patterson¹, Madeline J. Price¹, Qiang Zhang², Christopher D. Scharer¹, Jeremy M. Boss¹

¹ Department of Microbiology and Immunology, Emory University, Atlanta, GA

² Department of Environmental Health, Rollins School of Public Health, Atlanta, GA

Cell division is required for the initiation of B cell differentiation, the regulation of isotype class switching, and ultimately entry of cells into the plasma cell (PC) lineage. Division coupled changes in the expression of transcription factors, such as interferon regulatory factor-4 (IRF4), that coordinate the PC transcriptional program also occur; however, little is known regarding how these factors coordinate cell division and exit from the cell cycle after differentiation. To begin to address this gap in knowledge, we assessed the cell division kinetics of wildtype (WT) B cells responding to the T cell independent antigen lipopolysaccharide (LPS). Interestingly, we found that WT B cells undergo at least 8 divisions before differentiating into PCs. Computational modeling of division rates over time defined a proliferative burst between 48 and 60 hours after LPS injection that is characterized by a rapid increase in the rate of cell division. IRF4-deficient B cells divided but failed to undergo the proliferative burst resulting in fewer activated B cells. Indeed, IRF4 is expressed at low levels during these divisions before being upregulated in post-mitotic CD138⁺ PCs after 8 divisions. Notably, while more than 40% of WT B cells reached division 8+ by 72 hours, IRF4-null B cells appeared to be stalled at divisions 2-5. Taken together, these data suggest that IRF4 regulates the rate of cellular proliferation during B cell differentiation and that this role may be concentration-dependent.

Session 3:

Neurological Disorders

11:20AM

REGULATION OF THE POTASSIUM CHLORIDE COTRANSPORTER-2 (KCC2) ON SPINAL MOTONEURONS FOLLOWING PERIPHERAL NERVE INJURY

E.T. Akhter^{1,2}, S. Sarin², A.W. English¹, F.J. Alvarez²

¹ Department of Cell Biology, Emory University

² Department of Physiology, Emory University

Following many types of injury, the potassium chloride cotransporter-2 (KCC2) on neurons is dysregulated. KCC2 controls intracellular chloride levels; decreased KCC2 activity alters the driving force for GABA/glycine inhibitory synapses and increases excitability in the affected neurons and networks. This phenomenon has been well described in dorsal horn sensory-associated interneurons following peripheral nerve injury (PNI), which is associated with the development of neuropathic pain. KCC2 is also downregulated in motoneurons axotomized in PNI, but this phenomenon's significance and mechanisms have received less attention. Previous studies in the spinal cord point to microglia and BDNF-TrkB signaling as responsible for KCC2 downregulation in dorsal horn interneurons. However, blocking microglia activation specifically in the ventral horn and deleting BDNF-TrkB signaling both genetically and pharmacologically did not prevent KCC2 downregulation in motoneurons axotomized after similar nerve injuries. Moreover, we found that KCC2 expression recovers only after motoneurons re-innervate peripheral muscle. In addition, RNA-scope indicates that KCC2 disappearance in axotomized motoneurons is likely controlled at the mRNA level. Therefore, we conclude that the mechanisms that control KCC2 in motoneurons after nerve injuries are quite distinct from those previously reported in the dorsal horn.

EXPLORING THE IMPACT OF *SCN8A* DIIS4 VOLTAGE-SENSOR DYSFUNCTION ON MOTOR FUNCTION AND NERVE CONDUCTION

George Andrew S. Inglis¹, Jennifer C. Wong¹, Kameryn M. Butler¹, Jackie Thelin², Olivia Mistretta³, Arthur English³, Andrew Escayg¹

¹ Department of Human Genetics, Emory University, Atlanta, Georgia

² College of Arts and Science, Emory University, Atlanta, Georgia

³ Department of Cell Biology, Emory University, Atlanta, Georgia

Mutations in the S4 voltage-sensor domains (VSDs) of the voltage-gated sodium channel gene, *SCN8A*, cause a spectrum of clinical phenotypes, including ataxic gait, intellectual disability, hypotonia, and epilepsy. To better understand how VSD mutations contribute to the diversity of patient phenotypes, we targeted the *Scn8a* DIIS4 VSD in a CRISPR/Cas9 mutagenesis screen. Here we present data on three mouse lines with novel *Scn8a* mutations: an in-frame 9bp deletion ($\Delta 9\text{bp}$), an in-frame 3bp insertion (+3bp), and a 35bp frameshifting deletion that results in a null allele ($\Delta 35\text{bp}$). Homozygotes from each line exhibit distinct movement abnormalities, suggesting that each mutation has a unique impact on $\text{Na}_v1.6$ function. Furthermore, heterozygotes for each *Scn8a* mutation display increased resistance to induced seizures, suggesting that these mutations are loss-of-function. While $\Delta 9\text{bp}$ and 3bp heterozygotes are visually indistinguishable from their WT littermates, they exhibit deficits in motor coordination, grip strength, and hearing. $\Delta 9\text{bp}$ and 3bp homozygotes also exhibit significant decreases in sciatic nerve conduction velocity. Strikingly, $\Delta 35\text{bp}$ mutants do not display any of these deficits, suggesting that hypomorphic *Scn8a* mutations have a more profound effect on mobility and related comorbidities than null alleles. By examining the consequences of variable VSD dysfunction, we aim to use these models to develop novel strategies for the treatment of patients with *SCN8A* VSD mutations.

LRRK2: LURKING BETWEEN THE BRAIN AND GUT

Mary K Herrick¹, Madelyn C Houser¹, Lindsey Sniffen¹, Jianjun Chang¹, Malú G Tansey¹

¹ Department of Physiology, Emory University School of Medicine, Atlanta GA

Links between Parkinson's disease (PD) and the gastrointestinal system have become increasingly common leading to the hypothesis that intestinal inflammation contributes to increased intestinal permeability that promotes systemic inflammation and affects neuroinflammation and neurodegeneration associated with PD pathogenesis. Mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) are known as the greatest genetic contributor to PD. While pathogenic mechanisms of LRRK2 are not well understood, LRRK2 has been shown to regulate inflammatory processes and our group has shown its expression is increased in PD immune cells. Elevated LRRK2 kinase activity due to increased protein expression or the G2019S mutation, the most common LRRK2 PD mutation, drives dysregulation resulting in increased inflammatory cytokines and inflammation. To directly investigate the role of increased LRRK2 protein and increased G2019S-mediated kinase activity on PD-associated neuroinflammation and neuropathology in a model of intestinal inflammation, we employed BAC transgenic mice overexpressing wildtype or G2019S LRRK2 subjected to acute DSS-induced colitis. We found that G2019S mice are more susceptible to acute DSS-induced colitis and exhibit increased CD8 T cell infiltration to the brain, increased activated microglia, and alterations in colonic tight junction proteins indicative of a leaky gut. Ongoing studies are determining if LRRK2 kinase inhibitors rescue phenotypes.

THE CILIARY PROTEIN ARL13B REGULATES AXON GUIDANCE IN THE MOUSE HINDBRAIN

Sarah Suci^{1,2}, Julien Ferent³, Sandii Constable², Laura Mariani^{2,5}, Eva Anton⁴, Frederic Charron³, Tamara Caspary²

¹ Genetics and Molecular Biology Graduate Program, Emory University, Atlanta, GA

² Emory University, Atlanta, GA

³ IRCM, Montreal, Quebec, Canada

⁴ University of North Carolina, Chapel Hill, NC

⁵ Neuroscience Graduate Program, Emory University, Atlanta, GA

The ciliopathy Joubert Syndrome (JS) presents with physical anomalies, intellectual disability, and is diagnosed by the molar tooth sign (MTS). The MTS results from cerebellar hypoplasia in conjunction with axon guidance defects in the white matter tract known as the superior cerebellar peduncles (SCPs). Mutations in cilia-associated genes including *ARL13B* cause JS. *ARL13B* regulates transcription-dependent Shh signaling, which requires cilia to regulate cell-fate specification and cerebellar precursor proliferation. *Arl13b* mutations in mice lead to constitutive but low-level transcription-dependent Shh signaling, which is consistent with cerebellar hypoplasia in JS. Shh signaling uses a distinct, transcription-independent pathway to regulate axon guidance, and so we hypothesized that aberrant Shh signaling might provide a common mechanism for the MTS. To examine *Arl13b*'s potential role in transcription-independent Shh signaling, we examined SCP guidance in mouse brains lacking either *Arl13b* or all Shh signaling in projection neurons. We observed significant guidance defects in SCPs lacking Shh signaling or *Arl13b*. These data indicate *Arl13b* regulates axon guidance in projection neurons that use Shh as a guidance cue, implicating a cilia-associated gene in axon guidance. Taken together, our data suggest that disruption of Shh signaling may be a common mechanism underlying the MTS phenotype seen in JS.

Session 4:

**Epigenetics and
Gene Expression**

2:40PM

CRISPR SYSTEMS DIVERSIFY FUNCTIONS WITH SUBTLE RNA VARIATIONS

Hannah K. Ratner,^{1,2,3} Andrés Escalera-Maurer,^{4,5} Anaïs Le Rhun,^{4,5} Siddarth Jaggavarapu,^{2,3,6} Jessie E. Wozniak,^{1,2,3} Emily K. Crispell,^{1,2,3} Emmanuelle Charpentier,^{4,5,7} David S. Weiss^{1,2,3,6}

¹ Department of Microbiology and Immunology, Microbiology and Molecular Genetics Program, Emory University, Atlanta

² Emory Vaccine Center, Emory University

³ Yerkes National Primate Research Center, Emory University

⁴ Max Planck Unit for the Science of Pathogens, Berlin, Germany

⁵ Helmholtz Centre for Infection Research, Department of Regulation in Infection Biology, Braunschweig, Germany

⁶ Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine

⁷ Institute for Biology, Humboldt University, Berlin, Germany

CRISPR-Cas systems are widespread in prokaryotes and function as adaptive immune systems that use small RNAs (crRNAs) to guide Cas protein(s) to recognize and cleave harmful foreign nucleic acids. There is increasing evidence for broader roles of these systems. The CRISPR-Cas9 system of pathogenic *Francisella novicida* is required for virulence. We determined that Cas9 enables virulence by repressing the transcription of four endogenous genes. This regulation is mediated by a non-canonical small RNA (scaRNA), rather than a crRNA, that guides Cas9 to bind DNA targets and block RNA polymerase. scaRNA binds endogenous targets without lethally cleaving the bacterial chromosome because of reduced scaRNA:DNA complementarity. Using these parameters, we reprogrammed scaRNA to repress genes of interest and found that crRNAs of both native *F. novicida* CRISPR systems, Cas9 and Cas12, could be similarly commandeered to direct transcriptional repression. The specificity of these systems is determined by distinct RNAs, allowing DNA-binding complexes and DNA-cleaving complexes to exist simultaneously in a host bacterium. Natural CRISPR-mediated transcriptional interference by cleavage capable Cas proteins likely represents a broad paradigm of regulatory functionality, which is potentially critical to the physiology of numerous Cas9- and Cas12-encoding pathogenic and commensal organisms.

EXPOSURE TO POLYBROMINATED BIPHENYL (PBB) ASSOCIATES WITH DNA METHYLATION DIFFERENCES ACROSS THE GENOME

Sarah W. Curtis¹, Varun Kilaru², Dawayland O. Cobb², Metrecia L. Terrell³, Carmen Marsit⁴, Michele Marcus^{3,4,5}, Karen N. Conneely⁶, Alicia K. Smith²

¹ Genetics and Molecular Biology Program, Emory University

² Department of Gynecology and Obstetrics, Emory University

³ Department of Epidemiology, Emory University

⁴ Department of Environmental Health, Emory University

⁵ Department of Pediatrics, Emory University

⁶ Department of Human Genetics, Emory University

In 1973, 6.5 million people were exposed to polybrominated biphenyl (PBB), an endocrine-disrupting compound. Highly-exposed individuals have numerous health problems, but the mechanism is unknown. Other endocrine-disrupting compounds are associated with epigenetic differences, but no epigenetic studies have been done for PBB. In this study, DNA from 658 individuals with PBB exposure was interrogated with the MethylationEPIC BeadChip. Each of the ~850,000 CpG sites was tested against current PBB levels. After multiple test correction, 1890 CpG sites associated with total PBB levels. These CpGs were enriched in enhancer and insulator regions. To understand the function of these enriched enhancers, PBB-associated CpGs were interrogated for transcription factor binding sites and links to gene expression. They were more likely to be in ARNT and ESR2 transcription factor binding sites, and there was significant overlap between PBB-associated CpGs and estrogen-associated CpGs. PBB-associated CpGs were also enriched for CpGs linked with gene expression (eQTM). These eQTMs were associated with the expression of genes in immune pathways. Taken together, these results indicate that exposure to PBB is associated with differences in epigenetic marks that suggest that it is acting similarly to estrogen and is associated with dysregulated immune system pathways.

A LysR-TYPE TRANSCRIPTIONAL REGULATOR IMPACTS MULTIPLE PHENOTYPES IN *ACINETOBACTER BAUMANNII*

Aimee R. Paulk Tierney¹, Philip N. Rather^{1, 2}

¹ Department of Microbiology and Immunology, Emory University School of Medicine

² Research Service, Department of Veterans' Affairs, Atlanta VA Health Care System

Acinetobacter baumannii is a nosocomial multidrug resistant Gram-negative pathogen that exhibits phenotypic heterogeneity resulting in two colony variants, termed opaque (VIR-O) and translucent (AV-T). Each variant shows different patterns of gene expression, resulting in several phenotypic differences including increased quorum sensing signal production, motility, and virulence in the VIR-O variant. Cells of each variant interconvert at high frequency, and therefore our group has focused on identifying genes that regulate this switch. These efforts led to the recent identification of *ABUW_1132* (hereafter, *1132*), a gene predicted to encode a LysR-type transcriptional regulator (LTTR). Deletion of *1132* in the strain AB5075 results in a 35-fold decrease in VIR-O to AV-T switching as well as a 104-fold increase in AV-T to VIR-O switching. Surprisingly, the *1132* deletion also results in the total loss of quorum sensing signal secretion, a phenotype that is enhanced in the wild-type VIR-O variant. Additionally, both the deletion and overexpression of *1132* increases motility. These findings demonstrate a critical role for *1132* in *A. baumannii*'s virulence switch and possible involvement of *1132* in other regulatory pathways that impact quorum sensing and motility.

CROSS-SPECIES GENOME-WIDE PROFILING REVEALS DEPLETION OF CHARACTERISTIC ENHANCER HISTONE MODIFICATIONS AT ACCESSIBLE CHROMATIN SITES IN PLANTS

Kelsey A. Maher^{1,2}, Dongxue Wang³, Benjamin G. Barwick⁴, Roger B. Deal¹

¹ Department of Biology, Emory University

² Graduate Program of Biochemistry, Cell, and Developmental Biology, Emory University

³ Department of Biochemistry, Emory University

⁴ Department of Hematology and Oncology, Emory University

Transcriptional regulation is a universal mechanism for a wide array of biological processes and is driven in large part by genetic enhancer elements. While these regulatory elements have been well-studied in animal species, their plant counterparts remain poorly characterized. While high-throughput profiling of animal genomes has yielded great success in identifying genetic enhancers through secondary characteristics – histone posttranslational modifications (PTMs), chromatin accessibility, and the production of enhancer RNAs (eRNAs) – it remains an open question whether these metrics can be used to locate regulatory regions of plant genomes. Here, we compare the enrichment of the four histone PTMs most commonly associated with animal enhancers – H3K27ac, H3K27me3, H3K4me1, and H3K4me3 – between *Drosophila melanogaster*, *Homo sapiens*, *Arabidopsis thaliana*, and *Oryza sativa* genomes in single cell-type datasets. Sites of accessible chromatin were identified through ATAC-seq or DNase-seq and were analyzed as putative enhancer regions. Through the intersection of these data it becomes clear that there are distinct differences between the epigenetic makeup of plant and animal genomes. While it is known that animal promoters and enhancers have bidirectional transcription, this analysis revealed that plant promoters and enhancers have a distinct pattern, and only exhibit histone PTM deposition and transcription in the sense direction. While further investigation is merited, this pattern appears to span the evolutionary divergence between monocots and dicots, and may speak to a fundamental difference between the transcriptional machinery of plant and animal kingdoms.

A ROLE FOR EPIGENETIC MECHANISMS IN HOMOLOGOUS CHROMOSOME RECOGNITION DURING MEIOSIS

Christine Doronio^{1,2}, William G. Kelly²

¹ Genetics and Molecular Biology Program, Emory University

² Biology Department, Emory University

During meiosis, homologous chromosomes must correctly identify one another in order for proper alignment to occur. Improper alignment can lead to large chromosomal rearrangements and aneuploidies that result in improper development and embryonic lethality. Currently, little is known about how homologs identify each other to the exclusion of other chromosomes. During meiosis the patterns of transcription that occur are unique for each chromosome, and likewise create unique patterns of epigenetic modifications such as the methylation of Lysine 36 on Histone H3 (H3K36me). In humans, H3K36me is recognized by the chromodomain containing protein MRG15, the homolog of which in *C. elegans* is named MRG-1. *C. elegans* lacking MRG-1 display homolog pairing and alignment defects yet the specific role of MRG-1 in homolog recognition is unknown. Our hypothesis is that histone modifications that result from meiotic transcription, including H3K36me, provide a “barcode” used to distinguish chromosomes during homolog searching and identity recognition is facilitated through MRG-1. After examining the role of MRG-1 in mutant germlines, our data demonstrates that germ cells lacking MRG-1 exhibit numerous meiotic defects such as increased sterility, synaptic delay, and improper crossover formation. Conversely, germlines lacking H3K36me exhibit similar defects. These results suggest epigenetic modifications and proteins that recognize them play an important role in homolog recognition during meiosis.

Session 5:

**Immunity and
Pathogens**

4:05PM

VIRUS-HOST INTERACTIONS DETERMINE INFLUENZA A VIRUS CO-INFECTION DEPENDENCE

Kara Phipps¹, Hui Tao¹, Ketaki Ganti¹, Anice C. Lowen¹

¹ Department of Microbiology and Immunology, Emory University School of Medicine

The influenza A virus (IAV) genome segmentation enables genetic diversification by reassortment, which occurs when two IAVs co-infect the same cell and exchange segments. Segmentation imposes constraints, however, as each gene is necessary for productive infection. The factors which determine co-infection dependence remain to be characterized. Frequency of co-infection dependence was evaluated by measuring reassortment levels between phenotypically similar, genetically barcoded viruses in various cell lines. A/guinea fowl/HK/WF10/1999 (WF10) viruses resulted in high levels of reassortment in mammalian MDCK cells, indicative of an acute dependence on co-infection for productive infection; this dependence was lessened in DF-1 chicken fibroblasts. Reassortment frequency also differed with strain: A/mallard/Minnesota/199106/99 (MN99) viruses reassorted less than WF10 viruses in MDCK cells. *In vivo* studies using aforementioned strains in avian and mammalian hosts corroborated these findings. RNA quantification studies revealed that co-infection boosts viral replication in multiple virus-host combinations and ~30% of single virion infections with WF10 in MDCKs lack the full viral genome. Studies of reassortment with MN99:WF10 chimeric viruses indicated a potential role for the HA glycoprotein and viral polymerase in co-infection dependence. Overall, our findings indicate that multiple infection is a prevalent feature of IAV infection and its frequency is determined by virus-host interactions.

EPIGENETIC PRIMING ACCELERATES MEMORY B CELL REACTIVATION

Madeline J. Price¹, Sakeenah L. Hicks¹, Anna K. Kania¹, Tian Mi, Christopher D. Scharer¹, Troy D. Randall², Jeremy M. Boss¹

¹ Department of Microbiology and Immunology and the Emory Vaccine Center, Emory University School of Medicine

² Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham

Immunological memory is one of the hallmarks of the adaptive immune response. However, the molecular mechanisms by which memory cells are primed to respond are currently unknown. Using a mouse model of influenza infection, memory and naïve B cells were purified to identify changes in gene expression and chromatin accessibility. Naïve B, IgM+ and IgG+ memory B cells have distinct gene expression profiles. There is a significant upregulation of total mRNA content in memory B cells, suggesting an “antigen experience” signature is present following primary stimulation. Bone marrow plasma cell (PC) transcription factors are increased in memory B cells compared to naïve B cells by gene set enrichment analysis. Additionally, memory B cells display open chromatin in regulatory regions that map to PC-specific transcription factors. To confirm the importance of these molecular changes, naïve and memory mice were challenged with a heterosubtypic influenza infection. Memory mice form germinal centers earlier and to a higher frequency than naïve mice. Memory mice also form more NP+IgG+ plasma cells, indicating that these mice are more capable of differentiating into secondary germinal center B cells and PC upon challenge. These data describe a molecular basis for enhanced the differentiation capacity of memory B cells.

IDENTIFICATION OF A β -LACTAMASE THAT CONTRIBUTES TO INTRINSIC β -LACTAM RESISTANCE IN *CLOSTRIDIODES DIFFICILE*

B. K. Sandhu^{1,2}, S. E. Anderson², A. N. Edwards², E. C. Woods², S. M. McBride²

¹ Genetics and Molecular Biology Graduate Program, Emory University

² Department of Microbiology and Immunology, Emory Antibiotic Resistance Center, Emory University School of Medicine

Clostridioides difficile causes severe antibiotic-associated diarrhea and colitis. *C. difficile* is an anaerobic, Gram-positive spore former that is highly resistant to β -lactams, the most commonly prescribed antibiotics. Antibiotic resistance in *C. difficile* allows the pathogen to replicate and cause disease in antibiotic-treated patients. However, the mechanisms of β -lactam resistance in *C. difficile* are unknown. Our data demonstrate that *C. difficile* produces a β -lactamase, an enzyme that cleaves and inactivates β -lactams – a common resistance mechanism in other bacterial species. We identified an operon encoding a lipoprotein of unknown function and a β -lactamase that was highly upregulated in response to β -lactam antibiotics. An in-frame deletion of the operon abolished β -lactamase activity in *C. difficile* strain 630 Δ *erm* and exhibited decreased resistance to some, but not all, β -lactams. We found that the activity of the β -lactamase, named BlaD, is dependent upon the redox state of the enzyme, and transport of BlaD out of the cytosol is facilitated by the N-terminus. We are currently investigating whether the lipoprotein aids in BlaD transport. The identification and characterization of the *C. difficile* β -lactamase and other mechanisms of β -lactam resistance in *C. difficile* may enable identification of novel targets for the prevention of *C. difficile* β -lactam resistance.

THE EVOLUTION OF VIRULENCE IN A HETEROGENEOUS HOST POPULATION

P. Signe White^{1,4}, Angela Choi², Arthur Menezes², Rishika Pandey³, McKenna Penley⁴, Levi Morran⁴

¹ Population Biology, Ecology, and Evolution, GDBBS, LGS, Emory University

² College of Arts and Sciences, Emory University

³ Department of Genetics, University of Georgia

⁴ Department of Biology, Emory University

To survive and reproduce, parasites must adapt to the immune heterogeneity of their host environment. Individuals within a host population vary in their susceptibility to infection. Previous work has shown that some parasites specialize on the most common host genotype in a host population. However, if the host population has an equal ratio of susceptible to immune individuals (1:1), parasite generalism may evolve. Here, we used experimental evolution to determine how host specialization and generalism evolve. We began by creating *Caenorhabditis elegans* host populations of differential immunity in varying ratios. After passaging our parasite, *Serratia marcescens*, through 20 host generations, we then analyzed changes in virulence via host mortality assays. Compared with the ancestral genotype, we found that parasites adapted to the most common host in all treatments (i.e. increased virulence on that genotype), regardless of host genotype. The evenly mixed host populations were variable in mortality rates when infected with evolved bacteria. Our experiments show that parasite specialization is influenced in part by variation in host immune genotype. Next steps include sequencing parasite populations throughout the experiment to determine underlying genes that correspond to a relative increase or decrease in virulence.

ABUNDANT EXPRESSION OF CCR5 ON EARLY HOFBAUER CELLS MAY INCREASE HIV-1 SUSCEPTIBILITY

Dominika Swieboda^{1,2}, Erica Johnson¹, Ioanna Skountzou², Rana Chakraborty³

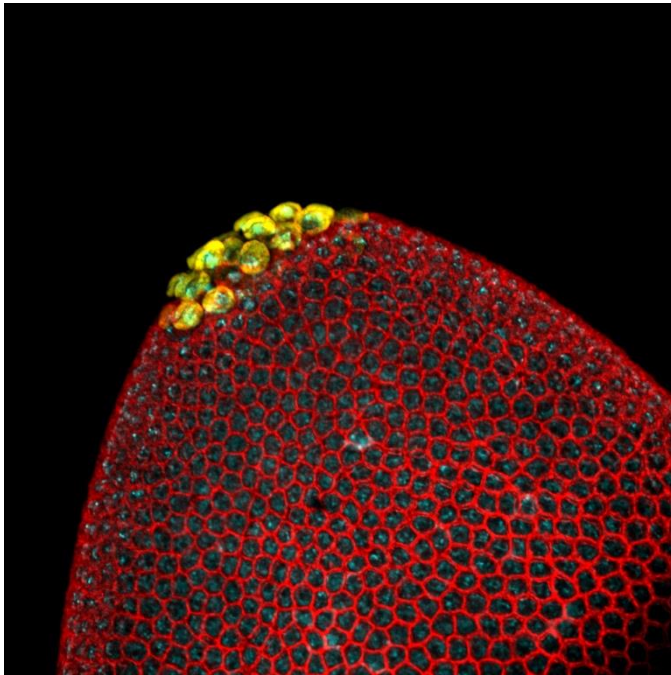
¹ Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine

² Department of Microbiology and Immunology, Emory University School of Medicine

³ Department of Pediatrics and Adolescent Medicine, Division of Infectious Diseases, Mayo Clinic, School of Medicine

Maternal antiretroviral therapy reduces, but does not eliminate, vertical transmission of HIV-1. Placental macrophages (Hofbauer cells, HCs) may be key mediators of *in utero* transmission. Studies have demonstrated that HIV-1 replication can be regulated by cytokines and interferons (IFNs), and certain maternal coinfections enhance susceptibility and viral replication *in vitro* by altering HC polarization. Early-gestation HCs have yet to be evaluated in the context of HIV-1 permissivity. We determined the prevalence of HCs positive for HIV-1 co-receptors throughout gestation and evaluated expression of HIV-1 restriction factors SAMHD1, Tetherin, Trim5 α , TREX-1, and APOBEC3G. HCs were treated with polarizing cytokines or IFNs, and changes in receptor and restriction factor expression were determined. Confoundingly, the frequency of HCs positive for CCR5 decreases with gestational age; only 60% of term HCs expressed CCR5, while close to 100% of early-gestation HCs were positive for this receptor. HIV-1 restriction factors were present at baseline, and upregulation of restriction factors in HCs isolated from early-gestation matched or exceeded that of term samples. Placental macrophages in early pregnancy may be susceptible to HIV-1 infection due to abundant expression of CCR5. Infection susceptibility may be counterbalanced by robust basal and cytokine-induced expression of key HIV-1 restriction factors.

Poster Presentation Abstracts



ICI Image Contest Runner-Up:

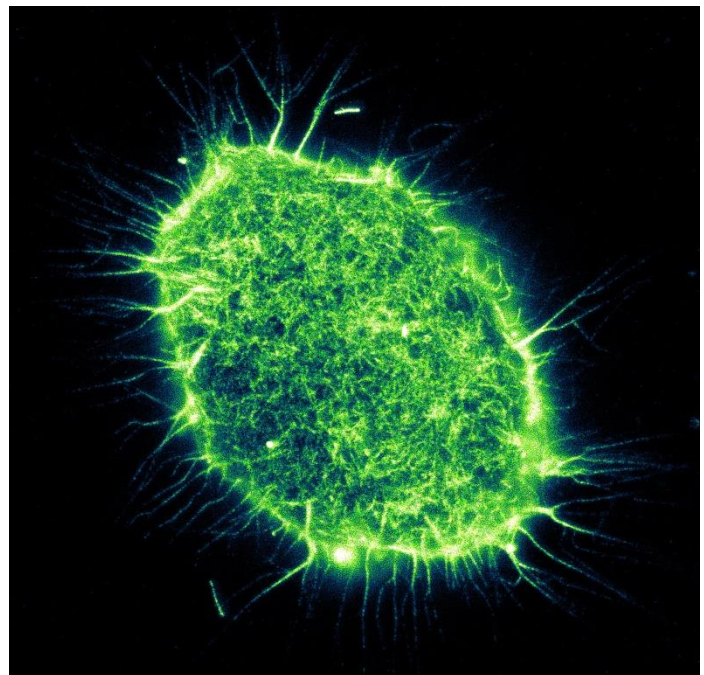
**Joanna Perez,
Lerit Lab, BCDB**

Confocal image showing pole cell budding in early *Drosophila* embryos. Nuclei is stained with DAPI (cyan). Cell boundaries staining is shown in red and germline specific marker, Vasa, is shown in yellow.

ICI Image Contest Runner-Up:

**Brian Pedro,
Marcus Lab, CB**

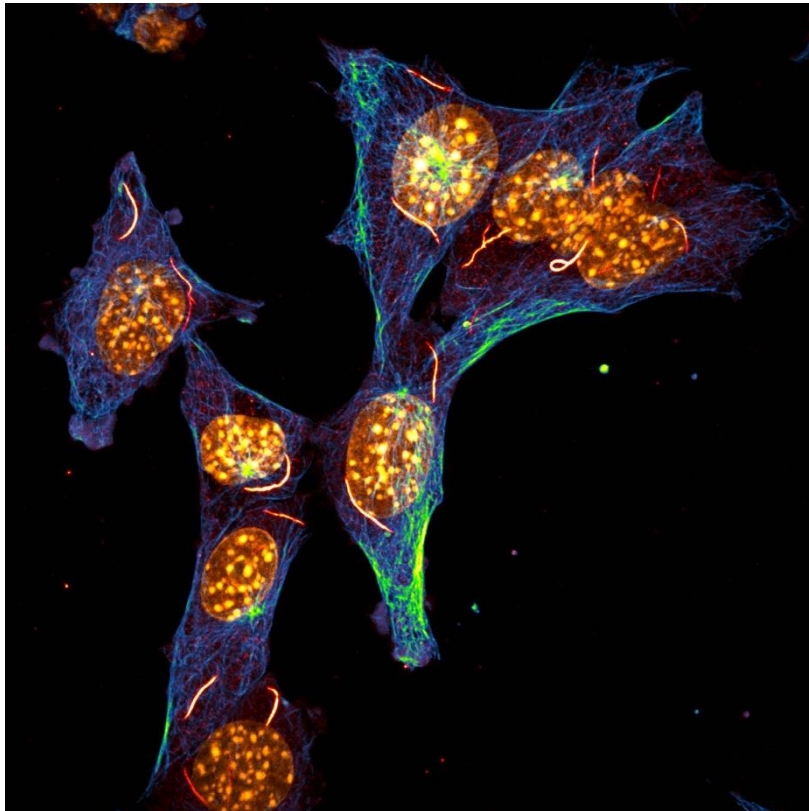
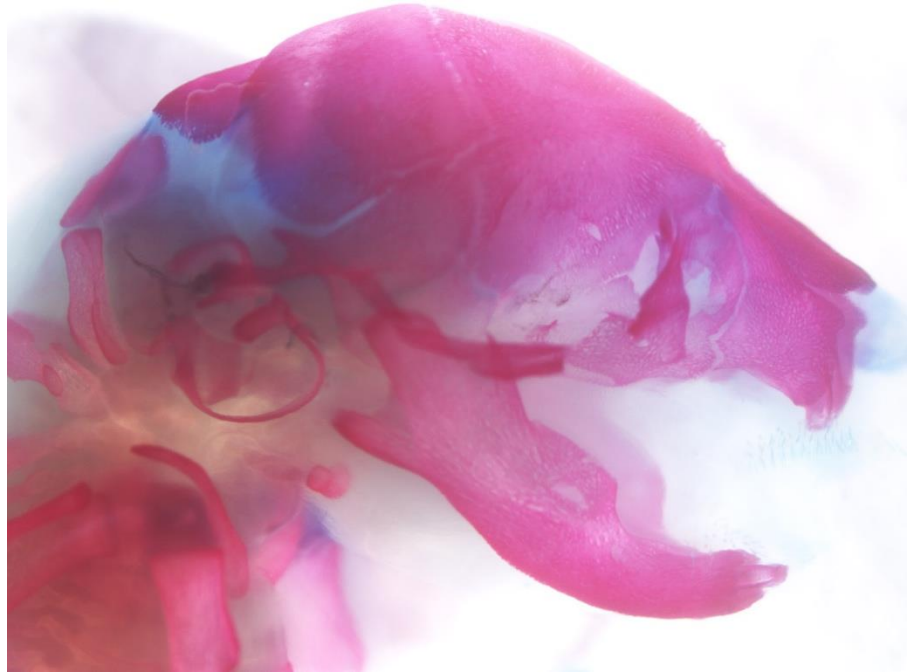
A single H1299 non-small cell lung cancer cell, labeled with SiR-Actin live-cell dye and imaged via STED super-resolution microscopy.



**ICI Image Contest
Runner-Up:**

**Alyssa Scott,
Katz Lab, GMB**

Skeletal Prep- This is a whole-mount skeletal preparation of a postnatal day 0 mouse that has been stained with Alcian Blue and Alizarin Red to identify cartilage and bone, respectively.



ICI Image Contest Runner-Up:

**Cara Schiavon,
Kahn Lab, CB**

MEFs fixed and stained for nuclei,
microtubules, and Rods and
Rings, imaged by confocal

Poster Presentations

Session 1: 12:30 - 1:30PM - Odd-numbered posters

Session 2: 1:30 - 2:30PM - Even-numbered posters

Poster	Name		Program	Poster	Name		Program
44.2	Anderson	Sarah	MMG	33	Lopez	Alejandro	NS
1	Assefa	Ezana	GMB	34	Lustberg	Daniel	MSP
2	Beaver	Jacob	MMG	65	Mattison	Kari	GMB
3	Berry	Jameson	CB	35	McCaffery	Jessica	IMP
4	Bixler	Brianna	GMB	36	Modeste	Erica	MSP
5	Brooks	Kelsie	MMG	37	Moller	Abraham	MMG
6	Camp	Chad	MSP	38	Moody	Jasmine	GMB
7	Cato	Michael	BCDB	39	Peterson	Raven	BCDB
8	Christian	Courtney	BCDB	17	Pollak	Rebecca	GMB
9	DiCandia	Michael	GMB	41	Prince	Stephanie	NS
10	Downs	Anthony	MSP	42	Ravi	Meghna	NS
11	Engstrom	Amanda	BCDB	43	Robinson	Beverly	GMB
12	Enriquez	Ana	MMG	44.1	Rodriguez Stewart	Roxana	MMG
13	Ferdous	Salma	GMB	45	Root	Jessica	NS
14	Fiedler	Sarah	GMB	46	Russell	Athena	GMB
15	Ford	Bijean	IMP	47	Scott	Alyssa	GMB
16	Garza	Kristie	NS	48	Sequeira	Michelle	NS
40	Grewenow	Stephanie	GMB	49	Shapiro	Lindsey	NS
18	Habib	Jakob	IMP	50	Shartouny	Jessica	IMP
19	Herting	Cameron	MSP	51	Sherman	Edgar	MMG
20	Holder	Dylan	GMB	52	Shields	Cara	CB
21	Houser	Madelyn	IMP	53	Shurzinske	Amanda	GMB
22	Jalloh	Binta	GMB	54	Smith	Taylor	GMB
23	Jeffers	Lauren	BCDB	55	Srinivas	Pooja	MSP
24	Jeong	Nuri	NS	56	Sterrett	Maria	BCDB
25	Johnson	Ashlyn	NS	64	Summerbell	Emily	CB
26	Kania	Anna	GMB	57	Suster	Izabela	BCDB
27	Kietzman	Henry	NS	58	Terry	Doug	GMB
28	Koyen	Allyson	CB	59	Weir	David	CB
29	Kuehner	Janise	GMB	60	Wilde	Shyra	MMG
30	Kundinger	Sean	BCDB	61	Woods	Megan	MMG
31	Lewis	Jordan	PBEE	62	Woon	Ellen	NS
32	Li	Yixiang	CB	63	Wynne	Meghan	NS

REGULATION OF A VIRULENCE SWITCH BY AN ANTIBIOTIC RESISTANCE LOCUS IN *ACINETOBACTER BAUMANNII*

Sarah E. Anderson¹ and Philip N. Rather^{1,2}

¹ Department of Microbiology and Immunology and Emory Antibiotic Resistance Center, Emory University, Atlanta, GA

² Research Service, Atlanta VA Medical Center, Decatur, GA

Acinetobacter baumannii is a Gram negative nosocomial pathogen that exhibits widespread antibiotic resistance. Many strains of *A. baumannii*, including the highly virulent AB5075, undergo a high frequency switch between virulent and avirulent subpopulations. These subpopulations are easily distinguished on 0.5x agar plates with oblique illumination. Under these conditions, the virulent variant appears opaque (O), whereas the avirulent variant appears translucent (T). AB5075 produces multiple subpopulations of O variants, which differ in terms of their rate of switching to T. While “normal” O variants switch to T at a rate of about 30% in twenty-four hours, low-switching O (LSO) variants switch at a rate that is approximately 1,000-fold reduced. Whole genome sequencing revealed a duplication in an integron encoded on an antibiotic resistance plasmid in the normal O variant relative to the LSO. Overexpression of this region in the LSO background was sufficient to induce hyperswitching from O to T. Overexpression of truncated DNA fragments from the integron was used to identify the region responsible for inducing switching. The intergenic region between *aadB* and *intl* in the integron was sufficient to induce switching. We hypothesize that this region encodes a novel sRNA or small peptide that stimulates O to T switching.

EXCISION OF THE PATHOGENIC CAG REPEAT IN HUNTINGTON'S DISEASE IN PLURIPOTENT STEM CELLS USING A CAS9 NICKASE CAN BE INHERITED THROUGH THE GERMLINE VIA DERIVED MALE GERM CELLS

Ezana Assefa^{1,2,3}, In-ki Cho^{1,2}, Anthony W. S. Chan^{1,2}

¹ Yerkes National Primate Research Center

² Department of Human Genetics, Emory University School of Medicine

³ Genetics and Molecular Biology Program, Emory Laney Graduate School

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat (TNR) expansion in the first exon of the huntingtin gene (*HTT/IT15*). The age of onset for HD is inversely correlated to the size of CAG repeats; repeats larger than 40 form an expanded polyglutamine (polyQ) tract, which can form oligomeric aggregates in HD-patient brains. The severity of HD is compounded by the pathogenic instability of TNR in both somatic and germline cells. The huntingtin protein (HTT) has the highest expression and TNR mosaicism in the brain and testes, correlating to selective neurodegeneration and male germline transmission (i.e. anticipation). We hypothesize that somatic and germline expansions can be prevented by editing pathogenic *HTT* using the CRISPR-Cas9 system. Multiple pairs of guide RNAs (gRNAs) were designed to target polyQ tracks flanking exon 1 of *HTT*. Two HD patient-derived induced pluripotent stem cell (iPSC) lines and two transgenic HD rhesus macaque stem cell lines will be used in this study. TNR instability will be compared across all cell lines during *in vitro* spermatogenesis and neurogenesis, and we expect gene-edited cell lines will show comparable instability levels to the wild-type (WT) cell lines. Finally, spermatids derived from gene-edited rhesus macaque stem cells will be used to create preimplantation embryos for the derivation of embryonic stem cells (ESCs) to determine if gene-edited repeat tracts can be inherited, potentially providing a novel approach in HD therapeutics.

ZIKA VIRUS MICRONEEDLE VACCINATION CONFERS LONG-TERM PROTECTION TO IMMUNE-PRIVILEGED COMPARTMENTS

Jacob T. Beaver¹, Lisa K. Mills¹, Edward S. Esser¹, Elizabeth Q. Littauer¹, Nadia Lelutiu¹, Olivia Q. Antao¹, Rumi Habib¹, Vidisha Singh¹, Dahnide T. Williams¹, Andrey Romanyuk², Mark Prausnitz², Elena V. Vasilieva¹, Richard W. Compans¹, Ioanna Skountzou¹

¹ Department of Microbiology & Immunology, Emory University School of Medicine

² School of Chemical and Biomolecular Engineering, Georgia Institute of Technology

Zika virus remains a critical international health issue, yet there is no commercial vaccine available and few studies seek to characterize how vaccinations protect immune privileged compartments, such as eyes and brain tissues. We observed Zika virus pathogenesis can be monitored by clinical scoring methods specifically aimed at ocular and motor/neural symptoms, as ocular conjunctivitis and joint-pain are two of the more common symptoms of ZIKV infections. We demonstrate skin vaccination using inactivated virus delivered by microneedle patches (MNP) improves antibody breadth and quality, antigen presentation timeline, and protection against ocular and motor/neural symptoms, as well as infection-induced tissue damage. The efficacy demonstrated via MNP versus IM vaccinations occurs in both prime and prime/boost schedule. A booster MNP vaccine further accelerates convalescence and reduces damage to tissues of the ocular tract. Given that ZIKV infections have shown proclivity for immune privileged compartments, such as eyes and brain, the development of a protective vaccine against ZIKV should demonstrate protection for these compartments, while minimizing the risk of auto-reactive antibodies that lead to nervous system damage and auto-immunity. Our results demonstrate that vaccination against ZIKV using MN patches offers protection for these critical tissue compartments, as well as providing less auto-immune antibodies.

TUMOR CELL-DIRECTED ONCOLYSIS BY REOVIRUS IS ENHANCED BY DOXORUBICIN-VIRUS CONJUGATION

Jameson T. L. Berry¹, Bernardo A. Mainou¹

¹ Division of Pediatrics Infectious Disease, Emory University

Triple-negative breast cancer (TNBC), characterized by the lack of estrogen, progesterone, and HER2/Neu receptors, constitutes 10-20% of all breast cancer cases. Treatment is largely limited to cytotoxic chemotherapy. Combination therapy can increase treatment efficacy. Reovirus, an oncolytic virus, preferentially infects and kills transformed cells. In a high-throughput screen of small molecule inhibitors, we identified doxorubicin as an enhancer of reovirus infectivity in the TNBC cell line MDA-MB-231. To better control doxorubicin delivery and enhance reovirus oncolytic potential, we chemically conjugated doxorubicin to reovirus virions (reo-dox). While reo-dox attachment to MDA-MB-231 cells is slightly impaired, viral replication is largely unaffected. Infection of MDA-MB-231 cells with reo-dox increased cytopathicity with faster induction of cell death than virus alone. MDA-MB-231 cells infected with reo-dox, but not virus alone, induced DNA double-strand breaks and activation of DNA damage response. Together, our findings show that reo-dox exhibits superior toxicity in TNBC cells than virus alone. Future studies will define the mechanism of enhanced cytopathicity of reo-dox and oncolytic efficacy of dox-conjugated reovirus *in vivo*. Delivery of small molecule inhibitors via conjugation to reovirus particles may provide an effective new method to directly target and kill cancer cells while minimizing toxicity to healthy cells and tissues.

PARENT-OF-ORIGIN MAINTENANCE OF TRANSCRIPTION-FACTOR MEDIATED INTERACTIONS FROM GAMETES TO THE PRE-IMPLANTATION MOUSE EMBRYO

Brianna Jeanne Bixler¹, Yoon Hee Jung¹, Hannah Gold¹, Victor G. Corces¹

¹ Department of Biology, Emory University

Gametes are the foundational material of the embryo, which undergoes highly-orchestrated and dynamic gene expression programs prior to implantation. It is known that genomic sites bound by transcription factors in the gametes also remain accessible in the preimplantation embryo. First, we hope to characterize which transcription factors remain bound from the gametes to the pronuclei of the zygote. ATAC-seq is a population-based sequencing method that allows us to determine and distinguish the binding of transcription factors and nucleosomes across the genome by the size and frequency of Tn5 insertion. SNPs between strains of mice allow us to distinguish the maternal and paternal genomes. With ATAC-seq datasets of the zygote, the sperm, and the MII oocytes of two distinct mouse strains coupled with Hi-C, ChIP-seq, and RNA-seq of the preimplantation embryo, we can correlate these transcription factors with zygotic gene activation and the increasingly ordered chromatin structures of the pre-implantation embryo.

TRANSMITTED/FOUNDER VIRUS-LIKE VARIANTS ARE ARCHIVED IN THE RESERVOIR

Kelsie Brooks¹, Dario Dilemnia¹, Daniel Wilkins¹, Daniel Claiborne², Samantha McNally¹, Eric Hunter^{1,3}

¹ Emory Vaccine Center, Emory University, Atlanta, GA

² Ragon Institute of MGH, MIT and Harvard, Cambridge, MA

³ Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA

Heterosexually transmitted HIV infections are predominantly initiated by a single viral variant identified as the transmitted/founder virus (TFV). TFV impacts on the reservoir of latently infected cells remain understudied; the reservoir is a significant barrier to HIV cure. We have identified the TFV(s) in 13 HIV+ individuals and investigated intrahost TFV evolution through amplification and sequencing of the viral *env* gene during chronic, treatment-naïve infection. Additionally, to sample the reservoir, we amplified and sequenced *env* genes from peripheral white blood cells collected following treatment initiation while viral load was suppressed to <100 copies/mL. Chronic infection viral variants demonstrated considerable evolution from the TFV in all individuals. Reservoir variants included both variants evolved from the TFV, and variants contemporaneous with the TFV. In 12 individuals infected by a single TFV, the closest genetic variant to the TFV was amplified from the reservoir, with an exact match observed in two individuals. These variants may represent archiving of viruses present early in infection that remained in latently infected cells despite treatment. Archiving of variants in the reservoir indicates that earlier treatment initiation, known to improve treatment outcomes, may additionally limit viral diversity of the reservoir for more effective targeting with HIV cure strategies.

LOSS OF *GRIN2A* PROMOTES EPILEPTIC ACTIVITY AND HIPPOCAMPAL HYPEREXCITABILITY

Chad R. Camp,¹ Jacob White,² Russell Sanchez,² Stephen F. Traynelis,¹ Sookyong Koh²

¹ Department of Pharmacology, Emory University

² Department of Pediatrics, Emory University

De novo mutations in the *GRIN2A* gene, which encodes the GluN2A subunit of the N-methyl-D-aspartate receptor, are linked to several forms of epileptic encephalopathy (EE). EE is a group of devastating epilepsies, which involve intractable seizures and different brain abnormalities. The prognosis for EE patients is poor, as our current pharmacological options mainly dampen symptoms, without rectifying aberrant neuronal circuitry. 56% of epilepsy-related *GRIN2A* mutations are loss-of-function, displaying diminished receptor function and/or surface expression. These results are intriguing, as one might hypothesize that the loss of excitatory synaptic subunit would decrease excitability, rather than promote epileptiform activity. To understand this paradox, we have used *Grin2a* +/- and -/- mice as a model for loss-of-function *GRIN2A* variants. Preliminary data indicate that *Grin2a* -/- (2AKO) mice have a lower threshold for febrile-induced seizures compared to wildtype (WT) mice and exhibit an increased frequency of epileptiform burst-firing activity in the CA1 region of the hippocampus. These data indicate that the loss of *Grin2a* in mice is sufficient to elicit an epileptic phenotype. By using this mouse model, we can begin to elucidate both the cellular and temporal mechanisms driving this increased excitability, uncovering novel avenues for circuit-correcting therapeutics.

DEVELOPMENT OF SMALL MOLECULE MODULATORS OF LIVER RECEPTOR HOMOLOG-1

Michael Cato¹, Emma D'Agostino¹, Jeffery Cornelison¹, Suzanne Mays¹, Autumn Flynn¹, Denise Okafor¹, Nathan Jui¹, Eric Ortlund¹

¹ Department of Biochemistry, Emory University

Liver receptor homolog-1 (LRH-1) is a nuclear receptor that is an attractive target for treating metabolic disorders as it exhibits anti-diabetic properties when activated in mice and is implicated in glucose homeostasis, steroidogenesis, cholesterol transport, and bile acid homeostasis. Our lab has used structure-activity relationship studies to identify regions of the LRH-1 ligand-binding pocket (LBP) that can be targeted to modulate its activity. From these studies, we have developed an agonist ("Cpd33") that binds with high affinity (K_d : ~1 nM) but contains a styrene that decreases biological stability and hinders further modifications intended to explore other regions of the LBP that may be targeted to enhance drug efficacy. We therefore have substituted the styrene with an aniline substituent to develop a new compound ("Cpd33-aniline") that binds and activates LRH-1 with high affinity and potency. Interestingly, the aniline substituent adopts an orientation proximal to the activation function surface (AFS), a binding interface that recruits coactivator proteins responsible for exerting LRH-1 transactivation effects. This uniquely positions us to target a region of the binding pocket closely associated with LRH-1 activation to uncover interactions that modulate LRH-1 activity to develop compounds that will be useful for probing LRH-1 biology and treating metabolic disorders.

MAINTENANCE OF MUSCLE MYOSIN DURING AGING

Courtney Christian¹, Hiroshi Qadota¹, Guy Benian¹

¹ Department of Pathology, Emory University

As longevity increases, age-related diseases will become a greater public health concern. Sarcopenia is the age-related decline in muscle mass and function without any underlying disease. The molecular mechanisms responsible for this pathology remain unknown. Over the course of a lifetime, striated muscles are exposed to both thermal and chemical stressors. It is hypothesized that chaperone proteins responsible for initially folding myosin motors in striated muscles also re-fold and maintain myosin after the muscle is exposed to stress. Here we show that the myosin chaperone UNC-45 and its co-chaperone HSP-90 decrease during aging in the model organism *Caenorhabditis elegans*. This decline in chaperone proteins correlates with decreased assembled thick filaments in muscle cells. We also see a decrease in UNC-45 in an *hsp-90* loss of function mutant, suggesting a role for HSP-90 in UNC-45 stabilization. This leads us to investigate the possibility that during aging a diminished HSP-90 contributes to UNC-45 degradation, which then leads to a loss of myosin and thick filaments. A better understanding of how myosin and its chaperone proteins are regulated and affected by aging will lead to better prevention and treatment of sarcopenia and, possibly, the age-related decline of heart muscle function.

INVESTIGATION OF THE FACTORS THAT REGULATE SPO0A ACTIVITY IN *CLOSTRIDIoidES DIFFICILE*

Michael A DiCandia,¹ Joshua B Jones,¹ Grace L Swaim,² Adrienne N Edwards,¹ Shonna M McBride¹

¹ Department of Microbiology and Immunology, Emory University School of Medicine

² Yale University, New Haven, Connecticut

The human pathogen *Clostridioides difficile* is an anaerobic, Gram-positive bacterium that relies on spore formation for transmission. In *C. difficile*, the transcription factor Spo0A is the master regulator of sporulation. Despite the clinical significance of spores in *C. difficile* pathogenesis, little is known about the mechanisms that regulate sporulation initiation. We hypothesize that in *C. difficile*, sporulation initiation is governed through direct interactions of Spo0A with multiple phosphotransfer proteins, though the Spo0A residues that facilitate these interactions remain poorly understood. Here, we investigate the function of Spo0A through site-directed mutagenesis of highly conserved Spo0A residues. We identified multiple sites that are important for Spo0A activity and sporulation. As predicted, we observed the mutation of a highly conserved aspartate residue suspected to be the site of activation (D61A) results in loss of spore formation. We identified two Spo0A mutations, V23A and P65A, that confer an asporogenous phenotype and are therefore critical for sporulation. Three additional residues, A40S, A92S, and K113A were identified that result in severely impaired sporulation frequencies, while K97A was found to have an increased sporulating phenotype. Further, we isolated a compensatory mutation in the P65A strain (P65A*-1) that restores sporulation. Additional characterization of this suppressor mutation is expected to reveal an essential interacting partner of Spo0A and will help elucidate the mechanisms governing sporulation initiation in *C. difficile*.

TRIHXYPHENIDYL RESCUES THE DEFICIT IN DOPAMINE NEUROTRANSMISSION IN A MOUSE MODEL OF DYT1 DYSTONIA

Anthony M Downs, Xueliang Fan, H.A. Jinnah, Ellen J Hess

Departments of Pharmacology and Neurology, Emory University School of Medicine

Trihexyphenidyl, a nonselective muscarinic receptor antagonist, is the small molecule drug of choice for the treatment of DYT1 dystonia, but it is poorly tolerated due to significant side effects. A better understanding of the mechanism of action of trihexyphenidyl is needed for the development of improved treatments. Using *ex vivo* fast scan cyclic voltammetry and *in vivo* microdialysis, we tested the hypothesis that trihexyphenidyl normalizes striatal dopamine release in a mouse model of DYT1 dystonia. THP increased striatal dopamine release and efflux as assessed by *ex vivo* voltammetry and *in vivo* microdialysis respectively. We performed additional experiments to further examine the mechanisms by which trihexyphenidyl increases dopamine release. Trihexyphenidyl required nicotinic receptors but not glutamate receptors to increase dopamine. Dyt1 mice were more sensitive to the dopamine release decreasing effects of nicotinic acetylcholine receptor antagonism (IC_{50} : WT= 29.46nM, Dyt1= 12.26nM) and less sensitive to acetylcholinesterase inhibitors. These studies reveal that trihexyphenidyl enhances striatal dopamine. These data suggest that nicotinic acetylcholine receptor neurotransmission is altered in Dyt1 mice, that nicotinic receptors indirectly mediate the differential effects of trihexyphenidyl in Dyt1 mice, and that nicotinic receptors may be suitable therapeutic targets for DYT1 dystonia.

TAU INDUCES NEURODEGENERATION IN ALZHEIMER'S DISEASE THROUGH THE SEQUESTRATION AND INHIBITION OF LSD1 FUNCTION

Amanda K. Engstrom¹, Alicia C. Walker¹, Rohitha A. Moudgal¹, Dexter A. Myrick¹, David J. Katz¹

¹ Department of Cell Biology, Emory University

Alzheimer's Disease (AD) is characterized by the aberrant accumulation of β -amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau (NFTs). However, the molecular mechanism by which NFTs lead to neuronal cell death remains unclear. Previously, we demonstrated that the histone demethylase, LSD1, is autonomously required for neuronal survival, and is mislocalized to somatodendritic NFTs in AD patients. These data raise the possibility that NFTs sequester LSD1 in the cytoplasm, interfering with its continuous requirement to repress inappropriate transcription. Consistent with this, LSD1 is depleted from the nucleus in neurons of P301S Tauopathy mice. Furthermore, *Lsd1* heterozygosity exacerbates the expression changes induced by the Tau P301S transgene. If LSD1 is the main target of pathological Tau, then modulating LSD1 levels in the Tau P301S mice should be sufficient to enhance or suppress Tau mediated neurodegeneration *in vivo*. Strikingly, making P301S mice heterozygous for *Lsd1* reduces survival, exacerbates paralysis, and increases hippocampal neurodegeneration, while overexpressing LSD1 in hippocampal neurons, after the formation of NFTs, blocks Tau induced neurodegeneration for months. Taken together, our results suggest that pathological Tau induces neurodegeneration through the sequestration and inhibition of LSD1 function. This novel mechanism provides a highly promising target for therapeutic intervention in AD.

MYCOBACTERIUM TUBERCULOSIS MODULATION OF NOTCH LIGAND EXPRESSION IMPEDES DENDRITIC CELL-T CELL CROSSTALK AND LIMITS TH₁₇ POLARIZATION

Ana Beatriz Enriquez¹, Jonathan Kevin Sia¹, Melanie Quezada¹, Jyothi Rengarajan^{1,2}

¹ Emory Vaccine Center, Emory University

² Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is among the leading causes of death worldwide. While IFN- γ and CD4⁺ Th₁ T cell responses are necessary to mount an immune response to Mtb, they are not sufficient to provide protection. Studies from several groups, including our own, have highlighted an important role for IL-17 and Th₁₇ responses in immunity to Mtb infection. Previous research from the laboratory has shown that Mtb restricts Th₁₇ responses by dampening dendritic cell (DC) responses and has identified CD40-mediated co-stimulation as critical for generating Th₁₇ responses in response to Mtb. We showed that exogenous CD40 engagement on Mtb-infected DCs enhances Th₁₇ polarization and reduces Mtb burden in the lungs in a vaccination model. However, the DC mechanisms that mediate CD40-dependent Th₁₇ polarization and protection have not been defined. Here we show that Notch signaling in DCs modulates DC-T cell crosstalk and influences T cell polarization during infection. Engaging the CD40 pathway on Mtb-infected DCs increases the mRNA and protein expression of Notch ligands DLL4 and Jagged1. Blockade of Jagged1 during a DC-T cell co-culture lowers Th₁ responses while blockade of both DLL4 and Jagged1 significantly limits Th₁₇ polarization. These results reveal that during infection, Mtb restricts expression of Notch ligands, which impedes Th₁₇ responses during TB.

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF LSD1 WITHIN THE MURINE EYE

Salma Ferdous^{1,2}, Isabelle Gefke², Jeffrey H. Boatright^{2,3}, Hans E. Grossniklaus², John M. Nickerson^{1,2}

¹ Genetics and Molecular Biology Graduate Program, Emory University

² Department of Ophthalmology, Emory University

³ Atlanta Veterans Administration Center of Visual and Neurocognitive Rehabilitation

Purpose: To assess the role of lysine specific demethylase 1 (Lsd1) in proper murine ocular development. Lsd1 is an epigenetic protein that demethylates H3K4 mono- and dimethylation (H3K4me1/2) and plays a role in neuronal development. Inhibition of Lsd1 is known to attenuate rod photoreceptor development; however, its general role in ocular development is unknown.

Methods: Immunohistochemistry and western blotting for Lsd1 and H3K4me1/2 expression was conducted on murine eyes across numerous developmental time-points to determine localization and relative levels.

Results: Lsd1 and H3K4me1/2 had highest retinal expression at post-natal day 2 (P2), concurrent with retinogenesis and terminal differentiation and expression gradually decreased until P36. Lsd1 and H3K4me1/2 are expressed universally within the developing retinoblast at uniformly high levels. By P36, variation in Lsd1 expression is seen among different retinal subtypes, however H3K4me1/2 remained uniformly expressed. Additionally, Lsd1 was variably expressed in lens, cornea, and retinal pigment epithelium (RPE).

Conclusions: Lsd1 may play a critical role in retinal cell differentiation and formation of their unique transcriptomes. In nocturnal animals, rods and cones have inverse chromatin architectures which may account for their distinct Lsd1 expression patterns. This may be causative or consequential. Future studies will delete Lsd1 from these cell types.

DYNEIN INDEPENDENT ROLE FOR DYNEIN LIGHT CHAIN IN MEIOTIC PROGRESSION

Sara Fielder^{1,2}, William G. Kelly²

¹ Graduate Program in Genetics and Molecular Biology, Emory University

² Biology Department, Rollins Research Center, Emory University

Homologous chromosome pairing and meiotic synapsis are essential processes that are required in both sexes to prevent aneuploidy in offspring. Despite the importance and conservation of synapsis, not every aspect is the same between the two sexes. My results indicate that male and female *C. elegans* have different requirements for dynein in regulating synapsis. Dynein dependent forces have been proposed to test whether a potential match is correct, and once a match has been established, synapsis (SYP) proteins load between the homologs. Knockdown of the light chain (DLC-1) results in formation of a SYP polycomplex in females. Unexpectedly, DLC-1 depletion in males shows grossly normal synapsis. More surprisingly, mutants in the heavy chain also don't show SYP polycomplexes in female meiosis. This indicates there is a previously undescribed function for DLC-1 in synapsis. A consensus binding motif for the mammalian DLC-1 ortholog has been reported, and we identified a potential binding motif in a SYP protein. Additionally, knock down of an axis component of the synaptonemal complex results in many small SYP polycomplexes instead of one large complex as in the DLC-1 knockdown. All this suggests that DLC-1 directly interacts with SYP and may have a role in SYP regulation.

TRANSMIGRATION INTO THE CF AIRWAY MICROENVIRONMENT ALTERS MONOCYTE PHENOTYPE AND METABOLISM

Bijean Ford, Camilla Margaroli, Osric Forrest, Chris Driggers, Milton Brown, Rabindra Tirouvanziam

Center for CF and Airways Disease Research, Department of Pediatrics, Emory University, Atlanta, GA

The airways of patients with cystic fibrosis (CF) are chronically inflamed, due in part to unrelenting recruitment of polymorphonuclear neutrophils (PMNs) from blood into the airways. Prior studies by our group showed that PMNs recruited to the CF airway lumen acquire a pathological phenotype featuring active elastase-rich granule release, acquisition of immunoregulatory activities, and metabolic licensing (dubbed "GRIM" PMNs). Recently, we showed that GRIM PMNs can be produced in vitro by transmigration of blood PMNs toward CF sputum supernatant (CF SSN) through a small airway epithelium (Forrest, J Leukoc Biol, 2018). Monocytes can potentially exert inhibitory effects on PMN-driven inflammation, however, their ability to survive upon migration into CF airways and their fate therein is unclear. Our goal was to determine whether blood monocytes survive and alter their phenotype and metabolism upon in vitro transmigration. Our results suggest the CF airway environment supports the survival of transmigrated monocytes and confers an activated phenotype to them, similar to that seen with transmigrated PMNs. In particular, transmigrated monocytes appear poised for clearance functions with modulation of metabolism and inhibitory receptors, however, high levels of other markers may indicate a contradictory ineffectiveness in this environment, thereby leaving GRIM PMNs unchecked.

GAMMA NEURAL ACTIVITY PRODUCES UNIQUE NEURO-IMMUNE RESPONSE

Kristie Garza^{1,2}, Alyssa Pybus², Matthew Attokaren², Sitara Sankar², Levi Wood^{2,3}, Annabelle Singer^{1,2}

¹ Neuroscience Graduate Program, GDBBS, Emory University

² Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology

³ Woodruff School of Mechanical Engineering and Institute of Bioengineering and Bioscience, Georgia Institute of Technology

Recruiting microglia, the primary immune cells of the brain, is considered a potential treatment for a wide range of neurological disorders. Exogenously driving gamma neural activity through flickering lights at 40Hz leads to changes in microglia morphology, suggesting microglial recruitment. These changes in microglial morphology are suggested to decrease amyloid plaque load in a mouse model of Alzheimer's disease. While this paradigm has potential clinical applications, there is little known about the mechanism underlying gamma-induced microglia changes. We know microglia release and respond to changes in cytokine expression. Here, we show that 40Hz flicker leads to changes in pro-inflammatory cytokine expression. Further, we focus on internal signaling pathways as a potential mechanism changing cytokine expression and show that 40Hz flicker changes phosphoprotein levels in both the MAPK pathway and NFkB pathway, two pathways known to regulate cytokine release among other functions. Indeed, changes in protein phosphorylation occur rapidly following 40Hz flicker stimulation onset while changes in cytokine expression occur later. These results are the first to show how gamma frequency electrical neural activity affects neuro-immune signaling.

IDENTIFYING FUNCTIONAL PROPERTIES OF CORTICAL DENDRITE TARGETING INTERNEURONS

Stephanie Grewenow^{1,2}, Matthew J Rowan²

¹ Genetics and Molecular Biology Graduate Program, Emory University

² Department of Cell Biology, Emory University

Determining the roles of neuron subpopulations in the mammalian brain, given their distinct physiological features, is a major goal in neuroscience. Our ultimate goals are to understand how these different neuronal populations influence learning and memory, as well understand how the physiology of these distinct cell types are affected in neurological disorders. The mammalian neocortex is the region of the brain responsible for high-order cognition and sensorimotor behavior. Deficits associated with these functions can arise when there is an imbalance between neuronal excitation and inhibition. GABAergic interneurons are the primary inhibitory neuron contained within the neocortex. These cells create distinct activity patterns for information processing by modulating excitatory signal input. It is known that interneurons precisely control action potential spike timing in target principle neurons. It is less understood whether interneurons regulate additional information processes, such as synaptic plasticity. Dendrite targeting interneurons are likely involved in cortical plasticity due to their inhibitory activity on cells with multiple converging inputs. We aim to identify mechanisms that these interneurons use to regulate circuit function to further our understanding of synaptic plasticity and its role in information storage processes such as cognition and memory.

IFNAR SIGNALING AUGMENTS PLASMACYTOID DENDRITIC CELL ACTIVATION DURING COSTIMULATION INDEPENDENT REJECTION

Jakob Habib¹, Dave Mathews¹, Ying Dong¹, Allison Stephenson¹, Abheek Ghosh¹, Cindy Breeden¹, Andrew Adams¹

¹ Emory Transplant Center, Department of Surgery, Emory University School of Medicine

Costimulation blockade (CoB) is a promising new transplant immunosuppression strategy offering improved long-term patient and allograft survival without the nephrotoxicity of calcineurin inhibitors. However, increased risks of acute rejection have impeded widespread adoption of CoB. Type I interferons (IFN), produced mainly by plasmacytoid dendritic cells (pDCs), induce systemic inflammation that may prime the adaptive immune system for acute rejection. Here we examine the contribution of signaling through the type I IFN receptor (IFNAR) to CoB-resistant allograft rejection. We performed fully MHC-mismatched skin grafts from Balb/C donors to C57BL/6 recipients. Mice were treated with CoB alone (CTLA4-Ig + anti-CD154) or in conjunction with anti-IFNAR. Untreated mice rejected rapidly (MST=11d), CoB treatment improved survival but resulted in CoB-independent rejection (MST=23d), and CoB+anti-IFNAR significantly improved survival (MST>60d). IFNAR was highly expressed on murine pDCs relative to conventional DCs and T cells. CoB+anti-IFNAR reduced MHC I and CD80 expression on pDCs compared to CoB alone. CoB+anti-IFNAR also led to a reduction in activation (CD44+) and effector functions (IFN γ +TNF α +) of CD8+ T cells compared to CoB alone. These data suggest that signaling through IFNAR augments pDC activation and is associated with CD8+ T cell activation and effector functions in order to promote CoB-resistant rejection.

INTERLEUKIN-1 SIGNALING REGULATES BLOOD-BRAIN BARRIER INTEGRITY IN GLIOBLASTOMA

Cameron Herting^{1,2}, Alyssa Duffy², Zhihong Chen², Victor Maximov², Dmitry Shayakhmetov³, Dolores Hambardzumyan²

¹ Molecular and Systems Pharmacology, Emory University Laney Graduate School

² Department of Pediatrics: Hematology-Oncology, Emory University School of Medicine

³ Department of Immunology, Emory University School of Medicine

Glioblastoma is the most common and aggressive primary brain tumor characterized by poor overall survival and limited treatment options. A hallmark of glioblastoma is aberrant angiogenesis that results in disruption of the blood-brain barrier and development of glioblastoma-associated cerebral edema. Typically, edema is managed by the corticosteroid dexamethasone which is known to normalize the blood-brain barrier. The effects on angiogenesis, however, are unclear. Interleukin-1 (IL-1) signaling inhibition has been shown to modulate edema formation and angiogenesis in other neuroinflammatory conditions, but not in glioblastoma. In this work, we demonstrate that IL-1 signaling does not impact the production of the angiogenic factor VEGF by bone marrow-derived macrophages and microglia *in vitro*. Moreover, we demonstrate no effect of dexamethasone treatment on VEGF levels in murine PDGFB-driven glioblastoma *in vitro* and *in vivo*, suggesting that the restoration of blood-brain barrier integrity by dexamethasone is VEGF-independent. Genetic ablation of IL-1 β and subsequent MRI analysis is shown to reduce formation of edema *in vivo*, a result confirmed by serial sectioning and histological analysis. Genetic ablation of IL-1R1 *in vivo* is shown to reduce blood-brain barrier permeability in a Hoechst dye-based vessel leakage assay. This suggests specific IL-1 inhibition may be an attractive alternative to dexamethasone.

ARABIDOPSIS MBD9 IS REQUIRED FOR SELECTIVE H2A.Z DEPOSITION

Paja Sijacic¹, Dylan H. Holder^{1,2}, Marko Bajic^{1,2}, Roger B. Deal¹

¹ Department of Biology, Emory University

² Graduate Program in Genetics and Molecular Biology, Emory University

The SWR1 chromatin remodeling complex, which deposits the histone variant H2A.Z into nucleosomes, is well characterized in yeast and animals but has yet to be purified in plants. We used the conserved SWR1 subunit ACTIN RELATED PROTEIN 6 (ARP6) as bait in tandem affinity purification experiments to isolate associated proteins from *Arabidopsis thaliana*. We identified all 11 conserved subunits found in yeast SWR1 and the homologous mammalian SRCAP complexes. We also identified several additional proteins not previously associated with SWR1, including Methyl-CpG-BINDING DOMAIN 9 (MBD9). We used ChIPseq on *arp6* and *mbd9* mutant plants to find that MBD9 is required for proper H2A.Z incorporation at thousands of discrete sites, which represent a subset of the regions normally enriched with H2A.Z. Our data affirm the conservation of the SWR1 complex across eukaryotes and also provide new insights into the mechanisms that target H2A.Z to chromatin.

**EXPERIMENTAL COLITIS PROMOTES PARKINSONIAN
NEUROPATHOLOGY AND EXACERBATES TOXIN-MEDIATED
NEURODEGENERATION**

Madelyn C. Houser¹, W. Michael Caudle², Jianjun Chang¹, Yuan Yang¹, Sean D. Kelly¹, Danielle Oliver¹, Sanam Golshani¹, Kathleen M. Shannon³, Ali Keshavarzian⁴, Malú G. Tansey¹

¹ Department of Physiology, Emory University School of Medicine

² Department of Environmental Health, Emory University Rollins School of Public Health

³ Department of Neurology, University of Wisconsin School of Medicine and Public Health

⁴ Department of Internal Medicine, Rush Medical College

The etiology of Parkinson's disease (PD) remains uncertain, but gastrointestinal dysfunction frequently manifests years before the onset of PD motor symptoms, intestinal inflammation is present in PD patients, and inflammatory bowel disease patients are at increased risk of developing PD. This has prompted theories that gut inflammation can promote PD pathology in the brain. This phenomenon and mechanisms that might drive it have not been established experimentally, however. To address this, we induced colitis in a mouse model and evaluated the effects on inflammation and neuron function in the brain. We discovered that the induction of colitis promoted T cell infiltration and sustained inflammation and oxidative stress in brain regions involved in PD and perturbed neuronal activity there without causing significant terminal loss. When mice were exposed to the neurotoxin MPTP after colitis, they exhibited more severe neurodegeneration than with the toxin alone. These effects were particularly pronounced in mice that lacked the Regulator of G-Protein Signaling 10 which rendered them more vulnerable to inflammation- and oxidative stress-mediated neuropathology. Our findings confirm that intestinal inflammation can induce PD-related neuropathology and sensitize to further neurological insults, particularly in the context of genetic susceptibility, providing support for gut-to-brain theories of PD pathogenesis.

A POLY(A) RNA BINDING PROTEIN CRITICAL FOR NEURODEVELOPMENT REGULATES SPLICING SEX LETHAL IN *DROSOPHILA*

Binta Jalloh¹, John Christopher Rounds¹, Brianna Brown¹, Tina Lence², Jean-Yves Roignant², Anita Corbett¹, and Kenneth Moberg¹

¹ Department of Cell Biology, Emory University, Atlanta, GA

² Institute of Molecular Biology, Johannes Gutenberg University, Mainz, Germany

Regulation of gene expression at the RNA level is carried out by important molecular machines called RNA binding proteins (RBPs). Over 500 RNA binding protein encode genes important for brain development and function. Because RBPs control all aspects of RNA biology, it is not surprising that mutations in gene encoding RBPs have been linked to brain dysfunction. One particular RBP that has been linked to a form of autosomal recessive, non-syndromic human intellectual disability is ZC3H14. Loss of the ZC3H14 ortholog, dNab2, within *Drosophila* neurons impairs behavior, short-term memory, and sex bias towards females. Intriguingly, these mutant fly brains exhibit longer poly (A) tails and alters splicing of specific RNAs in our RNA-Seq data. The splicing of a gene called *sex lethal* (Sxl), which determines whether the fly develops as a male or female. Biochemical data shows dNab2 mutant females have low protein expression of female specific sex lethal protein but show elevated expression of the male specific isoforms of Sxl mRNA transcripts compared to dNab2 mutant male flies. These preliminary data led us hypothesis that dNab2 plays a role in m⁶A RNA methylation and splicing to regulate neuronal function and sex termination in the dosage compensation pathway of *Drosophila*.

CHRONIC ALCOHOL SIGNIFICANTLY AFFECTS PULMONARY FUNCTION BOTH AT BASELINE AND IN RESPONSE TO ENDOTOXEMIA

Lauren A. Jeffers¹, Prestina Smith¹, Michael Koval^{1,2}

¹Pulmonary Medicine, ²Cell Biology, Emory University School of Medicine

Acute respiratory distress syndrome (ARDS) is an inflammatory disease characterized by significant pulmonary edema and pathologic alveolar flooding due to disrupted fluid homeostasis and increased alveolar permeability. Chronic alcohol abuse exacerbates acute lung injury and is an independent risk factor to develop ARDS. Alveolar epithelial permeability is regulated by claudin-family proteins that are incorporated into tight junctions (TJ) structures. We have found that chronic alcohol negatively impacts alveolar barrier function *in vitro* by changing claudin expression and integration into TJs. Defining molecular mechanisms responsible for the alcohol-induced increased incidence of ARDS is critical to understand ARDS pathology. We used an Evans Blue (EB) permeability assay to assess changes in alveolar and vasculature permeability due to alcohol with endotoxin as a 'second-hit' to mimic the effects of pneumonia and sepsis by either intraperitoneal (IP) or intratracheal (IT) administration, respectively. In alcohol-fed mice, the alveolar barrier was impaired by endotoxin, allowing permeation into the airspaces. We found continued defects in barrier function in primary alveolar cells cultured from alcohol-fed after differentiation and persistent barrier disruption in mice in an alcohol withdrawal experiment after 6 weeks. Current studies are targeting the TJ protein Claudin-5 to rescue alcohol-induced barrier dysfunction *in vivo*.

ALTERED HIPPOCAMPAL CA1 NEURAL ACTIVITY IN VIRTUALLY NAVIGATING 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

Nuri Jeong¹, Stephanie M. Prince¹, Abigail L. Paulson², Lu Zhang², Matty K. Attokaren², Solange Amigues², John H. Tipton², Annabelle C. Singer^{1,2}

¹ Neuroscience Graduate Program, Emory University

² Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory School of Medicine

Deficits in spatial learning are early signs of Alzheimer's disease (AD). Hippocampal theta (4-12 Hz) oscillations and gamma (30-100 Hz) coupled to theta cycle are prominent features during active spatial exploration and are known to be important in spatial information processing. However, it remains unknown how theta-gamma oscillatory hippocampal network activity is altered in mouse models of AD. Previously, in the 5XFAD mouse model of AD, we demonstrated reduced gamma power during quiescence, specifically high-frequency sharp-wave ripples critical for hippocampal learning. Since gamma-coupled to theta oscillations are important for learning and memory, we suspected similar deficits during theta oscillations in 5XFAD mice. To test this hypothesis, we recorded local field potentials and single-unit activity with a 32-channel silicon probe in the hippocampal pyramidal layer of mice navigating in a virtual environment. We subsequently identified putative inhibitory interneurons and pyramidal cells based on spike waveform properties and evaluated their spiking activity during theta oscillations. We found a statistically significant difference in cell-type-based modulation of spiking by theta phase in 5XFAD mice. This study investigating changes in oscillatory activity of different CA1 cell types may elucidate novel changes in neural activity and spatial learning impairment in AD.

ASTROCYTE PROTEINS ARE ENRICHED IN FRONTOTEMPORAL DEMENTIA

Ashlyn Johnson^{1,2}, Jingting Dai^{2,3}, Eric Dammer^{2,3}, Duc Duong^{2,3}, James Webster², Rhythm Shah², Nicholas Seyfried^{2,3}, Chadwick Hales^{2,4}

¹Neuroscience Graduate Program, Emory University

²Center for Neurodegenerative Disease, Emory University School of Medicine

³Department of Biochemistry, Emory University School of Medicine

⁴Department of Neurology, Emory University School of Medicine

Frontotemporal dementia (FTD) is a genetically and phenotypically diverse neurodegenerative disorder characterized by behavioral disinhibition, language deficits, and memory impairments. 36-50% of FTD cases are neuropathologically characterized by accumulations of hyperphosphorylated microtubule associated protein tau (MAPT), and mutations in MAPT can lead to an inherited form of FTD (FTD-MAPT). We used quantitative proteomics to sequence total brain homogenates from multiple tauopathies and control cases. Weighted gene co-expression network analysis (WGCNA) and cell subtype profiling revealed significant enrichment of astrocyte related proteins in FTD-MAPT compared to both control cases and other tauopathies. Many of these enriched astrocyte proteins are localized to the astrocyte endfeet. Here, we validate enrichment of astrocyte proteins in FTD-MAPT via western blot and immunohistochemistry. Future studies will investigate the role of enriched astrocyte endfeet proteins in the perivascular space.

H3K27ME3-SPECIFIC DEMETHYLASES RESTRAIN B CELL DIFFERENTIATION

Anna K. Kania¹, Christopher D. Scharer¹, Muyao Guo¹, Jeremy M. Boss¹

¹Department of Microbiology and Immunology, Emory University

B cell terminal differentiation into antibody secreting plasma cells must be properly regulated to ensure robust humoral immune responses against foreign but not self-antigens. While the role of transcription factors in this process is well-established, the epigenetic enzymes that participate in B cell differentiation is still not fully understood. The role of active demethylation of H3K27me₃, by enzymes such as UTX and JMJD3, in B cells is still to be elucidated. To determine the requirement of these enzymes, we stimulated naïve B cells *ex vivo* in the presence of an H3K27me₃ demethylase inhibitor or vehicle control and assessed B cell differentiation. Inhibition of UTX and JMJD3 increased frequency of CD138⁺ plasmablasts after 3 days of *ex vivo* culture without impacting cell division or ability of plasmablasts to secrete antibodies. Genome wide expression analysis of cells cultured in the presence or absence of the inhibitor revealed a skewing of differentially expressed genes that were repressed following inhibition. Gene Set Enrichment Analysis (GSEA) revealed that genes involved in NF-κB-mediated TNF-α signaling as well as IL6 and STAT3 signaling were downregulated in inhibitor treated cells. Similar to what was observed after inhibitor treatment, genetic deletion of these enzymes *in vivo* using *Utx^{fl/fl} Jmjd3^{fl/fl} Cd19^{Cre/+}* led to an increase in CD138⁺ plasmablasts. These data support a that UTX and JMJD3 regulate the epigenetic dynamics of B cell terminal differentiation.

β 1-INTEGRINS ARE NECESSARY FOR MEDIAL PREFRONTAL CORTEX DEVELOPMENT AND FUNCTION**Henry W. Kietzman**^{1,2,3}, Lauren P. Shapiro^{2,3,4}, Shannon L. Gourley^{1,2,3,4,5}¹ Graduate Program in Neuroscience, Emory University² Department of Pediatrics, Emory University School of Medicine³ Yerkes National Primate Research Center, Emory University⁴ Molecular and Systems Pharmacology, Emory University⁵ Department of Psychiatry, Emory University School of Medicine

Proper development of the brain relies on periods of structural remodeling. These structural adaptations require coordinated signaling between the extracellular environment and the actin cytoskeleton. Composed of an α subunit responsible for extracellular ligand binding and a β subunit that activates intracellular signaling, integrin receptors provide such a link. Here we tested the hypothesis that the β 1 subunit, localized to synapses and abundant in the prefrontal cortex, would be essential to prefrontal cortical development, which extends into adolescence. We used viral-mediated gene silencing to reduce β 1-integrins in the medial prefrontal cortex (mPFC), delivering viral vectors just prior to adolescence or in adulthood. Early-life, but not adult-onset, β 1-integrin silencing eliminated dendritic spines, the sites of excitatory plasticity in the brain. To assess functional consequences, we focused on goal-directed decision making. The mPFC is necessary for acquiring and consolidating associations between actions and their outcomes. We trained mice to nose poke for food reinforcers, then decreased either: 1) the contingency between nose poking and food, or 2) food value. Early-life β 1-integrin silencing occluded sensitivity to action-outcome associations in both conditions, causing habits. Meanwhile, depression- and anxiety-like behaviors were intact. Our findings suggest that developmental β 1-integrins are necessary for mPFC maturation and function.

EZH2 MEDIATES RESISTANCE TO CISPLATIN IN SMALL CELL LUNG CANCER THROUGH NUCLEOTIDE EXCISION REPAIR

Allyson Koyen¹, Matthew Madden¹, Jie Xu¹, David Yu¹

¹Department of Radiation Oncology, Winship Cancer Institute, Emory University

Small cell lung cancer (SCLC) is the most aggressive form of lung cancer, with a five-year survival rate of 7%. Cisplatin-based chemotherapy is the first line treatment for SCLC; however, many patients develop treatment resistance and experience tumor recurrence. Targeting proteins critical to the repair of cisplatin DNA lesions is one strategy for overcoming acquired cisplatin resistance in SCLC, but many proteins that mediate repair have yet to be identified. To address this, we performed a synthetic lethal siRNA screen in cisplatin resistant SCLC cells and identified EZH2 as one of the strongest mediators of cisplatin resistance. EZH2 localizes to sites of DNA damage and interacts in a complex with DDB1, and DDB2, members of the nucleotide excision repair (NER) pathway. Knockdown of EZH2 and DDB1 together are epistatic in the sensitization of SCLC to cisplatin and UV. Specifically, knockdown of EZH2 promotes the degradation of DDB2, the protein responsible for detecting UV and cisplatin lesions, occurring independently of EZH2 methyltransferase activity on H3K27. Finally, EZH2 expression correlates with cisplatin resistance across SCLC cell lines. Together, this data suggests that EZH2 functions as a novel regulator of NER, and that EZH2 is a promising target for cisplatin resistant SCLC.

USING 3D HUMAN ORGANOID MODEL TO STUDY NEURONAL-SPECIFIC EPIGENETIC LANDSCAPING IN BRAIN DEVELOPMENT AND NEURODEGENERATIVE DISORDERS

Janise N Kuehner¹, Feng Wang¹, Emily Bruggeman¹, Chongchong Xu², Zhexing Wen², Bing Yao¹

¹ Department of Human Genetics, Emory University School of Medicine

² Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine

Genetic and epigenetic spatiotemporal regulation of gene expression in the mammalian central nervous system is critical in how the brain develops, functions and forms its complex circuitry between differentiated neurons. During mammalian embryonic neurodevelopment, epigenetic mechanisms, such as DNA methylation and demethylation dynamics have important roles in controlling neural progenitor cell proliferation and new neuron production. Ectopic alteration of these key epigenetic marks often leads to severe brain disorders such as Alzheimer's disease (AD). The precise regulation of DNA methylation and demethylation in early human neurodevelopmental stages, particularly in a cell type-specific manner, remain elusive. To begin to answer these questions, we are in the process of using human induced pluripotent stem cells to generate organoids, which are 3-dimensional cultures serving as an excellent model to recapitulate human brain development and disease pathology *in vitro*. In this study, we will perform genome-wide analysis of DNA methylation and demethylation at several developmental stages of organoid culture that are derived from normal and AD patients to identify key epigenetic loci related to normal development and their alteration in AD. Using isolation of nuclei tagged in specific cell types (INTACT), we will determine the epigenetic landscape in excitatory and inhibitory neurons related to AD pathogenesis.

PROTEOMIC ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS IN ARGININE-RICH LOW COMPLEXITY RNA BINDING PROTEINS THAT AGGREGATE IN NEURODEGENERATIVE DISEASE

Sean R. Kunding^{1,2}, Isaac Bishof^{1,2}, Duc M. Duong^{1,2}, Nicholas T. Seyfried^{1,2}

¹ Department of Biochemistry, Emory University School of Medicine

² Center for Neurodegenerative Diseases, Emory University School of Medicine

U1 small nuclear ribonucleoprotein 70 kDa (U1-70K) and other RNA binding proteins (RBPs) harbor disordered low-complexity domains containing highly repetitive basic (K/R) and acidic (D/E) residues, referred to as a basic-acidic dipeptide (BAD) domain. These domains are important in oligomerization and nuclear granule assembly and enhance RBP aggregation in Alzheimer's Disease (AD). Post-translational modifications (PTMs) including phosphorylation and methylation within BAD domains regulate key RNA processing steps. However, the identification of PTMs within BAD domains utilizing a trypsin digestion is challenging due to the sheer number of arginine and lysine residues. Additionally, peptide fragmentation by collision induced dissociation (CID) proves extremely inefficient at fragmenting multiply-protonated peptides. To overcome these constraints, we employed a middle-down proteomic approach applying limited trypsin digestion followed by electron transfer dissociation (ETD) mass spectrometry to map previously unidentified sites of phosphorylation and methylation within the BAD domain of U1-70K and other RBPs from human embryonic kidney (HEK) cell nuclear extracts. Ultimately this approach can be leveraged to map unique, neurodegenerative disease-specific PTM profiles within BAD domains and DPRs, in both brain and spinal fluid.

**EVALUATING HILL-ROBERTSON INTERFERENCE IN
CAENORHABDITIS ELEGANS USING EXPERIMENTAL EVOLUTION**

Jordan Lewis¹, McKenna Penley¹, Levi Morran¹

¹ Department of Biology, Emory University

Hill-Robertson Interference hypothesizes that genetic recombination can be advantageous to an organism by interrupting natural linkage disequilibria that occurs within a population. This allows organisms a mechanism through which natural selection can act more efficiently on less favorable alleles. However, theoretically recombination could also separate positive associations in the genome and break apart advantageous combinations of alleles. These dynamics could be even more important in determining the selective advantage or disadvantage of outcrossing in mixed mating populations where outcrossing frequency can be altered by virulent parasites. In this experiment we utilized experimental evolution with 4 genetically diverse populations of *Caenorhabditis elegans* to evaluate the effects of genetic recombination in the presence of selection pressure from a pathogen, *Serratia marcescens*, when both asexual and sexual reproduction is possible. The experiment was carried out to 15 generations and fitness assays will be done on the ancestral populations, intermediate populations, and ending populations as well as genetic sequencing to quantify changes that took place within the populations.

ACETYLATED KRUPPEL-LIKE FACTOR 5 AND TRANSFORMING GROWTH FACTOR- β MEDIATED DRUG RESISTANCE IN PROSTATE CANCER

Yixiang Li¹, Baotong Zhang¹, Jin-Tang Dong¹.

¹ Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University

Prostate cancer is the second leading cause of cancer related death in the United States. As the most aggressive form of prostate cancer, metastatic Castration Resistant Prostate Cancer (mCRPC) is primarily treated with chemotherapy including docetaxel. However, the effect of chemotherapy is limited by development of lethal drug resistant diseases in almost all patients. Human Krüppel-Like Factor 5 (KLF5) was identified as a tumor suppressor in prostate cancer and underwent acetylation by P300 acetyltransferase recruited by Transforming Growth Factor- β . We found that KLF5 alone doesn't induce docetaxel resistance in DU145 and PC3 cells. However, KLF5 is indispensable in TGF- β induced docetaxel resistance. We further found that TGF- β induced docetaxel resistance depends on acetylation of KLF5 on lysine 369. Mass spectrometry analysis revealed that JUNB, a downstream target of c-Jun N-terminal kinase, is a direct binding target of KLF5. Knocking down of JUNB inhibits acetylated KLF5 induced docetaxel resistance. These suggested that acetylated KLF5 mediated drug resistance through interaction with JUNB. Our study introduced a novel mechanism of acetylated KLF5 mediated docetaxel resistance in prostate cancer and contributed to an advanced therapy combination for advance prostate cancer patients.

INFLUENCE OF DESCENDING CORTICAL PROJECTIONS ON SPINAL REFLEX EXCITABILITY IN POST-STROKE INDIVIDUALS

Alejandro J Lopez^{1,2}, Jiang Xu^{2,3}, Steven P Eicholtz², Michael R Borich², Trisha M Kesar²

¹ Neuroscience Graduate Program, Graduate Division of Biological and Biomedical Sciences, Emory University

² Department of Rehabilitation Medicine, Division of Physical Therapy, Emory University

³ Department of Rehabilitation Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Combining peripheral nerve stimulation (PNS) with transcranial magnetic stimulation (TMS) at specific inter-stimulus intervals (ISIs) can index the excitability of descending corticofugal projections onto spinal lower motor neurons (LMNs). Previous studies have shown that post-stroke individuals have decreased ipsilesional corticospinal tract (CST) output and abnormally elevated paretic limb spinal reflex excitability. However, the influence of descending corticofugal projections on spinal LMNs for post-stroke individuals remains unknown. The purpose of this study was to evaluate the excitability of these projections in post-stroke individuals. Ten neurologically-unimpaired, adult participants and eight post-stroke participants completed a single experimental session. Unconditioned (UC) Hoffmann's reflexes (H-reflexes) were obtained by delivering PNS to the posterior tibial nerve that elicited an H-reflex in the target soleus muscle. Subsequently, TMS-conditioned H-reflexes were collected in an analogous manner. Short-latency facilitation (SLF) of the H-reflex occurs when a subthreshold TMS pulse is sent *after* PNS, and long-latency facilitation (LLF) occurs when a subthreshold TMS pulse is delivered *before* PNS. Our results indicate that post-stroke individuals show a significant reduction in SLF ($p=.01$) and LLF ($p=.03$) compared to able-bodied participants. Our future work will aim to determine how the excitability of descending corticofugal projections onto spinal motoneurons is modulated by rehabilitation interventions.

INVESTIGATING THE ROLE OF THE LOCUS COERULEUS NOREPINEPHRINE SYSTEM IN THE EXPRESSION OF NOVELTY-INDUCED ANXIETY

Daniel Lustberg¹, Rachel Pearcy¹, David Weinshenker¹

¹ Department of Human Genetics, Emory University School of Medicine

The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the brain and regulates arousal, attention, decision making, and emotion. The LC projects to corticolimbic circuits that govern diverse stress-induced behavioral responses, and the firing rate of LC-NE neurons increases in response to stressors and novel environments. Pharmacological or optogenetic activation of the LC-NE system elicits anxiety-like behavior in animal models, and dysfunction of the LC-NE system contributes to the pathophysiology of anxiety disorders in humans. Given the involvement of the LC-NE system in anxiety and novelty detection, we hypothesized that innate novelty-induced anxiety-like behavior (neophobia) would be impaired in mice lacking the gene encoding the NE biosynthetic enzyme dopamine β -hydroxylase (*Dbh*). We compared neophobic behavior of NE-deficient *Dbh*^{-/-} mice and their NE-competent *Dbh*^{+/-} littermates and observed a profound reduction in novelty-induced anxiety-like behavior in *Dbh*^{-/-} mice that was associated with decreased neuronal activity (measured by Fos induction) in several LC target regions. Administration of a DBH inhibitor or adrenergic receptor antagonists diminished neophobic responses in control mice, and novelty-induced anxiety was restored in *Dbh*^{-/-} mice by the synthetic NE precursor DOPS. These findings suggest that the LC-NE system is required for the expression of unconditioned neophobic behavior.

IDENTIFICATION AND ANALYSIS OF *ATP6V0C* VARIANTS IN EPILEPSY PATIENTS

Kari A. Mattison,¹ Sarah W. Leung,² Cristina da Silva,³ Kathryn Garber,^{1,3} Anita H. Corbett,² Andrew Escayg¹

¹ Department of Human Genetics, Emory University, Atlanta, GA

² Department of Biology, Emory University, Atlanta, GA

³ EGL Genetics, Tucker, GA

Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures due to an imbalance in neuronal excitability and inhibition. Genetic factors, with diverse biological functions, are thought to be responsible for approximately 70-80% of epilepsy cases. The application of next-generation sequencing to clinical genetic testing is accelerating the discovery of novel epilepsy-causing genes. Utilizing a rich resource of clinical whole-exome sequence data from EGL Genetics, we have identified a putative *de novo* epilepsy causing variant (A138P) in *ATP6V0C* which encodes a membrane bound subunit of the vacuolar H⁺-ATPase (V-ATPase). Two additional small deletions, predicted to cause frameshifts, have been reported in the literature in patients with epilepsy and/or developmental delay. We are evaluating the effect of the three patient variants on V-ATPase function using a pH sensitive growth assay in a *Saccharomyces cerevisiae* model. Future studies will further characterize these epilepsy variants, as well as the effect of population variants on V-ATPase function. Overall, our work will establish the role of *ATP6V0C* as an epilepsy gene and may lead to the discovery of other V-ATPase subunits that contribute to epilepsy.

HETEROLOGOUS ADENOVIRAL IMMUNIZATIONS PROVIDE STERILIZING PROTECTION AGAINST *P. VIVAX* IN A SURROGATE MURINE MODEL

Jessica McCaffery¹ Jairo Andres Fonseca^{1,2}, Luis E. Muñoz¹, Elena Kashentseva³, Balwan Singh¹, Igor P. Dmitriev³, David T. Curiel³, Fidel Zavala⁴, Alberto Moreno^{1,2}

¹ Emory Vaccine Center, Yerkes National Primate Research Center, Emory University

² Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine

³ Cancer Biology Division, Department of Radiation Oncology, Washington University School of Medicine

⁴ Department of Molecular Microbiology and Immunology and Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University

Heterologous prime-boost immunization regimens including DNA, MVA or adenoviral vectors (Ad) have been reported to improve protective efficacy over traditional vaccine candidates. However, immunization Ad vectors is sufficient to induce anti-vector antibodies that reduce vaccination efficacy of homologous Ad boosts. Here we described the immunogenicity and efficacy of a heterologous Ad immunization regimen including the recombinant human Ad5/3 and the simian SAd36, tested in mice. These vectors were used to deliver a chimeric multi-stage *P. vivax* protein based on the circumsporozoite protein (CSP) and the merozoite surface protein 1 (MSP1). Ad immunizations were followed by two protein boosts with the corresponding chimeric proteins. There regimens were compared to mice receiving only protein immunizations and unvaccinated mice. Heterologous Ad regimens induced high titers of antibodies capable of recognizing the native structure of both *P. vivax* CSP and MSP1 by immunofluorescence, a balanced IgG1/IgG2a response, and significantly improved IL-2 production by CD4 and CD8 T cells when the SAd36 was used for priming. When protection was assessed using murine *P. berghei* parasites transgenic for *P. vivax* CSP repeat region, we observed sterilizing protection was observed in 8/10 mice immunized with the Ad5/3-SAd36-protein regimen, and 9/10 mice immunized with the SAd36-Ad5/3-protein regimen.

A PROTEOMIC ANALYSIS OF SEX-SPECIFIC DIFFERENCES LINKED TO ALZHEIMER'S DISEASE RISK

Erica Modeste, Eric Dammer, Duc M. Duong, James J. Lah, Allan I. Levey, Nicholas T. Seyfried

Department of Biochemistry and Neurology, Emory University School of Medicine

Alzheimer's disease (AD) is an age-dependent neurodegenerative disease that is increasing in prevalence worldwide. Despite the growing burden of AD, current treatments have limited effects on the progression of the disease. In addition to age, there are many factors that increase an individual's risk to develop AD. For example, women are disproportionately affected by AD compared to men. Although clinical explanations for this difference have been postulated, research at the molecular and biochemical level remains sparse. To bridge this gap in knowledge, we used a proteomic approach to analyze post-mortem human brain tissues of the dorsolateral prefrontal cortex from two independent cohorts (Banner/SunHealth and Mt. Sinai). The three different groups of patients were defined as normal β -amyloid load (normA β) / No Cognitive Impairment (NCI), high β -amyloid load (highA β) / NCI, and AD. Patients indicated as highA β /NCI had comparable proteomic A β levels of AD patients but did not have any cognitive impairments. Proteomic analysis revealed two proteins that were driving sex differences amongst the patients: CD99 and UBA1. CD99 and UBA1 were found to be differentially expressed between sexes and significantly correlated to AD pathology. Sex-based differences in the human brain proteome were also benchmarked against proteomics generated from male and female mouse models of AD (5xFAD). Our findings begin to resolve sex-driven protein expression preserved across mouse and human brain tissue and inform a deeper mechanistic understanding of the biological underpinning of female risk for AD.

POPULATION GENOMIC AND EVOLUTIONARY STUDIES OF PHAGE RESISTANCE AND HOST RANGE IN *STAPHYLOCOCCUS AUREUS*

Abraham G. Moller^{1,2}, Robert A. Petit III¹, Michelle N. Hargita¹, Michelle Su^{1,2}, Timothy D. Read¹

¹ Division of Infection Diseases, Department of Medicine, Emory University

² Microbiology and Molecular Genetics, Graduate Division of Biological and Biomedical Sciences, Emory University

Staphylococcus aureus is an increasingly antibiotic resistant human pathogen that causes diseases ranging from skin infections to septic shock. The goal of our research is to understand the *S. aureus* species-wide genetic determinants of phage resistance (ability of bacteria to survive phage challenge) and host range (maximum number of strains a particular phage can infect). We hypothesize that barriers to infection at the cell surface play the most significant role in determining *S. aureus* phage resistance and host range and that phage resistance mutations involving such barriers are costly and unstable without phage selection. Our project has two main aims – 1) identifying phage resistance mutations through laboratory selection and analyzing their natural evolutionary histories, and 2) identifying species-wide phage resistance and host range determinants. Regarding the first aim, we have selected and sequenced at least ten mutants resistant to two virulent phages. For the second aim, we have screened 126 diverse strains for resistance to seven phages. Using this information, we can rationally design phage cocktails that target the broadest host range of *S. aureus* strains and address the impact of phage on *S. aureus* evolution.

PIX-1, A RHO-GEF, THAT DIRECTS SITE-SPECIFIC ASSEMBLY OF INTEGRIN ADHESION COMPLEXES IN STRIATED MUSCLE

Jasmine Moody,¹ Hiroshi Qadota,¹ April R. Reedy,¹ N. Shanmugam,¹ Leila Lesanpezeshki,² Siva A. Vanapalli,² Yohei Matsunaga¹, Guy M. Benian¹

¹ Dept. of Pathology, Emory University

² Dept. of Chemical Engineering, Texas Tech University

Integrin adhesion complexes (IACs) consist of integrin and a complex of proteins crucial for adhesion of cells to extracellular matrix. In non-muscle cells these IACs are called focal adhesions and in muscle are called costameres. Much is known about the composition of IACs, but little is known about the mechanisms involved in when and where IACs will form. *C. elegans* striated muscle has IACs at M-lines, dense bodies, and attachment plaques at muscle cell boundaries. We discovered that loss of function of *pix-1* results in the absence of IACs at muscle cell boundaries, but not at M-lines and dense bodies. PIX-1 consists of SH3, RhoGEF and coiled coil domains and is orthologous to □PIX of humans. *pix-1* mutants are slower moving and produce less force than wildtype, demonstrating the importance of the boundary structures in force transmission. Antibodies to PIX-1 localize to all 3 IAC structures. Mutation in a highly conserved residue of the RhoGEF domain produces a PIX-1 protein of normal size and abundance but results in lack of boundary structures suggesting the importance of RhoGEF activity. Analysis of mutants in a putative *pix-1* pathway shows that the RhoGEF activates the Rac protein CED-10 and acts via PAK kinases.

NANOWIRES THAT INTERACT WITH $\beta 1$ INTEGRIN INCREASE TRANSEPITHELIAL PERMEABILITY AND INDUCE CYTOSKELETON REARRANGEMENT

Raven J. Peterson^{1,2}, Colin R. Zamecnik³, Tejal A. Desai³, Michael Koval^{1,2,4}

¹ Biochemistry, Cell and Developmental Biology Graduate Program

² Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Emory University

³ Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco

⁴ Department of Cell Biology, Emory University

Epithelial barrier function has two regulated components, the transcellular path mediated by transcytosis, and the paracellular path between cell-cell contact sites regulated by tight junctions (TJs). We previously determined that stimulation of epithelial cells by contact between the apical plasma membrane with nanostructured films (NSFs) increases transepithelial permeability to large macromolecules through both the transcellular and paracellular pathways via stimulation of apically localized integrin $\beta 1$. NSFs act by engaging large, heterogeneous patches of the apical plasma membrane, making it difficult to precisely define molecular mechanisms linking specific surface proteins to changes in epithelial barrier function. Thus, to specifically investigate whether $\beta 1$ integrin is directly involved in increased barrier permeability, we developed an anti-integrin nanowire system consisting of anti- $\beta 1$ integrin antibodies conjugated to functionalized polycaprolactone nanowires, which served as a platform to specifically cluster apically localized $\beta 1$ integrin to determine whether integrin stimulation has the capacity to regulate epithelial barrier function. Treatment of epithelial monolayers with anti-integrin nanowires decreased barrier function as measured by transepithelial resistance and paracellular flux. These functional effects were associated with nanowire-induced changes in the localization of TJ associated proteins, the integrin-associated actin binding protein talin, and rearrangement of the actin cytoskeleton.

METABOLIC DYSFUNCTION AS A CONTRIBUTOR TO 3Q29 DELETION SYNDROME PHENOTYPES

Rebecca M Pollak¹, Timothy Rutkowski², Stephanie Grewenow¹, Tamika Malone², Ryan Purcell³, Kimberly Pachura⁴, Grace Wynn⁴, Tamara Caspary², Paul Dawson⁴, Dean P Jones⁵, Michael E Zwick², Jennifer G Mulle²

¹ Genetics and Molecular Biology Program, Laney Graduate School, Emory University

² Department of Human Genetics, School of Medicine, Emory University

³ Department of Cell Biology, School of Medicine, Emory University

⁴ Department of Gastroenterology, Department of Pediatrics, School of Medicine, Emory University

⁵ Division of Pulmonary, Allergy and Critical Care Medicine, School of Medicine, Emory University

3q29 deletion syndrome (3q29Del) is caused by a rare (~1:30,000) 1.6 Mb heterozygous deletion. Individuals with 3q29Del have reduced birth weight (15.04 oz, $p=1.5E-6$). This phenotype is recapitulated in the mouse model of 3q29Del created by Emory Investigators (1.81 grams, $p=8.8E-12$) and may be a signature feature of the syndrome. Based on the function of genes in the interval, we hypothesized that 3q29Del may harbor an unidentified metabolic disorder. We used metabolic chambers to measure gross features of metabolism in our mouse model, including food and water consumption, activity, and energy expenditure. On a standard diet, the 3q29Del mice did not significantly differ from their WT littermates for any of the parameters measured. After correcting for weight, 3q29Del mice did not eat significantly less than their WT littermates, suggesting that the 3q29Del-associated weight deficit is not due to decreased feeding. 3q29Del mice do not move more than their WT littermates, and energy expenditure, as measured using indirect calorimetry, was not different. To further interrogate metabolic differences, we are currently performing untargeted metabolomics to identify molecular pathways that are disrupted by the presence of the 3q29 deletion, as these dysregulated pathways could provide clues to the mechanisms underlying 3q29Del-associated phenotype development.

MONOSYNAPTIC CONNECTIONS UNDERLIE GAMMA DEFICITS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

Stephanie Prince¹, Abigail Paulson², Lu Zhang², Matthew Attokaren², Solange Amigues², John Tipton², Annabelle Singer^{1,2}

¹ Neuroscience Graduate Program, Emory University

² Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology

Alzheimer's disease (AD), the most common form of dementia, is characterized by neurodegeneration and synapse loss. Alterations in electrophysiological activity have also been found in both humans with and animal models of AD. Yet, it remains unclear how neurobiological changes might disrupt neural circuits to produce altered neural activity and ultimately, cognitive impairment. Prior work has shown that gamma activity (30-100 Hz), a facilitator of learning and memory, is altered in multiple mouse models of AD and humans with AD. Gamma is generated by connections between interneurons and pyramidal cells, and thus we hypothesized that connections between these cell-types would be altered in a mouse model of AD (5XFAD). To test our hypothesis, we recorded local field potential and single unit spiking activity from hippocampus as 5XFAD and wild-type (WT) mice performed a spatial task in virtual-reality. After classifying single units into pyramidal cells and interneurons, we identified monosynaptically connected or synchronous pairs. Interestingly, we found weaker pyramidal-to-interneuron excitatory connections, and significantly less correlated synchronous interneuron pairs in the 5XFAD mice. This work has broad applications in revealing how neural circuits for memory are disrupted in AD, and in bridging the gap between molecular pathology, neural activity, and cognitive deficits.

STEROID HORMONE CONCENTRATIONS ARE ASSOCIATED WITH CHANGES IN POSTTRAUMATIC STRESS DISORDER SYMPTOMS AND FEAR PSYCHOPHYSIOLOGY DURING PREGNANCY AND IN THE POST-PARTUM PERIOD

Meghna Ravi¹, Tanja Jovanovic^{1,2}, Vasiliki Michopoulos^{1,2,3}

¹ Neuroscience Graduate Program, Emory University

² Department of Psychiatry and Behavioral Sciences, Emory University

³ Division of Developmental and Cognitive Neuroscience, Yerkes National Primate Research Center

Steroid hormones have been implicated in conferring increased risk for posttraumatic stress disorder (PTSD) in traumatized women compared to men. Furthermore, PTSD is associated with higher physiological expression of fear under safe conditions. However, the exact role that estradiol, progesterone, and cortisol play in conferring risk for PTSD and dysregulation of fear psychophysiology in traumatized women remains unclear. Because concentrations of steroid hormones fluctuate during pregnancy, we examined the association between varying levels of these steroid hormones on fear-potentiated startle (FPS) and PTSD symptoms over the course of pregnancy and one-month post-partum in traumatized African American women. Results indicated that progesterone concentrations at one-month post-partum correlated with lower FPS to a safety signal ($r=-0.88$; $p<0.05$). Cortisol concentrations were also associated with lower FPS to a safety signal one-month post-partum ($r=-0.88$; $p=0.05$). Our results suggest that increases in cortisol and progesterone seen in the third trimester of pregnancy are associated with decreased fear-related physiology to safety cues in traumatized women.

ROLE OF THE CPEB BINDING PROTEIN ORB2 IN ASYMMETRIC STEM CELL DIVISION

Beverly V Robinson¹, Dorothy A Lerit¹

¹ Department of Cell Biology, Emory University School of Medicine

Proper asymmetric neuronal stem cell division is important in the developing brain in order to ensure a population of self-renewing stem cells and differentiating cells. In normal *Drosophila melanogaster* neuroblasts (NB), asymmetric cell division is regulated in part through functionally unequal, or asymmetric, centrosomes. Centrosome asymmetries are generated through the asymmetric enrichment of the various proteins that comprise the pericentriolar material (PCM), which dictates the microtubule-organizing activity of the centrosome. Previous data suggests that the RNA-binding protein Orb2, a cytoplasmic polyadenylation element binding (CPEB) protein homolog, may play a role in regulating NB cell divisions. To determine the mechanism by which Orb2 acts during asymmetric stem cell division, we compared the interphase localization of the downstream PCM effector, gamma-tubulin, between apical and basal centrosomes of wild-type versus *orb2* null NBs using confocal microscopy. Normally, gamma-tubulin is specifically enriched on the active, apical centrosome and depleted from the transiently inactive basal centrosome. Our preliminary data suggest that there is a loss of centrosome asymmetry in the *orb2* mutant NBs. We found that 64% (9/14 interphase NBs) of *orb2*⁻ NBs have gamma-tubulin enriched at both centrosomes (p-value<0.001). We conclude that loss of *orb2* impairs the transient inactivation of the basal centrosome.

UNDERSTANDING THE MECHANISM OF CELL DEATH INDUCTION IN TRIPLE-NEGATIVE BREAST CANCER CELLS BY ENGINEERED REOVIRUS

Roxana M. Rodríguez Stewart¹, Angela K. Berger², Jaime Guberman³, Bernardo A. Mainou^{1,2}

¹ Microbiology and Molecular Genetics, Emory University, Atlanta, Georgia

² Department of Pediatrics, Emory University, Atlanta, Georgia

³ Emory University, Atlanta, Georgia

Triple-negative breast cancer (TNBC) constitutes approximately 15% of all breast cancer and is associated with worse prognosis when compared to other subtypes of breast cancer. There is a need for targeted therapeutics to treat this type of breast cancer, as current therapies are largely limited to cytotoxic chemotherapy. Mammalian orthoreovirus (reovirus) causes a mostly asymptomatic infection in humans and preferentially kills transformed cells. To engineer reovirus with enhanced cytopathic properties against TNBC cells, we coinfecting TNBC MDA-MB-231 cells with parental reoviruses T1L, T2J, and T3D. Following sequential serial passage, we isolated reassortant reovirus r2Reovirus. r2Reovirus has genomic segments predominantly from T1L with one gene segment from T3D and synonymous and nonsynonymous point mutations. Infection of MDA-MB-231 cells with r2Reovirus is more efficient and induces cell death with faster kinetics than parental reoviruses. Reovirus kills MDA-MB-231 cells in an apoptosis-independent manner. Our data show PARP cleavage during reovirus infection of these cells is carried out in a caspase 3-independent manner and viral replication is necessary for reovirus induction of cell death. These findings suggest reovirus is inducing MDA-MB-231 cell death in a non-canonical manner. Future work will assess how reovirus is killing these cells and what cellular protease promotes PARP cleavage during infection.

CASEIN KINASE II PHOSPHORYLATES RNA-BINDING PROTEIN FUSED IN SARCOMA (FUS) AND FUNCTIONALLY MODIFIES LIQUID-LIQUID PHASE SEPARATION *IN VITRO*

Jessica Root¹, Michelle Johnson¹, Georgia Taylor¹, Cody Keating¹, Thomas Kukar^{1,2,3}

¹ Department of Pharmacology, Emory University, School of Medicine

² Center for Neurodegenerative Disease, Emory University, School of Medicine

³ Department of Neurology, Emory University, School of Medicine

Fused in sarcoma (FUS) is a RNA/DNA binding protein involved in transcription, mRNA splicing, and DNA repair that shuttles between the nucleus and cytoplasm. Abnormal cytoplasmic accumulation of FUS is the key pathological characteristic of Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD) subtypes. However, it is unclear why FUS accumulates in these diseases. One possibility is that the ability of FUS to self-associate and demix from the cytosol to form droplets, through a process called liquid-liquid phase separation (LLPS), is dysregulated in disease. Previous studies have demonstrated that post-translation modifications (PTMs) like phosphorylation can regulate FUS LLPS. We have recently identified that FUS binds to serine-threonine kinase casein kinase II (CK2) conferring predicted novel phosphorylation sites. We hypothesize that CK2 phosphorylation of FUS plays an important role in modulating LLPS. To investigate the regulatory significance of these observations we purified His-MBP-tagged FUS and performed *in vitro* kinase activity assays and LLPS droplet formation assays. We find that CK2 is able to phosphorylate FUS leading to modulation of droplet density and solution turbidity. These data support the hypothesis that FUS is a novel substrate of CK2, and that CK2 phosphorylation has a functional effect on FUS LLPS *in vitro*.

DEVELOPMENT OF TARGETED, NON-GENOTOXIC CONDITIONING AGENTS FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION GENE THERAPY

Athena Russell¹, Allison Lytle, PhD², Jaquelyn Zoine³, Jordan Shields⁴, Chengyu Prince⁴, H. Trent Spencer⁴, Shanmuganathan Chandrakasan⁴, Christopher B. Doering⁴

¹ Program in Genetics and Molecular Biology, Laney Graduate School, Emory University

² Program in Molecular and Systems Pharmacology, Laney Graduate School, Emory University

³ Program in Cancer Biology, Laney Graduate School, Emory University

⁴ Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Emory University School of Medicine

Recent advances in clinical gene transfer into hematopoietic stem cells (HSC) provide the opportunity to develop curative therapies for many genetic diseases. However, conventional HSC transplantation (HSCT) protocols require myeloablative preparations harboring side effects including sterility, hormonal dysregulation and genotoxicity. These risks represent the primary barrier to clinical translation of HSCT gene therapy. Therefore, the development of targeted, non-genotoxic conditioning agents is critical. Our group is developing novel HSCT conditioning agents comprising immunotoxin bioconjugates. Targeted immunotoxin internalization induces cytotoxicity thereby creating physical space in the bone marrow niche for HSC engraftment while simultaneously providing immune suppression to the transgene product. We are developing HSCT gene therapy for the most common severe bleeding disorder, hemophilia A, wherein autologous HSC are engineered to express blood coagulation factor VIII (fVIII). Durable engraftment and hematopoietic reconstitution after HSCT enables stable production of fVIII and phenotypic correction in a murine model of hemophilia A. Our preliminary data illustrate targeted HSC depletion following antibody-toxin conditioning in both wild-type C57BL/6J and hemophilia A mice. Furthermore, we have demonstrated mixed hematopoietic chimerism and >95% myeloid engraftment. Further studies are underway to evaluate the safety, feasibility, and efficacy of non-genotoxic immunotoxin conditioning for hemophilia A gene therapy.

THE MATERNAL EPIGENETIC REPROGRAMMING FUNCTION OF THE HISTONE DEMETHYLASE LSD1 IS COREST DEPENDENT AND MAY CONTRIBUTE TO NEURODEVELOPMENTAL DISORDERS

Alyssa Scott¹, David Katz¹

¹ Emory University, Cell Biology, Atlanta, GA

Somatic cell nuclear transfer has established that the oocyte contains maternal factors with epigenetic reprogramming capacity. However, the function of these maternal factors during the gamete-to-embryo transition remains poorly understood. In *C. elegans*, LSD1/KDM1A (lysine specific demethylase 1) acts with the CoREST repressor complex to enable this transition by removing H3K4me1/2 and preventing the transgenerational inheritance of transcriptional patterns. In mouse, the loss of maternal LSD1 results in embryonic arrest at the 1-2 cell stage, with arrested embryos similarly failing to undergo the maternal-to-zygotic transition. Moreover, partial loss maternally results in striking phenotypes weeks after fertilization, including perinatal lethality and abnormal behavior in surviving adults. To explore the mechanism underlying these heritable defects further, we developed a new maternally hypomorphic LSD1 allele that predominantly affects the binding of LSD1 to CoREST. This new allele phenocopies our mouse model with reduced LSD1, suggesting that the maternal reprogramming function of LSD1 is CoREST dependent. Additionally, in our new model we find that the incidence of perinatal lethality is higher in early and advanced maternal age. This trend with maternal age is reminiscent of the epidemiological data in autism, raising the possibility that defective maternal epigenetic reprogramming can contribute to neurodevelopmental disorders.

INHIBITING CORTICOSTERONE SYNTHESIS ATTENUATES COCAINE-INDUCED HABIT FORMATION***Michelle K. Sequeira*^{1,2,3,4}, *A. Allen*^{1,3,4}, *Shannon L. Gourley*^{1,2,3,4,5}**¹ Department of Pediatrics, Emory University² Graduate Program in Neuroscience, Emory University³ Yerkes National Primate Research Center, Emory University⁴ Department of Behavioral Neuroscience and Psychiatric Disorders, Emory University⁵ Molecular and Systems Pharmacology Program, Emory University

Stress is both a cause and consequence of neuropsychiatric disorders, such as substance use disorders. Corticosterone (CORT) is the primary stress hormone in rodents (cortisol in humans). CORT synthesis and release upon stressor exposure ultimately affects decision making. For instance, CORT biases organisms away from goal-directed decision making – selecting actions based on the likelihood that they will result in desired outcomes – towards habits, which are insensitive to goals. Similarly, cocaine biases humans and experimental animals towards habits and notably, elevates CORT. Here, we tested the hypothesis that cocaine-induced CORT release causes habit biases. We first confirmed that cocaine increases circulating CORT in mice. Next, we administered a CORT synthesis inhibitor, metyrapone, prior to cocaine. Following a drug washout period, we tested mice in a response-outcome contingency degradation procedure used to characterize decision-making strategies. Cocaine induced habit biases, as expected, which we blocked by inhibiting CORT synthesis. Moreover, CORT exposure in separate mice was sufficient to induce nearly identical habit biases. These experiments indicate that CORT exposure may be a mechanism by which cocaine induces habit-based decision making.

INVESTIGATING THE ROLE OF THE CANNABINOID 2 RECEPTOR IN EPILEPSY

Lindsey Shapiro¹, Jennifer C. Wong¹, Andrew Escayg¹

¹ Department of Human Genetics, Emory University, Atlanta, GA

Epilepsy affects over 50 million individuals globally, making it one of the most common neurological disorders. Approximately 30% of epilepsy patients do not respond to currently available treatments, thus, the development of novel therapies is critical. The Cannabinoid 2 Receptor (CB2R) has received recent attention as a potential therapeutic target for several neurological diseases. However, we still have little knowledge of whether CB2R activity may contribute to seizure generation. Here, we evaluated the effect of reducing CB2R expression on acute seizure susceptibility in C57BL/6J mice as well as mice harboring a human epilepsy mutation. We demonstrated that the loss of CB2Rs increases seizure susceptibility in both mouse lines. Further, administration of the CB2R antagonist, SR144528, increased seizure susceptibility in wild-type mice. Together, our results indicate that reduced CB2R expression exacerbates seizure phenotypes, thus supporting a potential role for CB2Rs as novel treatment targets for epilepsy. Given the known role of CB2Rs in attenuating inflammation, our future work will investigate whether CB2R activation is protective in models of chronic epilepsy that are characterized by robust neuroinflammation.

OPTIMIZING ANTI-VIRAL FROG PEPTIDES FOR THERAPEUTIC USE

Jessica Shartouny¹, Joshy Jacob¹

¹ Emory Vaccine Center, Emory University

Influenza viruses are responsible for much of the annual disease worldwide, in both seasonal epidemics and sporadic pandemics. In cases where the influenza vaccine is ineffective or unavailable, antiviral drugs are relied upon to manage outbreaks and lessen illness in individuals. There are, however, few antiviral drugs available and influenza can become resistant to those that are. Antimicrobial peptides (AMPs) are one large potential pool of new therapeutics, as they are produced innately by most forms of life and often have broad activities. A frog-derived AMP, Yodha, is an effective inhibitor of H1N1 and H3N2 influenza viruses as well as several flaviviruses. The peptide appears to form large conglomerates of fibrils in solution when viewed under electron microscopy, which is a hindrance to in vivo delivery, however. We have designed and screened several panels of modified peptides based on Yodha's primary structure to examine the structure-function relationship of the antiviral activity. From these screens, we have determined that polymerization is not required for anti-viral activity and several modified peptides have been found to out-perform wildtype Yodha and are primary candidates for the development of a deliverable drug.

AMINOGLYCOSIDE HETERORESISTANCE IN GRAM-NEGATIVE PATHOGENS

Edgar X. Sherman, David S. Weiss

Antibiotic resistance threatens to reverse the strides of modern medicine by reducing our ability to combat infections, which is essential prophylactic care for medical procedures such as surgery. Therefore, it is crucial that we fully appreciate the mechanisms of antibiotic resistance and understand why antibiotics sometimes fail in therapy. Antibiotic treatment failure is poorly understood but may in part be explained by heteroresistance. Heteroresistance is the phenomenon where sub-populations of resistant bacteria co-exist within a susceptible population. Depending on the frequency, the resistant sub-population may go undetected through conventional clinical diagnostic methods, but still cause treatment failure, resulting in extended hospital stays, secondary complications, or even death. My work aims to elucidate the factors surrounding heteroresistance to aminoglycosides, an important class of drugs used to treat serious infections. Here, I report the prevalence of aminoglycoside heteroresistance among Gram-Negative pathogens along with data showing which heteroresistant strains were undetected in the clinic. I use computational analysis to describe potential mechanisms underlying aminoglycoside heteroresistance. Finally, I show that aminoglycoside heteroresistance mediates treatment failure *in vivo* using a mouse model. Altogether, these data indicate that aminoglycoside heteroresistance can lead to treatment failure and necessary that we understand how to detect and control heteroresistance.

BMI-1 AS A THERAPEUTIC TARGET IN RHABDOMYOSARCOMA

Cara E. Shields¹, Selma M. Cuya¹, Sarah Chappell¹, Komal Rathi², Shiv Patel¹, Sindhu Potlapalli¹, Robert W. Schnepf¹

¹ Aflac Cancer and Blood Disorders Center, Department of Pediatrics, Division of Pediatric Hematology, Oncology, and Bone Marrow Transplant, Emory University School of Medicine; Winship Cancer Institute; Children's Healthcare of Atlanta

² Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Children's Hospital of Philadelphia

Rhabdomyosarcoma (RMS) is an aggressive soft tissue sarcoma which affects mainly children. Alveolar rhabdomyosarcoma (ARMS) harbors currently undruggable PAX-FOXO1 fusion proteins and has a worse overall outcome, therefore underscoring the need to identify novel targets for this cancer. We discovered that the epigenetic regulator BMI-1 is overexpressed in ARMS cells. BMI-1 is a known oncogene in other cancers, though its role in ARMS remains undescribed. We examined RNA-Seq tumor datasets and determined that BMI1 is robustly expressed in ARMS tumors. Additionally, we confirmed that BMI-1 is also overexpressed in ARMS cell lines at the levels of RNA and protein. Next, we depleted BMI-1 using multiple shRNAs/siRNAs and found this led to a marked decrease in cell growth, coupled with increased levels of apoptosis. We then turned to pharmacological inhibitors PTC-209 and the newer PTC-028. Both compounds inhibited BMI-1 function and reduced cell proliferation in ARMS cell lines within the nanomolar range. Overall, both genetic and pharmacologic inhibition of BMI-1 strikingly decreased ARMS cell proliferation. Currently, we are further investigating this pathway. Targeting BMI-1 pharmacologically could provide a novel therapeutic option for patients with ARMS and may apply more broadly to other sarcomas.

HOST IMMUNE FUNCTION IMPACTS GENOME INSTABILITY IN AN OPPORTUNISTIC FUNGAL PATHOGEN

Amanda Shurzinske¹, Meleah A. Hickman¹

¹ Department of Biology, Emory University

Candida albicans is an opportunistic fungal pathogen that causes a range of infection, depending on host immune function. In healthy individuals, superficial infections, such as oral thrush and vaginal candidiasis occur. However, in individuals with compromised immune function, severe bloodstream infections occur and may result in death. While typically diploid, *C. albicans* has a highly labile genome, and many clinical isolates deviate from diploidy. To investigate how immune function impacts *C. albicans* genome stability and ploidy dynamics, we infected healthy and immunocompromised *C. elegans* hosts with diploid and tetraploid *C. albicans* strains. We find that *C. albicans* extracted from healthy hosts have a 100-fold increase in the loss of a heterozygous marker, a standard measure of genome stability, compared to *C. albicans* grown *in vitro* or extracted from immunocompromised hosts. Furthermore, we find that diploid strains maintained diploidy regardless of host immune function. However, tetraploid strains, undergo ploidy reduction in healthy hosts, but maintain ploidy in immunocompromised hosts. Taken together, these results suggest that host immune function serves as a stressor for *C. albicans*, leading to increased genome and ploidy instability. These types of genomic changes may be important for *C. albicans* adaptation to the host environment.

CHARACTERIZATION OF PRAP1 DURING MURINE GESTATION

Taylor M. Smith^{1,2}, Alexandra Wolfarth^{2,3}, Andrew S. Neish.²

¹ Genetics and Molecular Biology Graduate Program, Emory University

² Department of Pathology and Laboratory Medicine, Emory University

³ Immunology and Molecular Pathogenesis Emory University

PRAP1, a 17 kDa protein, remains largely uncharacterized. Expressed and secreted by the uterine endometrium, PRAP1 is a known marker for implantation in mice but function during murine gestation remains unknown. Lactotransferrin (LTF), a well characterized, antimicrobial protein, is a biochemical binding partner of PRAP1. LTF is secreted into milk, amniotic fluid, and uterine fluid. Using staged pregnancies in wildtype C47BL/6J mice, uterine tissue was collected daily during gestation and four days post-partum, then analyzed by qPCR, immunofluorescence with α -PRAP1 and α -LTF, and H&E staining. We found that *Prap1* transcript is highly expressed during gestation compared to non-ovulating females, gradually increasing until peaking at parturition and decreasing 1000x within 4 days post-partum. Immunofluorescence analysis mid gestation demonstrated PRAP1 is localized specifically to the anti-mesometrial epithelia that interfaces with the amnion. LTF is expressed at high levels in early pregnancy and post-partum. We hypothesize PRAP1 plays a role in pregnancy maintenance and has antimicrobial properties. Microbial differences in the uterus between wildtype and *Prap1*^{-/-} animals during gestation and post-partum will be assessed by 16S analysis. Ultimately, we will perform transcriptomics of gravid endometrium comparing wildtype conventional, wildtype germ-free, and *Prap1*^{-/-} endometrium in an effort to discover novel modulators of pregnancy.

ALTERNATIVE MECHANISMS OF RIBOSOME STALLING RESCUE IN THE GRAM-NEGATIVE BACTERIUM *FRANCISELLA TULARENSIS*

Pooja Srinivas¹, Tyler D. P. Goralski², Kenneth C. Keiler², Christine M. Dunham¹

¹ Department of Biochemistry, Emory University

² Biochemistry and Molecular Biology, Pennsylvania State University

Many current antibiotics target the bacterial ribosome, but have cross-reactivity with eukaryotic ribosomes, causing host toxicity. One approach to prevent this is the development of antibiotics that target essential bacterial-specific pathways, such as ribosome stalling rescue. Stalled ribosome complexes arise when the ribosome halts during translation due to the absence of an in-frame stop codon. *Trans*-translation, found in 99.9% of bacterial species, is the main rescue pathway. Most bacteria remain viable without *trans*-translation because they encode additional rescue systems. The pathogenic bacterium *F. tularensis* appears to only encode *trans*-translation, but *trans*-translation deletion strains remain viable. *How does F. tularensis survive without known ribosome rescue mechanisms present?* Transposon mutagenesis data identifies a novel release factor, ArfT, which allows *F. tularensis* survival in the absence of *trans*-translation. *In vitro* translation assays find that ArfT facilitates peptidyl-tRNA hydrolysis with either RF1 or RF2, in contrast to the model of ArfA-RF2-mediated rescue. These data suggest that ArfT-mediated ribosome rescue occurs via a novel mechanism. Studies include structural and biochemical characterization of the molecular mechanism of ArfT and release factor binding to stalled *F. tularensis* ribosomes. These studies will provide new insights on the fundamental mechanism and role of ribosome rescue in bacteria.

A ROLE FOR RNA EXOSOME CO-FACTORS IN HUMAN DISEASE EXAMINED IN BUDDING YEAST AND DROSOPHILA MODELS

Maria C. Sterrett^{1,3}, Julia L de Amorim^{1,2}, Liz Enyenihi^{1,3}, Sara W. Leung¹, Laurie Hess¹, Jillian S. Losh⁴, Sergine Brutus^{1,3}, Brittany Avin¹, Jennifer Potter-Birriel¹, Taylor Craig¹, Jillian C. Vaught⁴, Katherine Mills-Lujan¹, Ambro van Hoof⁴, Anita H. Corbett¹, Milo B. Fasken¹

¹ Department of Biology, Emory University

² Biochemistry, Cell, and Developmental Biology Graduate Program, Emory University

³ Initiative to Maximize Student Development Program, Emory University

⁴ Department of Microbiology and Molecular Genetics, University of Texas Health Science Center-Houston, Houston, Texas

The RNA exosome is an evolutionarily conserved, ribonuclease complex that processes/degrades numerous classes of RNA. The 10-subunit exosome complex is composed of three subunit cap, a ring of six PH-like subunits, and a 3'-5' ribonuclease subunit, at the base. Recently, mutations in four structural subunit genes, *EXOSC2*, *EXOSC3*, *EXOSC8* and *EXOSC9*, have been linked human diseases. Mutations in *EXOSC2* have been linked to a novel syndrome characterized by retinitis pigmentosa, hearing loss, premature aging, and mild intellectual disability. In contrast, mutations in *EXOSC3* and *EXOSC8* are linked to pontocerebellar hypoplasia, while mutations in *EXOSC9* are linked to cerebellar atrophy. To gain insight into the functional consequences of the mutations in *EXOSC2/3/8/9* identified in patients, we generated the corresponding mutations in the *S. cerevisiae* orthologs, *RRP4/40/43/45*, and examined their function in budding yeast. We hypothesize that differences in disease phenotypes could reflect altered interactions with RNA exosome co-factors. To explore this possibility, we assessed yeast genetic interactions between RNA exosome mutations in budding yeast and co-factor deletions (*MPP6*, *RRP47*, *RRP6*) and used a non-biased high copy suppressor screen to identify functionally important interactions. Ultimately, these studies will provide insight into both the function of the RNA exosome and the disease pathology.

MYO10 ABERRANT METHYLATION AND OVEREXPRESSION IN LEADER CELLS REGULATES LUNG CANCER COLLECTIVE CELL INVASION AND FIBRONECTIN PATTERNING

Emily Summerbell^{1,2}, Jessica Konen³, Jeanne Kowalski⁴, Paula Vertino⁵, Adam Marcus²

¹Graduate Program in Cancer Biology, Emory University

²Department of Hematology and Medical Oncology, Emory University

³Department of Thoracic/Head-and-Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

⁴Department of Biostatistics and Bioinformatics, Emory University

⁵Department of Radiation Oncology, Emory University

Collective cancer cell invasion is found in most solid tumors and directly contributes to cancer metastasis. Within these collective invasion packs, highly-invasive leader cells pioneer migration and invasion while highly-proliferative follower cells travel behind them. We demonstrate distinct gene expression, DNA methylation, and phenotypes of isolated lung cancer leader and follower cells. Myosin-X (MYO10) is a noncanonical myosin that drives filopodia formation and extension and is necessary for invasion/metastasis of breast cancer, prostate cancer, and melanoma. MYO10 is highly overexpressed in leaders but not expressed in followers, and MYO10 overexpression correlates with promoter DNA hypomethylation. MYO10 immunofluorescence shows localization at leader filopodia tips in 2D culture and 3D invading spheroids in four lung cancer cell lines. MYO10 knockdown notably decreases filopodia length, cell motility, and 3D spheroid invasion. Furthermore, leaders produce and secrete fibronectin, unlike followers. Immunofluorescence within invading spheroids shows the formation of long fibronectin pillars extending beyond leaders. MYO10 knockdown disrupts these fibronectin structures but does not decrease the amount of fibronectin secreted by leader cells. Fibronectin knockdown completely abrogates lung cancer spheroid collective invasion. Therefore, our data suggest that MYO10 regulates cancer cell collective invasion by creating long filopodia that regulate fibronectin reorganization and subsequent collective invasion.

CDK5-DEPENDENT PHOSPHORYLATION OF THE QKI RNA BINDING PROTEIN ENHANCES OLIGODENDROGLIA DIFFERENTIATION BY INTEGRATED REGULATION OF CODING AND NON-CODING RNAS

Izabela Suster¹, Andrew Bankston¹, Feng Wang², Shuya Wang¹, Li Ku¹, Bing Yao², Yue Feng¹

¹ Department of Pharmacology, Emory University School of Medicine

² Human Genetics, Emory University School of Medicine

The RNA-binding protein QKI plays pivotal roles in driving oligodendroglia (OL) and myelination in the central nervous system. Besides the well-characterized function of QKI in regulating its direct target mRNAs, emerging evidence suggests QKI has sophisticated roles in regulating non-coding RNAs. Alternative splicing generates three QKI isoforms with distinct nuclear-cytoplasmic distribution. We found that all QKI isoforms are phosphorylated by cyclin-dependent kinase 5 (CDK5), a critical player that advances early OL development. CDK5-dependent phosphorylation of QKI enhances OL differentiation in culture, partly through enhanced RNA binding activity of QKI, which up-regulates pro-differentiation mRNA targets. Interestingly, CDK5-dependent phosphorylation resulted in drastic nuclear translocation of cytoplasmic QKI isoforms. Phospho-mimetic QKI in the nuclei selectively increased expression of specific circular RNAs flanked by Quaking response elements (QREs) in a human OL cell line. However, circRNA biogenesis is not regulated by QKI in human neuronal cell lines, indicating strong neural lineage specificity for QKI regulated circRNAs. Ongoing studies are identifying regulatory RNAs and proteins sponged by these QKI-regulated circRNAs, thereby controlling OL function. Our studies provide the first evidence that CDK5-dependent phosphorylation of QKI integrates QKI regulation of coding mRNA targets and of noncoding circular RNA targets to advance OL development.

LOCAL ROLE FOR STEROIDS IN REGENERATIVE GROWTH IN *DROSOPHILA*

Doug Terry¹, Joanna Wardwell-Ozgo¹, Can Zhang¹, Phil Byun¹, Kenneth H. Moberg¹

¹ Department of Cell Biology, Emory University School of Medicine

A decrease in regenerative capacity, the ability to restore both function and 3D patterning of wounded tissue, with increasing developmental age is a common feature of wound healing. For example, perinatal humans and mice are capable of regenerating the distal portions of amputated digits, but this ability is quickly lost thereafter. Likewise, *Drosophila* larval imaginal discs have high regenerative capability that declines as they approach pupation. Genetic analysis of *Drosophila* wing disc regeneration using a temperature-controlled ablation system has demonstrated that a number of conserved pathways contribute to wing regrowth (e.g. Hippo, Wnt, JAK/STAT). Based on our previous work linking the Hippo and ecdysone (Ec) pathways in disc cells, we investigated Ec roles in regenerating wing discs. We find evidence that transcriptional activity of the Ec receptor (EcR) is upregulated at the site of injury in larval wing discs and required for efficient wing regrowth in adults. Moreover, local knockdown of the P450 enzyme Shade, which converts Ec to 20E, inhibits wing regeneration. Our data suggest that local synthesis of 20E and induction of the Ec hormone pathway may be a required element of the imaginal disc regenerative program in *Drosophila*.

PROCASPASE-3 REGULATES A CASPASE-INDEPENDENT, RIPK3-INDEPENDENT FORM OF CELL DEATH

David B. Weir¹, Lawrence H. Boise²

¹ Cancer Biology Graduate Program, Emory University, Atlanta, GA

² Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University, Atlanta, GA

Our laboratory has demonstrated that procaspase-3 regulates serum withdrawal-induced death through modulation of fibronectin secretion. However, the mechanism underlying this death remains unclear. To delineate the mechanism we have employed our inducible shRNA system for fibronectin knockdown and various types of cell death inhibition. Procaspase-3^{-/-} mouse embryonic fibroblasts (MEFs) exhibit increased serum withdrawal-induced death when fibronectin secretion is eliminated. Overexpression of Bcl-X_L blocks this death. Furthermore, cytochrome c is released from mitochondria in procaspase-3^{-/-} MEFs subjected to fibronectin knockdown and serum withdrawal. These data are consistent with the initiation of an intrinsic apoptotic death pathway. Therefore, we utilized the pan-caspase inhibitor QVD-OPh to ascertain whether this death is caspase-dependent. QVD-OPh blocks serum withdrawal-induced death in WT MEFs, consistent with caspase involvement in serum withdrawal-induced apoptosis. However, QVD-OPh does not affect death due to serum withdrawal in procaspase-3^{-/-} MEFs lacking fibronectin secretion. Thus, procaspase-3^{-/-} MEFs subjected to fibronectin knockdown and serum withdrawal die in a caspase-independent manner. To elucidate whether serum withdrawal-induced death in procaspase-3^{-/-} MEFs lacking fibronectin secretion occurs via necroptosis we have utilized the RIPK3 inhibitor GSK872. RIPK3 inhibition does not affect this death. Taken together, our data demonstrate that procaspase-3 regulates a caspase-independent, RIPK3-independent form of cell death.

INHIBITION OF INTERLEUKIN-6 INCREASES SUSCEPTIBILITY TO INVASIVE GROUP A STREPTOCOCCAL INFECTION**Shyra Wilde¹**, Kayleigh Olivares², Christopher LaRock¹¹ Department of Microbiology and Immunology, Emory University, Atlanta, GA² Wayne State University School of Medicine, Detroit, MI

Sepsis is a potentially deadly complication of infection that can be impacted by immune status. Group A Streptococcus (GAS; *S. pyogenes*) is a common pathogen that can cause invasive infections in otherwise healthy individuals. An 8-year-old boy under treatment with IL-1 and IL-6 inhibitors presented with high fever and tested blood culture positive for GAS. The infection resolved after IV antibiotics. Post-marketing surveillance data revealed a correlation between patients taking IL-1 and/or IL-6 inhibitors and GAS infection incidence. Whole-genome sequencing revealed that the patient was infected with an M4 strain of GAS lacking virulence factors such as capsule that are typically associated with infection severity; invasive infection is associated with a globally-disseminated M1T1 clone that has acquired several immune-evading virulence factors. The GAS isolated from this patient replicates in whole human blood and did so more rapidly in the presence of either IL-1 or IL-6 inhibitors. In a murine sepsis model, inhibiting IL-1 or IL-6 increased bacterial proliferation and decreased host survival. We find that M4, but not M1T1 GAS, is restricted in a manner dependent on IL-6, but not due to differences in IL-6 expression. Thus, GAS serotype and human immune status can be predisposing factors for severe disease.

COOPERATIVE ANTIBIOTIC RESISTANCE AND MULTISTABLE POPULATION DYNAMICS WITHIN A *C. ELEGANS* GUT MICROBIOME**Megan Woods**¹, N. Vega¹¹ Department of Biology, Emory University

Interactions between microbes are known to drive the response of individual species and communities to perturbation. However, more information regarding species-species interaction in a group context is needed to understand the effects on community diversity and resilience. Eight species isolated from the *C. elegans* gut microbiome are grouped in combinations of six species per group. The goal of this research was to determine what effects the specific makeup of a combination has on its resilience. This is tested by perturbing with Ampicillin after community establishment. Single species abundance and proportion along with total growth are observed and calculated directly after perturbation and outgrowth in fresh media. Community context provided protection to sensitive strains, increasing survival during stress and/or outgrowth after removal of drug. One combination exhibited multiple equilibria both directly after perturbation and after outgrowth, wherein diversity and abundance either returned to pre-disturbance norms or hardly any growth occurred at all. Within this set of microbes, interactions between species, potentially driven by secreted factors, are an important driver of resilience to perturbation. These results suggest that community-level response to antibiotic is driven by species-specific interactions, which has implications for therapeutic response and antibiotic resistance evolution in the community context.

THE CATALYTIC PI3K SUBUNIT, p110d, SUPPORTS GOAL-DIRECTED DECISION MAKING

Ellen P. Woon¹⁻⁴, Shannon L. Gourley¹⁻⁵

¹ Department of Pediatrics, Emory University

² Graduate Program in Neuroscience, Emory University

³ Yerkes National Primate Research Center, Emory University

⁴ Department of Behavioral Neuroscience and Psychiatric Disorders, Emory University

⁵ Graduate Program in Molecular and Systems Pharmacology, Emory University

Goal-directed response selection refers to the ability to perform actions based on their expected outcomes and relies upon the orbitofrontal cortex (OFC). Failure to demonstrate goal-directed behaviors can lead to deferral to more familiar, inflexible behaviors, or “habits.” One molecular factor likely involved in toggling between goal-directed actions and habits is phosphoinositide 3-kinase (PI3K), a membrane-associated signaling complex that regulates neuronal survival and plasticity. PI3K is composed of p110 catalytic subunits, which align with specific signaling pathways. Here, we investigated the p110d subunit, downstream of receptor tyrosine kinases. We first trained mice to respond for food reinforcers using parameters that induced either goal-directed or habit-based response strategies. Western blot analyses revealed that habit-biased mice had less p110d in the OFC and also ventral hippocampus, compared to goal-directed mice. We then utilized viral-mediated gene silencing to reduce p110d in the OFC, which delayed the ability of mice to use action-outcome associations to update food-seeking response strategies. Conversely, stimulating the tyrosine/tropomyosin receptor kinase B (trkB) enriched action-outcome memory. Our results suggest p110d coordinates goal-directed action, potentially via trkB, a finding of potential therapeutic relevance for illnesses in which habitual behaviors (such as habitual drug seeking) predominate despite adverse consequences.

THE MITOCHONDRIAL CITRATE TRANSPORTER SLC25A1 IS REQUIRED FOR SYNAPTIC FUNCTION AND LIPID HOMEOSTASIS

Meghan Wynne^{1,2*}, Cortnie Hartwig^{1,3*}, Stephanie Zlatic^{1*}, Amanda Freeman^{1,4*}, Avanti Gokhale^{1*} and Victor Faundez¹

¹ Department of Cell Biology, Emory University

² Graduate Program in Neuroscience, Emory University

³ Department of Chemistry, Agnes Scott College

⁴ Center for the Study of Human Health, Emory University

* These authors contributed equally

Mitochondria play crucial physiological roles throughout the body due to their involvement in energy production, calcium homeostasis, and lipid metabolism, among other functions. Mitochondrial dysfunction has been increasingly recognized in the etiology of diverse neurological disorders, but mitochondrial mechanisms contributing to disease pathogenesis remain debated. We demonstrate through immunomicroscopy and electrophysiological recording of the *Drosophila* neuromuscular junction that hemideficiency of the mitochondrial citrate transporter SLC25A1 is sufficient to alter synapse morphology and neurotransmission. Behavioral assays in *Drosophila* also demonstrated that SLC25A1 deficiency alters sleep patterns. To discern the mechanisms contributing to these synaptic phenotypes, we analyzed the proteome and transcriptome of human lymphoblast cells lacking SLC25A1 and identified increased expression of cholesterol synthesis pathway genes. Additionally, western blots from these cells showed increased levels of low-density lipoprotein receptor (LDLR), which transports exogenous cholesterol into cells. Finally, plate-based immunoassays of lymphoblasts lacking SLC25A1 revealed elevations in intracellular and secreted apolipoprotein E (ApoE), a mediator of lipid transport among cells whose isoform ApoE4 is the strongest genetic risk factor for Alzheimer's Disease. Although some findings need to be replicated in the central nervous system, we propose that SLC25A1 plays a role in synapse function in normal and disease states through lipid-dependent mechanisms.