14th Annual GDBBS DSAC Student Research Symposium

Thursday, January 19th, 2017

Cox Ballroom

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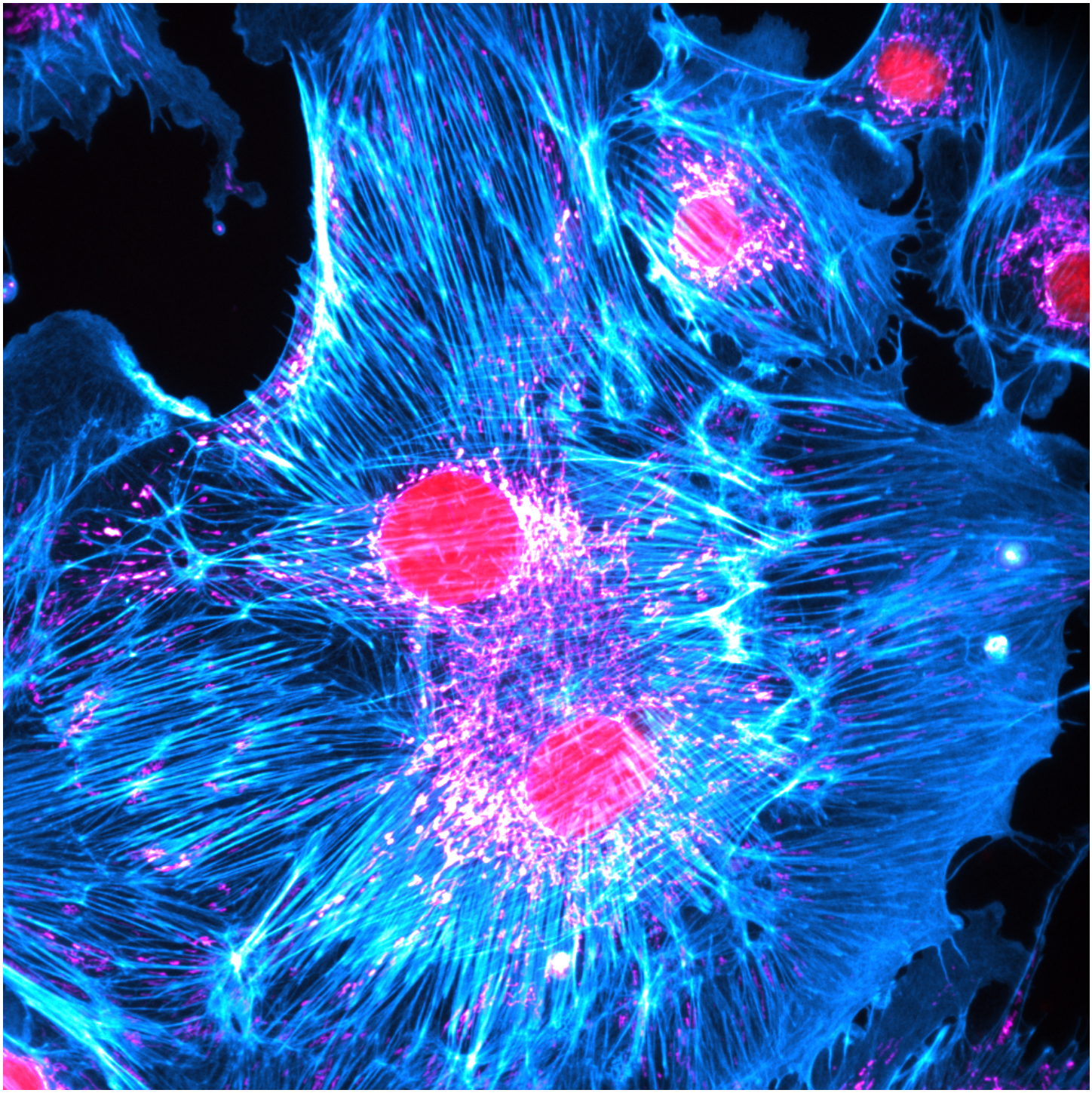
ICI Image Contest Winners

1st Place, ICI Image Contest



Stephanie Pollitt, Neuroscience

Skin cell with nuclei, mitochondria, and actin filaments labelled

The 14th Annual GDBBS DSAC Student Research Symposium
Thursday, January 19th, 2017
Cox Ballroom

8:15-8:45AM – Breakfast

Session 1: Gene Expression & Development 8:45-9:45AM

8:45 – Alicia Cutler (BCDB)
MYONUCLEAR NON-EQUIVALENCE IN NUCLEAR IMPORT PATHWAY ACTIVITY

9:00 – Gordon Dale (IMP)
GENE COVERSION IS THE DOMINANT MECHANISM OF SOMATIC HYPERMUTATION IN MICE AND HUMANS

9:15 – Marko Bajic (GMB)
INTEGRATIVE ANALYSIS OF SUBMERGENCE STRESS RESPONSE IN MEDICAGO ROOTS

9:30 – Kevin Morris (BCDB)
COORDINATION BETWEEN RNA REGULATORY FACTORS ZC3H14 AND THE THO COMPLEX IN NEURONAL MRNA PROCESSING

9:45-10:00AM – Break

Session 2: Infection 10:00-11:15AM

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IN VITRO EXPOSURE SYSTEM FOR STUDY OF AEROSOLIZED INFLUENZA VIRUS

10:15 – James Bowen (IMP)
SYSTEMS BIOLOGY UNCOVERS STAT5 ANTAGONISM BY WEST NILE VIRUS DURING INFECTION OF HUMAN DENDRITIC CELLS

10:30 – Kendra Quicke (MMG)
PLACENTAL MACROPHAGES ARE PERMISSIVE TO ZIKA VIRUS INFECTION

10:45 – Erica Harris (PBEE)
GUT MICROBE-MILKWEED PLANT INTERACTIONS AND THEIR IMPLICATIONS ON DISEASE RESISTANCE IN MONARCH BUTTERFLIES

11:00 – Ching-wen Chen (IMP)
2B4-MEDIATED COINHIBITION OF CD4+ T CELLS UNDERLIES MORTALITY IN EXPERIMENTAL SEPSIS

11:15-11:30AM – Break

Session 3: Translational Models *11:30AM-12:45PM*

11:30 – Jamie King (CB)
MECHANISMS OF TTK MODULATED EPITHELIAL TO MESENCHYMAL TRANSITION IN BREAST CANCER

11:45 – James Ross (CB)
DIFFERENTIAL EXPRESSION OF ONCOGENIC PROTEINS ACROSS TUMOR MICROENVIRONMENTS AND AT INFILTRATIVE MARGINS IN GLIOBLASTOMA

12:00 – Brittany Phillips (GMB)
RNA DECAY-MEDIATED REGULATION OF PABPN1: INSIGHT INTO THE MUSCLE-SPECIFIC BASIS OF OCULOPHARYNGEAL MUSCULAR DYSTROPHY

12:15 – Chloe Robbins (PBEE)
USING DNA METHYLATION DATA TO TEST HERITABILITY-BASED PREDICTIONS OF EVOLUTIONARY MODELS OF HUMAN AGING

12:30 – Marisa Grossman (PBEE)
INTRASPECIFIC COMPETITION DRIVES LOSS OF PYRETHROID RESISTANCE IN AE. AEGYPTI POPULATIONS

Poster Sessions & Lunch *12:45-2:15PM*

12:45-1:30 - Odd-Numbered Poster Presentations
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2:30 – Perez Diaz (NS)
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2:45 – Katherine Squires (MSP)
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3:00 – Madelyn Houser (IMP)
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3:15 – Cameron Herting (MSP)
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3:30 – Lauren Shapiro (MSP)
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Session 5: Immunity *4:00-5:15PM*

4:00 – Elizabeth Littauer (MMG)
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4:15 – Yanjun Feng (IMP)
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4:30 – Travis Rotterman (NS)
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4:45 – Emily Woods (MMG)
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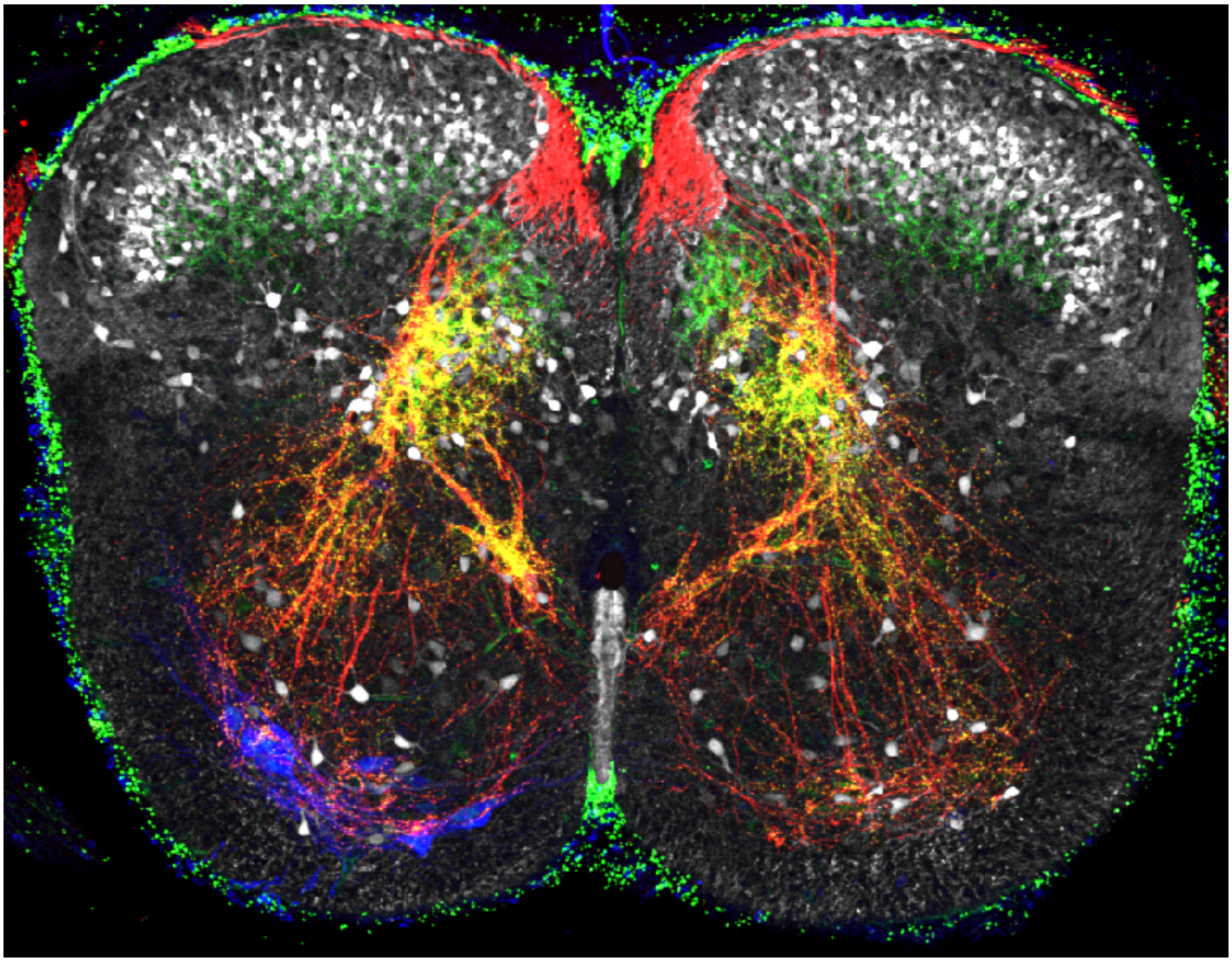
5:00 – Jenny Cosby (IMP)
BACTERIAL DELIVERY OF MOG EPITOPE SUPPRESSES AUTOIMMUNE DEMYELINATING DISEASE VIA MOG-SPECIFIC TOLERANCE MECHANISMS

Reception and Awards *5:15-6:30PM*

Oral Presentation Abstracts



2nd Place, ICI Image Contest
Travis Rotterman, Neuroscience

Postnatal day 5 spinal cord transverse section from a mouse model of spinal muscle atrophy (SMA). Motoneurons in the spinal cord were retrogradely labeled (blue) with a fluorescent tracer. Immunohistochemistry was used to identify primary afferent inputs (parvalbumin, red), vesicular glutamate transporter isoform 1 (VGLUT1, green), and an inhibitory interneuron population (calbindin, white).

Session 1:
Gene Expression & Development
8:45AM

Alicia Cutler, BCDB **8:45AM**

MYONUCLEAR NON-EQUIVALENCE IN NUCLEAR IMPORT PATHWAY ACTIVITY

Alicia Cutler[1], Jennifer Jackson[2], Anita Corbett[3], Grace Pavlath[4]

[1] Biochemistry Cell and Developmental Biology Graduate Program, Emory University, [2] Clinical Surgery, Boston University, [3] Biology Department, Emory University, [4] Pharmacology Department, Emory University.

Regional differences in transcription and protein expression within multinucleated myofibers are critical for proper muscle function. Regulation of import pathway activity in individual nuclei could be a mechanism to individually regulate transcription in nuclei within myofibers. Import into the nucleus is facilitated by nuclear transport receptors which recognize distinct nuclear localization signals (NLS) in the cargo protein and enter the nucleus through discrete import pathways. We examined import through the classical NLS pathway in primary mouse myotubes *in vitro* and found differences among myonuclei within a single cell. We further compared the activity of four well-defined pathways and identified three subsets of nuclei: nuclei with more than one active import pathway, nuclei with one detectable active import pathway, and nuclei with no detectable import. The classical NLS (cNLS) pathway was the predominant import pathway examined. We examined differences in cNLS reporter import among myonuclei across different stages of myogenesis to determine when differences among myonuclei are established. We found that cNLS import was high in myoblasts, fell drastically in myocytes, and gradually increased again in myotubes. Together, our results suggest that spatial and temporal regulation of distinct nuclear import pathways may be important in myofiber differentiation and regionalization.

Gordon Dale, IMP **9:00AM**

GENE COVERSION IS THE DOMINANT MECHANISM OF SOMATIC HYPERMUTATION IN MICE AND HUMANS

Gordon A. Dale[1]**,** Caitlin D. Bohannon[1], Daniel J. Wilkins[1], Dario Dilernia[1], Eric Hunter[1], Trevor Bedford[2], Rustom Antia[3], Ignacio Sanz[4], and Joshy Jacob[1*]

[1]Emory Vaccine Center, Yerkes National Primate Center, Emory University, Atlanta, GA, USA; [2]Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA, USA; [3]Department of Biology, Emory University, Atlanta, GA, USA; [4]Lowance Center for Human Immunology, Department of Medicine, Emory University, Atlanta, GA, USA.

Affinity maturation – the progressive increase in antibody affinities – is a hallmark of humoral immunity. Somatic hypermutation generates a plethora of antibody mutants in antigen-specific B cells, including those with mutations in immunoglobulin frameworks. Survival of mutants is dependent on the functional preservation of the immunoglobulin framework as well as the increasingly fine specificity of the complementarity determining regions (CDRs) to antigen during selection. Here we show that murine somatic mutations are introduced via gene conversion from other immunoglobulin gene segments from either the cis or trans allele. Similarly, analysis of two recent human immunoglobulin data sets reveals that a majority of mutations are traceable to other immunoglobulin gene segments. A subsequent analysis of the recently reported broadly-neutralizing, anti-malarial human antibodies with a LAIR1 insert revealed that gene conversion can also occur within the functional antibody rearrangement. Together this implies that the broad diversity generated in a humoral response is templated and genetically restricted. Further, this suggests that gene conversion allows B lymphocytes to maintain the integrity of the framework while simultaneously allowing selection for rare CDR mutants with increased affinity.

Marko Bajic, GMB **9:15AM**

INTEGRATIVE ANALYSIS OF SUBMERGENCE STRESS RESPONSE IN MEDICAGO ROOTS

Marko Bajic[1], Katie Hatch[2], and Roger Deal[2]

[1]Program of Genetics and Molecular Biology in the Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA; [2]Department of Biology, Emory University, Atlanta, GA

Plants are stationary organisms that must constantly respond to a changing environment in order to survive. Primary detection and response to flood stress occurs in the roots. However, how the specific cell types of the roots respond to these stresses is not completely understood. We have utilized two techniques, INTACT (Isolation of Nuclei TAgged in specific Cell Types) and TRAP (Tagged Ribosome Affinity Purification), to isolate nuclei and translating ribosomes from specific cell types of the plant *Medicago truncatula*, a legume model organism. We used ATAC-seq (Assay for Transposase-Accessible Chromatin sequencing) to characterize chromatin accessibility changes in *Medicago* root tips in response to 2 hours of submergence. Additionally, we performed RNA sequencing using nuclear RNA, total mRNA, and translating mRNA to characterize the transcriptional and translational response to submergence stress. Here we report the chromatin regions that are regulated in response to submergence stress. Additionally, we summarize how the response to hypoxia is regulated at the level of transcription and translation and how the targets of regulation compare between Medicago, tomato, and rice. The long-term goal of this research is to establish a comprehensive understanding of drought and flood stress response in crops and to use this information to develop hardier crops.

Kevin Morris, BCDB **9:30AM**

COORDINATION BETWEEN RNA REGULATORY FACTORS ZC3H14 AND THE THO COMPLEX IN NEURONAL MRNA PROCESSING

Kevin Morris[1], Nicholas Seyfried[2], and Anita Corbett[1].

Biochemistry, Cell and Developmental Biology Graduate Program, Emory University Laney Graduate School, Dept. of Biology[1], Emory University School of Medicine Proteomics Core[2], Emory University, Atlanta, GA.

Proper eukaryotic gene expression relies on the successful execution of nuclear RNA processing events coupled to efficient export of the resulting mRNPs. Defects in this process can lead to a wide range of disease. Our work focuses on understanding how inactivating mutations in the *ZC3H14* gene, which encodes a multifaceted yet understudied polyadenosine RNA-binding protein, lead to intellectual disability. Although ZC3H14 is ubiquitously expressed, the patients only display a non-syndromic form of intellectual disability. To better understand this enigma, we employed a proteomic approach to elucidate the role of ZC3H14 in the brain. In doing this approach, we discovered an interaction network between ZC3H14 and other RNA regulatory factors. Most noticeably, we discovered and have characterized an interaction between ZC3H14 and the mRNA processing complex, THO. Interestingly, inactivating mutations in genes encoding components of the THO complex also cause brain defects. Through our work, we have shown not only that these factors interact in the brain but that they coordinate the nuclear processing and export of critical neuronal transcripts. These factors influence the steady-state levels of transcripts possibly by influencing mRNA quality control, as defects in RNA processing emerge from depletion of these genes. Taken together, these results suggest a novel pathway for coordination of mRNA processing events that are critical for neuronal function.

Session 2:

Infection

10:00AM

Hannah Creager, MMG **10:00AM**

IN VITRO EXPOSURE SYSTEM FOR STUDY OF AEROSOLIZED INFLUENZA VIRUS

Hannah M. Creager[1,2], Hui Zeng[1], Joanna A. Pulit-Penaloza[1], Taronna R. Maines[1] Terrence M. Tumpey[1], Jessica A. Belser[1].

[1]Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA; [2]Microbiology and Molecular Genetics Graduate Program, Emory University, Atlanta, GA

Infection of adherent cell monolayers using a liquid inoculum represents an established method to reliably and quantitatively study virus infection, but poorly recapitulates the exposure and infection of cells in the respiratory tract that occurs during natural infection with aerosolized pathogens. We therefore sought to develop methodology to expose adherent mammalian cell monolayers to defined quantities of aerosolized influenza virus. In this study, we utilized a system previously shown to generate aerosols similar to those exhaled by infected humans. Calu-3 (human bronchial epithelial) cell cultures were placed inside an exposure chamber after removal of apical media and inoculated with ten-fold serial dilutions of aerosolized influenza viruses with distinct transmission phenotypes: one HPAI A(H5N1), one LPAI A(H7N9), and one seasonal A(H3N2) virus. All viruses were highly infectious under these conditions, replicating to high titer after exposure to fewer than five infectious virions. Growth of monolayers under air-liquid interface for three weeks before exposure to facilitate further cell differentiation and mucus production reduced viral infectivity up to 25-fold. By facilitating study of viral infectivity under conditions similar to those of natural infection and transmission, the methods described here have the potential to enhance our understanding of respiratory viruses.

James Bowen, IMP **10:15AM**

SYSTEMS BIOLOGY UNCOVERS STAT5 ANTAGONISM BY WEST NILE VIRUS DURING INFECTION OF HUMAN DENDRITIC CELLS

***James R Bowen*[1,2], Circe McDonald[1,2], and Mehul S Suthar[1,2]**

[1]Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA 30322, USA; [2]Emory Vaccine Center, Yerkes National Primate Research Center, Atlanta, GA 30329, USA.

West Nile virus (WNV) is a neurotropic Flavivirus and the leading cause of mosquito-borne encephalitis in the United States. Studies in mice have found dendritic cells (DCs) are pivotal for viral control and programming of antiviral immunity in a RIG-I like receptor (RLR) and type I interferon (IFN) dependent manner. To define the antiviral landscape during human WNV infection, we performed mRNA sequencing on infected monocyte DCs (moDCs) and found, in contrast to RLR and IFNβ signaling, WNV infection triggered a limited repertoire of antiviral response related genes. Despite induction of IFNβ during infection, we observed minimal expression of cytokines (IL-4, IL-12, IL-7, IL-15) or costimulatory molecules (CD80, CD86, CD40) that promote T cell immunity. Using weighted gene co-expression network analysis combined with cis-regulatory sequence analysis, we identified STAT5 as a regulatory node downstream of RLR and IFNβ signaling. Intriguingly, STAT5 signaling was not induced during WNV infection, and RIG-I agonist or IFNβ treatment failed to phosphorylate STAT5 in the presence of WNV infection. Combined, our data suggests that WNV dampens DC activation by antagonism of STAT5 signaling. Further studies are underway to better understand how RLR signaling promotes STAT5 signaling and to define the viral factors that antagonize STAT5.

Kendra Quicke, MMG **10:30AM**

PLACENTAL MACROPHAGES ARE PERMISSIVE TO ZIKA VIRUS INFECTION

***Kendra M. Quicke*[1,2], James R. Bowen[1,2]**, Erica L. Johnson[1], Circe E. McDonald[1,2], Huailiang Ma[2,3], Justin T. O'Neal[1,2], Augustine Rajakumar[4], Jens Wrammert[1,2], Bassam H. Rimawi[4], Bali Pulendran[2,3], Raymond F. Schinazi[1,5,], Rana Chakraborty[1], Mehul S. Suthar[1,2]

[1]Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, USA; [2]Emory Vaccine Center, Yerkes National Primate Research Center, Atlanta, GA, USA; [3]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA; [4]Department of Gynecology and Obstetrics, Division of Maternal Fetal Medicine and Reproductive Infectious Diseases, Emory University School of Medicine, Atlanta, GA, USA; [5]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Emory University School of Medicine, Atlanta, GA, USA.

The Zika virus (ZIKV) outbreak in Brazil has been directly linked to increased cases of microcephaly in newborns. Studies have identified viral RNA and viral particles in amniotic fluid, and fetal and newborn brain tissue. Additionally, pathology and viral RNA have been detected in placenta of mothers with reported ZIKV infection during pregnancy. These observations suggest ZIKV is capable of crossing this normally protective barrier. However, the mechanism of intrauterine transmission and the cell types involved remain unknown. Our work discovered productive infection of primary human placental macrophages (Hofbauer cells), and to a lesser extent cytotrophoblasts, which help form the placental lining. We demonstrate that ZIKV PRVABC59, a closely related strain to the epidemic strain in the Americas, infects and replicates in Hofbauer cells and may replicate in cytotrophoblasts with delayed kinetics. Viral replication coincides with induction of type I interferon, pro-inflammatory cytokines, and antiviral gene expression with little to no loss in cell viability. These findings will inform further studies aimed at understanding intrauterine transmission of ZIKV. Additionally, determining target cells of ZIKV replication and the antiviral responses induced during infection may aid development of antiviral therapies that target ZIKV at the placenta to limit infection of the fetus.

Erica Harris, PBEE **10:45AM**

GUT MICROBE-MILKWEED PLANT INTERACTIONS AND THEIR IMPLICATIONS ON DISEASE RESISTANCE IN MONARCH BUTTERFLIES

***Erica V. Harris*[1]**, Nicole M. Gerardo[1], and Jacobus C. de Roode[1]

[1] Department of Biology, O. Wayne Rollins Research Center, Emory University

There is increasing evidence that diet modulates gut-associated communities across the animal kingdom and that the gut microbiota affects disease resistance. Here, we use monarch butterflies (*Danaus plexippus*), their larval food plants (*Asclepias* spp.), and protozoan parasites (*Ophryocystis elektroscirrha*) to examine diet's interaction with the gut microbiota and natural parasite infection. Milkweeds with higher cardenolide concentrations reduce larval parasitic infection. However, the mechanism of protection is unknown. One possible mechanism is that the larval food plants affect the gut microbiota, which then reduce parasite growth and probability of infection. To test this hypothesis, the gut microbial communities of caterpillars fed on high-cardenolide food plants were characterized and manipulated. We compared the gut communities of caterpillars reared on two milkweed species (*A. incarnata* and *A. curassavica*) varying in cardenolide concentration. We dissected the larval midgut, and extracted, amplified, and sequenced the 16s rRNA gene. The comparison of gut bacterial communities of caterpillars fed on alternative milkweed diets revealed that the midgut bacterial community composition of uninfected caterpillars significantly differs based on the host plant species (ANOSIM, $R=0.2494$, $p<0.001$). We capitalized on these community differences manipulating the microbiota via fecal transplants and measuring parasite sporeload.

Ching-wen Chen, IMP **11:00AM**

2B4-MEDIATED COINHIBITION OF CD4+ T CELLS UNDERLIES MORTALITY IN EXPERIMENTAL SEPSIS

***Ching-wen Chen*[1,2,3],** Rohit Mitta[1]; Nathan J. Klingensmith[1]; Eileen M. Burd[4]; Greg S. Martin[5]; Craig M. Coopersmith[1,3]; Mandy L. Ford[1,2].

[1]Department of Surgery; [2]Emory Transplant Center; [3]Emory Critical Care Center; [4]Department of Pathology and Laboratory Medicine; [5]Division of Pulmonary, Allergy, Critical Care and Sleep, Department of Internal Medicine, Emory University, Atlanta, GA 30322

Sepsis is a leading cause of death in the U.S. yet no FDA-approved therapy for sepsis exists. Sepsis-related immune-incompetence is characterized by profound lymphocyte death and immune dysfunction. However, the mechanisms underlying lymphocyte loss of functionality remain poorly understood. 2B4 is a lymphocyte cosignaling molecule, mainly known to be expressed on NK cells and memory $CD8^+$ T cells, that has been shown to regulate T cell function in models of viral infection. Here we show that 2B4 signaling mediates sepsis lymphocyte dysfunction via its action on $CD4^+$ T cells, and further that disrupting 2B4 signaling improves sepsis survival. We observed that 2B4 expression is increased on $CD4^+$ T cells in both septic animals and human patients at early time points. Importantly, genetic loss or pharmacologic inhibition of 2B4 both significantly increased survival in a murine CLP sepsis model. We employed CD4- and CD8-specific conditional knockouts to show that 2B4 functions on $CD4^+$ T cell populations and modulates both adaptive and innate immune responses during sepsis. Our results illuminate a novel role for 2B4 coinhibitory signaling on $CD4^+$ T cells in mediating immune dysregulation, and overall suggest that reversing T cell dysfunction via the 2B4 pathway can improve survival during sepsis.

Session 3:

Translational Models

11:30AM

Jamie King, CB **11:30AM**

MECHANISMS OF TTK MODULATED EPITHELIAL TO MESENCHYMAL TRANSITION IN BREAST CANCER

Jamie L. King[1,2], Baotong Zhang PhD[1], Jin-Tang Dong PhD[1]

[1]Department of Hematology Oncology, Winship Cancer Institute, Emory University School of Medicine
[2]Cancer Biology Graduate Program, Emory University School of Medicine

TTK (MPS1) kinase has established roles in DNA repair, centriole duplication and spindle assembly. Additionally, TTK is overexpressed at the protein level in mesenchymal triple negative (TN) tumors and cell lines, which have undergone the epithelial to mesenchymal transition (EMT). Increased TTK expression is correlated with increased migration and metastasis. Upon silencing TTK expression in TN cells, we noted morphological and EMT marker changes indicative of reversion to an epithelial phenotype. We also noted an upregulation of the KLF5 transcription factor, an important molecule involved in EMT, upon TTK silencing. We hypothesize that TTK overexpression facilitates EMT through modulating KLF5 and micro-RNA 21 (miR-21) expression. In studying the signaling pathways altered upon TTK silencing, we have observed increased KLF5 expression but decreased miR-21 expression in parallel with loss of mesenchymal markers in TN cells. We have also observed selective decreases in SMAD3 signaling upon loss of TTK. Ongoing studies will examine how restoration of TTK variants (ex. Kinase dead vs. wild type) or restoration of miR-21 or KLF5 impacts the reversion to epithelial status in TN cells with loss of TTK. These studies will reveal novel mechanisms of metastatic signaling pathways for TTK in TN breast cancer.

James Ross, CB **11:45AM**

DIFFERENTIAL EXPRESSION OF ONCOGENIC PROTEINS ACROSS TUMOR MICROENVIRONMENTS AND AT INFILTRATIVE MARGINS IN GLIOBLASTOMA

James L. Ross[1,7], Lee AD Cooper[2,5,6,7,8], David Gutman[3,5,6],Merete Williams[1], Carol Tucker-Burden[1], Myles R. McCrary[6,8], Alexandros Bouras[9], Milota Kaluzova[4], William D. Dunn Jr.[2], Duc Duong[3], Constantinos G. Hadjipanayis[9], Daniel J. Brat[1,2,5,6]

[1]Departments of Pathology and Laboratory Medicine, [2]Biomedical Informatics, [3]Neurology, [4]Pediatrics, [5]Winship Cancer Institute, [6]Emory University School of Medicine, [7]Emory University Graduate Program in Cancer Biology, [8]Biomedical Engineering, Emory University / Georgia Institute of Technology, Atlanta, GA 30322.

Glioblastoma is highly heterogeneous and contains diverse microenvironments with uneven distributions of oncogenic alterations and signaling networks. Protein expression levels, including those of potential therapeutic targets, vary across environments and optimal treatments will require an understanding of these differential patterns. The diffusely infiltrative properties of GBM result in residual tumor at neurosurgical resection margins that is the source of relapse in nearly all cases, suggesting that therapeutic efforts should be focused here. To identify signaling networks and potential druggable targets across microenvironments and, in particular at tumor margins, we utilized 5-ALA fluorescence-guided surgical resection, followed by proteomic analysis of specific tumor regions. Reverse phase protein array was performed on 205 proteins isolated from the tumor margin, tumor bulk, and perinecrotic regions of 13 high-grade gliomas. We identified 37 proteins differentially expressed across microenvironments. Proteins strongly upregulated at the margin included protein kinases in the PI3K/Akt/mTOR pathway; among the most highly expressed were activated Akt(pT308) and Tyro3, which were both found to be specific to neoplastic cells by immunohistochemistry. Plasminogen Activator Inhibitor 1 (*SERPINE1*) was highly upregulated in perinecrotic regions, likely related to pro-thrombotic mechanisms. Our studies demonstrate that GBM tumor microenvironments have distinct protein expression profiles that may be biologically and therapeutically relevant.

Brittany Phillips, GMB **12:00PM**

RNA DECAY-MEDIATED REGULATION OF PABPN1: INSIGHT INTO THE MUSCLE-SPECIFIC BASIS OF OCULOPHARYNGEAL MUSCULAR DYSTROPHY

Brittany L Phillips[1], Luciano Apponi[1], Hyojung Choo[1], Ayan Banerjee[2], Anita H Corbett[2] and Grace K Pavlath[1]

[1]Department of Pharmacology, Emory University School of Medicine; [2]Department of Biology, Emory University

RNA binding proteins are critical for ensuring proper gene expression. Although many genes encoding RNA binding proteins are ubiquitously expressed, mutations in these genes often result in tissue-specific pathology. The ubiquitous poly(A) binding protein, PABPN1, plays well-characterized roles in polyadenylation. A GCN expansion mutation in a single copy of *PABPN1,* causing a modest Alanine expansion in the PABPN1 N-terminus, results in the muscle-specific disease Oculopharyngeal muscular dystrophy (OPMD). How this mutation leads to muscle-specific disease is unknown. Determining the unique properties of PABPN1 in muscle that predispose this tissue to OPMD pathology is critical for developing therapies. We have discovered that muscle-specific destabilization of *Pabpn1* mRNA contributes to low PABPN1 protein levels in muscle. These low PABPN1 levels may make muscle susceptible to mutations that reduce PABPN1 protein function while higher PABPN1 levels in non-muscle may compensate for reduced function. This muscle-specific regulation is mediated through the *Pabpn1* 3'UTR which contains an AU-rich element (ARE) that, when mutated, ameliorates muscle-specific regulation. The RNA binding protein HuR interacts with the *Pabpn1* 3'UTR and influences *Pabpn1* levels. These results show that tissue-specific regulation of RNA decay can underlie human disease and begin to dissect the key factors that mediate this regulation.

Chloe Robbins, PBEE **12:15PM**

USING DNA METHYLATION DATA TO TEST HERITABILITY-BASED PREDICTIONS OF EVOLUTIONARY MODELS OF HUMAN AGING

Chloe Robins[*†], Allan F McRae[‡***], Joseph E Powell[‡††], Howard W Wiener[§], Stella Aslibekyan[§], Elizabeth M. Kennedy[*], Devin M Absher[‡‡], Donna K Arnett[§§], Peter M Visscher[‡††], Grant W Montgomery[***], David J Cutler[*], Karen N Conneely[*]

[*]Department of Human Genetics, Emory University School of Medicine, [†]Graduate Program in Population Biology, Ecology, and Evolution, Laney Graduate School, Emory University, [‡]The Queensland Brain Institute, University of Queensland, [§]Department of Epidemiology, University of Alabama at Birmingham, [**]University of Queensland Diamantina Institute, University of Queensland, Translational Research Institute (TRI), [††]The Institute for Molecular Bioscience, University of Queensland, [‡‡]Hudson Alpha Institute for Biotechnology, [§§]College of Public Health, University of Kentucky, [***]QIMR Berghofer Medical Research Institute

The evolutionary theories of mutation accumulation (MA) and disposable soma (DS) provide possible explanations for the existence of human aging. To better understand the relative importance of these theories, we devised a test to identify MA- and DS-consistent sites across the genome using DNA methylation (DNAm) data. Two key characteristics of DNAm allowed us to do so. First, DNAm exhibits distinct and widespread changes with age, such that numerous sites across the genome are age-differentially-methylated (aDM). Second, many sites show heritable DNAm patterns within families. We extended heritability predictions of MA and DS to DNAm, predicting that MA-consistent DNAm sites will show increasing heritability with age, while DS-consistent sites will show the opposite. Variance components models were used to test for changing heritability of methylation with age at 48,601 aDM sites across the genome. 102 sites showed significant MA-consistent increases in heritability with age, while 2,266 showed significant DS-consistent decreases in heritability with age. These results suggest that both MA and DS play a role in explaining aging and aging-related changes, and that while the majority of DNAm changes observed in aging are consistent with random epigenetic drift, targeted changes exist and may mediate the effects of age-specific genes.

INTRASPECIFIC COMPETITION DRIVES LOSS OF PYRETHROID RESISTANCE IN AE. AEGYPTI POPULATIONS

***Marissa Grossman*[1]**, Valentin Uc-Puc[2], Adriana Flores[3], Pablo Manrique Saide[2], Gonzalo Vazquez-Prokopec[1]

[1]Emory University, [2]Universidad Autonoma de Yucatan, [3]Universidad Autonoma de Nuevo Leon

Control strategies for *Aedes aegypti* are being threatened by the evolution of "knock-down resistance," or *kdr*, which consists of point mutations in a sodium channel gene. These mutations may carry a fitness cost or reduce competitive ability when individuals are subjected to density dependent competition. To investigate these costs, we conducted experiments with susceptible and resistant field populations of *Ae aegypti*. We placed first instar larvae from each population into 2L buckets at two densities, 50 and 500 larvae, and created a third population consisting of a 50/50 mix of individuals from each population. We measured development time, immature survival, fecundity, phenotypic resistance, and *kdr* allele frequencies. The resistant population laid 4.3 times fewer eggs than the susceptible population, suggesting a significant fitness cost. More importantly, the insecticide assay revealed that individuals from the resistant population in the high density treatment were no longer resistant—93% were knocked-down, which is considered susceptible. Additionally, the frequency of one *kdr* allele in the resistant population at high density significantly decreased from 0.98 to 0.69 in only one generation of selection. Our results suggest that future control strategies could exploit the fitness and energetic cost of *kdr* to regain susceptibility into populations.

Session 4:

Neuroscience

2:30PM

Perez Diaz, NS **2:30PM**

THE ROLE OF THE SEROTONIN 5-HT_{2C} RECEPTOR IN COMPULSIVE AND ADDICTIVE BEHAVIORS

Perez Diaz, M.[1], Howell, L. L.[1,2] and M. E. Wilson[1,3]

[1]Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA, [2]Division of Neuropharmacology and Neurologic Diseases, Emory University, Atlanta, GA, USA, [3]Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, USA

Compulsivity, defined as a general inability to alter behavior with changing reinforcement contingencies, appears to be a core behavioral feature of addiction. However, it is unclear whether compulsivity is predictive of an individual's tendency to engage in addictive behavior or whether engaging in addictive behavior increases compulsivity. Serotonin 5-HT_{2C} receptor agonists decrease food consumption and reduce self-administration of psychostimulants, but no one has evaluated whether they can also reduce compulsive behavior. We measured compulsivity using a Discrimination Reversal Learning task prior to and after 6-month exposure to a highly palatable diet (food group) or methamphetamine self-administration (drug group). Prolonged exposure to both led to an increase in compulsivity and greater food or methamphetamine intake was predictive of larger increases in compulsivity. We also determined the effects of the selective 5-HT_{2C} receptor agonist WAY163909 (WAY) on compulsivity, food and METH intake. WAY treatments attenuated compulsivity and reduced consumption of the highly palatable diet and METH intake. Our study sheds light on the relationship between reinforcer intake and compulsivity and demonstrates the modulatory role that 5-HT_{2C} receptors play in these behaviors. This research was supported by USPHS Grants DA10344 (LLH), DA31246 (LLH), DK096983 (MEW) and P51OD11132 (Yerkes National Primate Research Center).

Katherine Squires, MSP **2:45PM**

ASHKENAZI HUMAN VARIANT IN REGULATOR OF G PROTEIN SIGNALING 14 (RGS14) DISRUPTS RGS14 SUBCELLULAR LOCALIZATION AND INTERACTION WITH BINDING PARTNERS

Katherine E Squires[1,3], Joshua L Traynelis[2], Serena Dudek[3], and John R Hepler[4].

[1]Molecular & Systems Pharmacology, [2]Emory College of Arts and Sciences, [3]NIEHS, [4]Department of Pharmacology

Long-term potentiation (LTP), the molecular correlate of learning and memory, is expressed throughout the hippocampus except for a small, understudied region known as CA2. Our previous studies have shown that a regulator of G protein signaling, RGS14, is a natural suppressor of: 1) LTP in CA2 neurons, and 2) hippocampal-based learning and memory. Further, RGS14 localizes to postsynaptic spines where LTP occurs. RGS14 contains various signaling domains, including a GPR motif that binds $G\alpha_{i1}$-GDP to form a stable signaling complex at the plasma membrane. Disruption of this complex reverses RGS14 suppression of LTP. Notably, the human population carries many missense variants of RGS14, one of which (R507Q) is in the GPR motif and found almost exclusively within the Ashkenazi Jewish population. We find that R507Q partially disrupts RGS14 binding to $G\alpha_{i1}$-GDP. The RGS14 GPR motif also contains a nuclear export sequence that regulates cytoplasmic-nuclear shuttling of RGS14. We find that R507Q targets RGS14 to the nucleus, likely by disrupting RGS14 interactions with a novel nuclear binding partner, Exportin 1. Taken together, these findings suggest that this Ashkenazi variant may confer a change in synaptic plasticity, and by extension, learning and memory.

Madelyn Houser, IMP **3:00PM**

EVALUATION OF GASTROINTESTINAL INFLAMMATION IN PARKINSON'S DISEASE

Madelyn C. Houser[1], Rema J. Henry[2], Jianjun Chang[1], Stewart A. Factor[3], Kathleen M. Shannon[4], Erin Hill-Burns[5], Haydeh Payami[5,6], Vicki S. Hertzberg[2], Malú G. Tansey[1]

[1]Department of Physiology, Emory University School of Medicine; [2]Center for Nursing Data Science, Nell Hodgson Woodruff School of Nursing, Emory University; [3]Department of Neurology, Emory University School of Medicine; [4]Department of Neurology, University of Wisconsin School of Medicine and Public Health; [5]Department of Neurology, University of Alabama at Birmingham, AL; [6]HudsonAlpha Institute for Biotechnology, Hunstville, AL

Parkinson's disease (PD) is a progressive disorder that currently is not diagnosed until characteristic motor impairments appear, at which time extensive, irreversible neurodegeneration has already occurred. An emerging paradigm of PD suggests that an extended prodromal period which includes gastrointestinal (GI) disease manifestations could present the opportunity for earlier diagnosis and intervention. We predicted that evaluation of intestinal inflammatory mediators could provide mechanistic information about PD-related GI dysfunction and possibly identify valuable early-stage biomarkers. We observed trends for PD-associated increases in levels of immune and angiogenesis factors in stool and significant indicators of inflammation in colonic biopsies from PD patients. PD patients reported anxiety, sleep disorders, and digestive problems more frequently than controls, and we detected associations between digestive problems and factors related to T cell recruitment. Interestingly, household controls reported digestive problems more frequently than non-household controls and exhibited similar tissue inflammatory markers. A history of smoking, known to affect PD risk, was associated with reductions in levels of multiple stool analytes. Our findings suggest that intestinal inflammation persists in PD, is associated with digestive problems and other non-motor manifestations of the disease, and could act as a key driver of neurodegeneration.

Cameron Herting, MSP **3:15PM**

MODELING ADULT PRONEURAL AND MESENCHYMAL GLIOBLASTOMA USING RCAS/TV-A TECHNOLOGY

Cameron Herting1 and Dolores Hambardzumyan[2]

[1] Molecular and Systems Pharmacology, Emory University School of Medicine; [2] Pediatric Hematology and Oncology, Emory University School of Medicine

Glioblastoma (GBM) is the most common and aggressive malignant glioma, characterized by genetic heterogeneity, resistance to treatment, and dismal prognosis. The bulk of research in GBM biology utilizes passaged cell lines and xenograft models that may neglect some aspects of the tumor-stroma interactions, particularly the actions of the immune system. In order to advance exact research into GBM tumorigenesis, progression and treatment, we utilized RCAS/t-va technology to develop mouse models of adult proneural and mesenchymal GBM. The proneural model was generated by overexpression of platelet-derived growth factor (PDGFB) while the mesenchymal model was generated by co-injecting RCAS-shNf1, RCAS-shp53 and RCAS-Cre into the subventricular zone of adult Ntv-a; Ink4a-Arf -/- ;Ptenfl/fl mice. Immunohistochemical analysis indicates the mesenchymal subtype displays a significantly higher proportion of activated macrophage and microglia within the tumor as measured by staining for the macrophage marker Iba1. Additionally, CD31 staining shows significantly higher vessel coverage and larger average vessel size in the proneural subtype. Stainings for Olig2, GFAP and CD44 mirror human data, supporting the validity of these models. Future analysis will elucidate additional subtype-specific molecular markers, differential gene expression and cellular characteristics and will allow for the development and accurate preclinical testing of subtype-specific targeted therapies.

Lauren Shapiro, MSP **3:30PM**

RHO-KINASE INHIBITION HAS ANTIDEPRESSANT-LIKE EFFICACY IN ADOLESCENCE MICE

***Lauren P. Shapiro*[1,2]**, Shannon L. Gourley[2,4,]

[1]Molecular and Systems Pharmacology, Emory University; [2]Department of Pediatrics, Emory University School of Medicine; Yerkes National Primate Research Center; [3]Graduate Program in Neuroscience, Emory University; [4]Department of Psychiatry, Emory University School of Medicine

Adolescence represents a critical period of neurodevelopment, defined by structural reorganization and synaptic maturation within the prefrontal cortex. Although these processes are critical for the transition to adulthood, structural instability may open a window of vulnerability to neuropsychiatric disorders including depression. Interventions that facilitate activity-dependent neural remodeling, as occurs during adolescence, may be advantageous. Here we evaluated the therapeutic-like potential of Rho-kinase (ROCK) inhibition, which can expedite activity-dependent dendritic spine plasticity. The brain-penetrant ROCK inhibitor fasudil had antidepressant-like effects in the forced swim test in adolescent mice and was comparable to ketamine and fluoxetine. Fasudil also decreased the latency to approach a palatable food in the novelty suppressed feeding task, a rapid antidepressant-like effect. Within the adolescent ventromedial prefrontal cortex (vmPFC), fasudil increased levels of the post-synaptic marker PSD-95, while pruning dendritic spines, resulting in adult-like spine densities. Fasudil stimulated several neurotrophin-related signaling factors in the vmPFC, including increasing the ratio of full-length:truncated tyrosine kinase receptor B (TrkB). Subsequent experiments utilizing viral vector-based and pharmacological manipulations indicated, however, that the antidepressant-like actions of fasudil are nonetheless attributable to the inhibition of the neuronal ROCK isoform, ROCK2.

Session 5:

Immunity

4:00PM

Elizabeth Littauer, MMG **4:00PM**

PREGNANCY MODULATES CELLULAR IMMUNE RESPONSES TO H1N1 INFLUENZA INFECTION

Elizabeth Q. Littauer[1,2], E. Stein Esser[1], Olivia Q. Antao[1,] Dahnide T. Williams[1], Richard W. Compans[1,2], and Ioanna Skountzou[1,2]

Department of Microbiology and Immunology[1], Emory Vaccine Center[2], Emory University, Atlanta, Georgia, USA

The 2009 H1N1 influenza pandemic demonstrated that pregnant women infected with influenza were at increased risk for severe respiratory distress, high incidence of hospitalization, preterm births and low birthweight neonates. We utilize a BALB/c pregnant mouse model which recapitulates clinical phenotypes shown during influenza infection of pregnant women. Pregnant mice sublethally infected ($0.5xLD_{50}$) with seasonal H1N1 A/California/07/09 exhibited reduced serum influenza-specific antibody avidity and HAI titers compared to infected non-pregnant controls despite equivalent activation of H1N1-specific antibody secreting cells (ASC). Pregnancy did not alter the frequency of activation of IL-4 and IFN-γ secreting T lymphocytes following H1N1 infection. Maturation of germinal center (GC)+ B cells in the spleen and migration kinetics of plasma cells from the spleen to the lungs were decreased in pregnant mice across time points 14 days post infection (d.p.i.). However, pregnancy increased the frequency of influenza-specific IgA ASC in the lungs 42 d.p.i. While pregnancy reduced the frequency of lung-resident CD4+ T cells, H1N1 A/Ca/07/09-specific IFN-γ secreting cells were increased in the lungs 42 d.p.i. These data suggest that the condition of pregnancy enhances cellular responses in the mucosa, perhaps to balance immunotolerance of fetal antigen or to improve maternal transfer of antibodies through breastmilk.

Yanjun Feng, IMP **4:15PM**

NK CELL IN CASP8-DEFICIENT MICE ENHANCES VIRUS-SPECIFIC T CELL ACCUMULATION

Yanjun Feng[1], Lisa Daley-Bauer[1], Linda Roback[1], Edward Mocarski[1]

[1]Department of Microbiology and Immunology, Emory University, Atlanta, GA 30322

Cell autonomous death is balanced against survival signaling to establish T cell levels during viral infection. It is known that mitochondrial cell death contributes to T cell contraction; however, the question remains about the role of extrinsic cell death in the T cell response. Caspase8 (Casp8) initiates extrinsic cell apoptosis while also suppresses RIP3-mediated necroptosis. We have employed wild type (WT), *Ripk3*$^{-/-}$ and *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ (DKO) mice to address the contribution of Casp8 in the antiviral T cell response to murine cytomegalovirus (MCMV) infection. We observed more abundant virus-specific CD8 T cells in DKO mice following expansion at 7 dpi. Importantly, DKO T cells respond the same as WT T cells when adoptively transferred into WT environment; however, adoptive transfer of DKO splenocytes into WT recipients results in greater expansion of the recipient (WT) T cell during infection, suggesting other cell types with Casp8 eliminated enhancing CD8 T cell level. Interestingly, removing Natural Killer (NK) cells in DKO mice reverses this enhancement of CD8 T cells. Altogether, even though Casp8 has no intrinsic impact on T cell in the course of expansion and contraction, Casp8-deficient NK cell results in greater T cell expansion during viral infection.

Travis Rotterman, NS **4:30PM**

THE REMOVAL OF PROPRIOCEPTIVE IA AFFERENT SYNAPSES FROM MOTONEURONS AFTER NERVE INJURY OCCURS THROUGH A MECHANISM DEPENDENT ON CHEMOKINE RECEPTOR CCR2

Travis M. Rotterman[1] and Francisco J. Alvarez[2]

[1]Laney Graduate School: Neuroscience Program, Emory University, Atlanta Georgia 30322, [2]Department of Physiology, Emory University, Atlanta Georgia 30322

Peripheral nerve injuries affect millions of individuals leaving them with permanent motor deficits such as loss of the stretch reflex. This deficit is largely due to the plasticity of spinal motor circuits in which the proprioceptive IA afferent inputs that monosynaptically connect to motoneurons are degraded. We hypothesize that this loss results from a central immune response in which blood-derived cells migrate into the CNS and differentiate into macrophages which specifically recognize injured afferent terminal for phagocytosis. We performed sciatic nerve transections in a transgenic mouse model in which a red fluorescent protein (RFP) was knocked in to a chemokine receptor, CCR2, known to be essential in the recruitment of peripheral macrophages during injury. Interestingly, we found a heterogenous cell population composed of T-cells and differentiating macrophage-like cell infiltrating the spinal cord 2-3 weeks following nerve injury. These cells surround axotomized motoneurons and appear to be directly involved in removing IA afferent synapses. Furthermore, we performed these experiments in CCR2 KO mice and found a decrease in infiltrating RFP+ cells and a significant preservation of IA afferent inputs across the dendritic arbor of motoneurons. These data strongly support the hypothesis that infiltrating peripheral macrophages are involved in motor circuit plasticity.

Emily Woods, MMG **4:45PM**

THE ROLE OF AN ABC TRANSPORTER MECHANISM IN LL-37 RESISTANCE IN *CLOSTRIDIUM DIFICILE*

Emily C. Woods[1], Shonna M. McBride[1]

[1]Emory University School of Medicine, Department of Microbiology and Immunology, Antibiotic Resistance Center, Atlanta, GA

Clostridium difficile is a bacterium that causes severe diarrheal disease. In order to cause disease in the gut, *C. difficile* must resist killing by host-produced antimicrobials, including LL-37, a cationic peptide produced in the colon and by neutrophils. Current epidemic strains of *C. difficile* have relatively high levels of LL-37 resistance. Exposing *C. difficile* to sub-lethal levels of LL-37 results in subsequently higher levels of LL-37 resistance, which strongly suggests that *C. difficile* has inducible LL-37 resistance mechanisms. However, the mechanisms that enable *C. difficile* to survive in the presence of LL-37 are unknown. We hypothesize that genes induced by LL-37 contribute to LL-37 resistance. In this study, we performed RNA-seq to identify genes induced by LL-37. One operon we identified, *CD1617-CD1619*, is highly upregulated in *C. difficile* in the presence of LL-37. This operon putatively encodes a GntR-family transcriptional regulator and an ABC transporter system. Unexpectedly, we discovered that a mutant of the transcriptional regulator and a mutant of the transporter are both more resistant to LL-37 and have increased expression of a second ABC transporter system (*cdd*). We propose that induction of this *cdd* transporter in the *CD1617* and *CD1618* mutants is a compensatory LL-37 resistance mechanism.

Jenny Cosby, IMP **5:00PM**

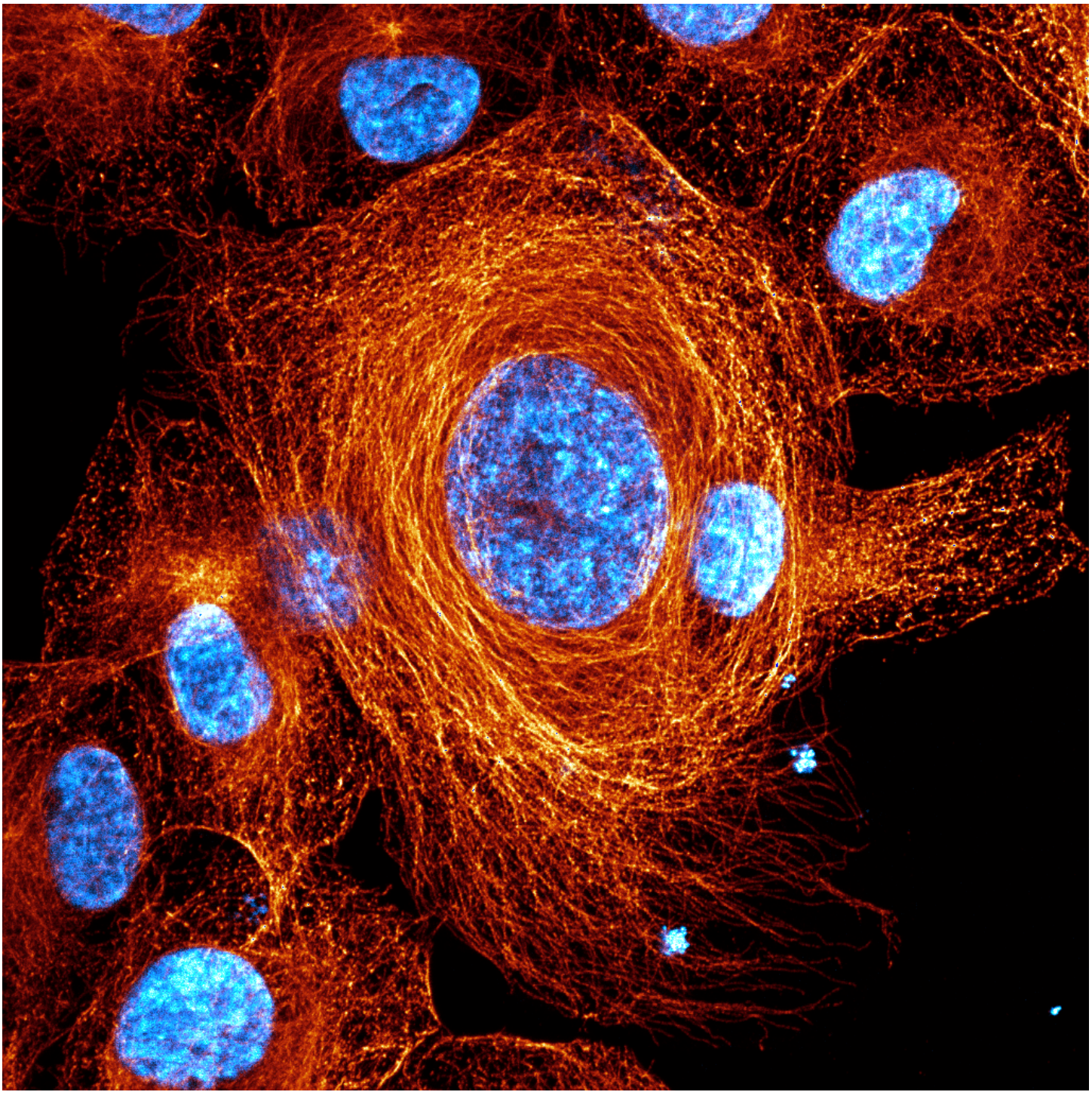
BACTERIAL DELIVERY OF MOG EPITOPE SUPPRESSES AUTOIMMUNE DEMYELINATING DISEASE VIA MOG-SPECIFIC TOLERANCE MECHANISMS

Jenny Cosby[1], Ryan Martinez[1], Brian Evavold[1]

[1]Emory University School of Medicine, Department of Microbiology and Immunology, Atlanta, GA

Molecular mimicry has been proposed to describe a relationship between infection and autoimmunity. Mimic epitopes are thought to expand self-reactive T cells to target self-antigens, leading to demyelinating disease. *Listeria monocytogenes* (Lm) as a vector allows for systemic antigen delivery, heavy bacterial burden for high antigen load, and strong T cell responses; therefore, we utilize a vaccine strain of *Listeria* containing myelin oligodendrocyte glycoprotein (MOG) to recapitulate molecular mimicry. Upon infection, myelin-specific T cells are minimally expanded (~4 times) compared to naïve controls and substantially less expanded than the ~57 times which occurs upon the classical induction method of demyelinating disease. Most of the MOG-specific tetramer+ T cells expanded via infection were Tregs. To determine whether MOG-specific tolerance limits disease initiation and molecular mimicry, we also investigated our infection model in a MOG deficient mouse that has altered thymic selection and an absence of MOG-specific suppressive Tregs. Only in the MOG deficient mice does Lm-MOG initiate EAE with substantial numbers of MOG-specific Teff upon primary infection. These data reveal two major findings: 1) MOG tolerance mechanisms are difficult to overcome and 2) deficient MOG tolerance mechanisms increase the risk for molecular mimicry induced autoimmune demyelinating disease.

Poster Presentation Abstracts



3rd Place, ICI Image Contest
Emily Summerbell, Cancer Biology
Immunofluorescence of microtubules and nuclei in lung cancer cells

Poster Presentations

Session 1: 12:45 - 1:30PM - Odd-numbered posters
Session 2: 1:30 - 2:15PM - Even-numbered posters

Poster	Name	Program	Poster	Name	Program
1	Amanda Engstrom	BCDB	35	Alyssa Scott	GMB
2	Sabrina Lynn	BCDB	36	Sarah Suciu	GMB
3	Kelsey Maher	BCDB	37	Shannon Torres	GMB
4	Tyler Moser-Katz	BCDB	38	Morgan Barham	IMP
5	Samantha Schwartz	BCDB	39	Lynette Chea	IMP
6	Jarred Whitlock	BCDB	40	Sarah Connolly	IMP
7	Mwangala Akamandisa	CB	41	Elina El-Badry	IMP
8	Briana Brown	CB	42	Osric Forrest	IMP
9	Alexander Chen	CB	43	Mojibade Hassan	IMP
10	Rachel Commander	CB	44	Andrew Jones	IMP
11	Nicholas Eyrich	CB	45	Michael LaMuraglia	IMP
12	Valentina Gonzalez-Pecchi and Lauren Rusnak	CB	46	Sonia Laurie	IMP
13	Allyson Koyen	CB	47	Amanda Mener	IMP
14	Kristin Limpose	CB	48	Maria White	IMP
15	Emily Summerbell	CB	49	Kelsie Brooks	MMG
16	Katelyn Ponder	CB	50	Jessica Coates	MMG
17	Fadi Pulous	CB	51	Ashley Cross	MMG
18	Alessandra Richardson	CB	52	Kara Phipps	MMG
19	Hope Robinson	CB	53	Lalita Priyamvada	MMG
20	Brian Pedro	CB	54	Roxana Rodríguez Stewart	MMG
21	David Weir	CB	55	Riley Perszyk	MSP
22	Kameryn Butler	GMB	56	Brandon Stauffer	MSP
23	Sarah Curtis	GMB	57	Matt Tillman	MSP
24	Christine Doriono	GMB	58	Laura Butkovich	NS
25	Salma Ferdous	GMB	59	Mary Herrick	NS
26	Sara Fielder	GMB	60	Carlie Hoffman	NS
27	Crystal Grant	GMB	61	Elizabeth Kline	NS
28	Robert Haines	GMB	62	Stephanie Pollitt	NS
29	PamelaSara Head	GMB	63	Ashley Swain	NS
30	George Inglis	GMB	64	Mfon Umoh	NS
31	Anna Knight	GMB	65	JR McMillan	PBEE
32	Trenell Mosley	GMB	66	Amanda Vincete-Santos	PBEE
33	Rebecca Pollak	GMB	67	Patricia Signe White	PBEE
34	J. Christopher Rounds	GMB			

Amanda Engstrom, BCDB **Poster #1**

LSD1 INHIBITION MAY CONTRIBUTE TO TAU-MEDIATED NEURODEGENERATION IN ALZHEIMER'S DISEASE

Amanda K. Engstrom[1]**,** Rohitha A. Moudgal[1]**,** Michael A. Christopher[1], Dexter Myrick[1], Benjamin G. Barwick[2], Allan I Levey[3], David J Katz[1]

[1]Dept. of Cell Biology, [2]Dept. of Microbiology and Immunology, [3]Dept. of Neurology, Emory University

Alzheimer's disease (AD) is an irreversible, progressive brain disorder caused by massive neuronal cell death in the frontal and temporal cortices and the hippocampus. AD is associated with the accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs). However, how NFTs contribute to neuronal cell death remains unclear. Recent data from our lab has demonstrated that the histone demethylase LSD1 is mislocalized with NFTs in AD cases. In addition, loss of LSD1 systemically in adult mice is sufficient to recapitulate many aspects of AD. These data raise the possibility that neurofibrillary tangles contribute to neuronal cell death, in AD by interfering with the continuous requirement for LSD1 to repress inappropriate transcription. Here we further test this model by removing one copy of *Lsd1* from P301S Tau mice, which contain a human transgene overexpressing an aggregation-prone mutant of tau. Our preliminary results show these mice exhibit a much faster and more severe neurodegeneration phenotype. This suggests that aggregated tau functions genetically through the loss of LSD1. As a result, it may be possible to target this step therapeutically to block the progression of AD.

Sabrina Lynn, BCDB **Poster #2**

IMPACT OF TIGHT JUNCTION REMODELING ON LUNG EPITHELIAL BARRIER FUNCTION

K. Sabrina Lynn, Barbara Schlingmann, Rachel Vance, Max Cornely, Michael Koval[1]

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

Survival of acute respiratory distress syndrome (ARDS) is chiefly attributed to the ability to maintain airspace fluid balance. The severity and risk of ARDS is magnified with chronic alcohol abuse. Currently, our lab is investigating the molecular mechanisms behind increased incidence of ARDS, with particular interest in the effects on tight junctions. Previous work in the Koval lab determined that chronic alcohol ingestion increases expression of tight junction protein claudin-5 by the alveolar epithelium, which is necessary and sufficient to decrease alveolar epithelial barrier function. This impairment of the alveolar epithelial barrier correlated to molecular rearrangement of claudin-18 into spike-like structures perpendicular to the cell junction interface. 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. This suggests a role for clathrin-mediated, dynamin-dependent endocytosis in TJ spike formation. 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.

Kelsey Maher, BCDB **Poster #3**

IDENTIFICATION OF OPEN CHROMATIN REGIONS FOR MULTIPLE PLANT SPECIES USING INTACT-ATAC-SEQ

Kelsey Ann Maher[1,2], Marko Bajic[1,3], Roger Bancroft Deal[1]

[1] Department of Biology, Emory University, Atlanta, GA, [2] Biochemistry, Cell, and Developmental Biology Program in the Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA, [3] Genetics and Molecular Biology Program in the Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA

Eukaryotic genomes are packaged with proteins to form a hierarchical structure, called chromatin. The degree to which regions of chromatin are compacted has a major impact on the ability of transcription factors (TFs) to bind their regulatory sequences, and affect transcription. The ability to probe the relative 'openness' of an organism's chromatin, therefore, is crucial to understanding both where these regulatory elements are located within the genome and how networks of TFs interact to regulate transcription in a sophisticated manner. Assay for Transposase-Accessible Chromatin-sequencing (ATAC-seq) probes chromatin openness with a hyperactive transposase; however, the transposase also reacts with organellar DNA, diminishing the amount of nuclear-mapping reads that can be used in downstream analysis for each sequencing reaction. This problem is amplified in plant species, which contain both mitochondria and chloroplasts, making chromatin accessibility profiling highly inefficient. Our lab has combined Isolation of Nuclei TAgged in specific Cell Types (INTACT) with ATAC-seq to selectively separate nuclei from other genetic organelles before transposase treatment, resulting in an 80% increase in nuclear-mapping reads. Using INTACT-ATAC-seq, we have profiled both the chromatin accessibility and the transcription factor networks of several important crop species to gain novel insight into transcriptional gene regulation in plants.

Tyler Moser-Katz, BCDB **Poster #4**

CD86 SIGNALING PROMOTES EVASION OF CELL DEATH IN MULTIPLE MYELOMA

Tyler Moser-Katz[1], Catherine M. Gavile[1], Lawrence H. Boise[1]

[1]Department of Hematology and Oncology, Emory University, Atlanta GA 30322

Multiple myeloma is the second most common hematologic malignancy with an estimated 30,000 new diagnoses and 12,000 deaths occurring in 2016. Despite recent therapeutic advances, myeloma remains incurable. Myeloma cells retain much of the characteristics of their normal plasma cell counterparts but have the capacity to proliferate while avoiding apoptosis. Genomic analysis from the lab has shown that myeloma patients expressing high levels of two surface proteins, CD28 and CD86 have poorer prognoses than patients with low levels of expression. Furthermore, our data show that interaction between CD28 and CD86 promotes survival in our myeloma cell lines. Our data also indicate that the cytoplasmic tail of CD86 may be mediating survival via regulation of different factors known to play a role in maintaining myeloma cell viability. To study CD86 intracellular signaling, we have developed stable myeloma cell lines that overexpress full-length CD86 as well as a mutant CD86 line which lacks the CD86 cytoplasmic tail (tail-less). We also have various truncation mutants that will enable us to delineate which domains of the CD86 cytoplasmic tail regulate the different factors that affect viability. Our data show that the cytoplasmic domain of CD86 regulates different aspects of myeloma survival.

Samantha Schwartz, BCDB **Poster #5**

REGULATION OF 2'-5'-OLIGOADENYLATE SYNTHETASE 1 (OAS1) BY SMALL DOUBLE-STRANDED RNAS

Samantha L. Schwartz[1, 2] and Graeme L. Conn[1, 2]

[1]Biochemistry, Cell and Developmental Biology (BCDB) Program, Emory University.

[2]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.

The innate immune system is our first line of defense against infecting pathogens. Misregulation of this system can cause increased susceptibility to infection and viral persistence, as well as diseases such as interferonopathies. The 2'-5'-oligoadenylate synthetase (OAS) enzymes are important innate immune sensors of cytosolic double-stranded RNA (dsRNA). Although structural studies have revealed much about OAS1 structure and regulation, it remains unclear how specific RNA features control the extent of OAS1 activation. We designed dsRNA hairpins containing OAS1 activation consensus sequences to examine the RNA features, and their context, that are necessary for potent activation. Remarkably, while a single point mutation on one strand resulted in *complete loss* of OAS1 activation, the equivalent mutation on the opposite strand led to *increased* OAS1 activation. Despite these stark differences in capacity to activate OAS1, both variants appear to bind with similar affinity. These findings suggest that dsRNAs can contain competing OAS1 binding sites with remarkably different capacities to activate the enzyme in a context-dependent manner. However, the signatures defining these sites as activating or non-activating are still unknown. Defining these molecular mechanisms will enhance our understanding of host-pathogen interactions and, e.g., reveal how viruses might evade detection by masking OAS1 activating motifs.

Jarred Whitlock, BCDB **Poster #6**

LIMB GIRDLE MUSCULAR DYSTROPHY 2L IS ASSOCIATED WITH A DEFFECT IN PHOSPHOLIPID SCRAMBLING.

Jarred M. Whitlock[1]**,** Kuai Yu[1], YuanYuan Cui[1], and H. Criss Hartzell[1].

Department of Cell Biology, Emory University, Atlanta, GA, USA.

ANO5 mutations are linked to variable myopathies, e.g. Limb Girdle Muscular Dystrophy type 2L (LGMD2L). ANO5 is a member of the anoctamin/TMEM16 superfamily of Ca^{2+}-activated Cl^- channels and regulators of Ca^{2+}-dependent phospholipid scrambling (Ca^{2+}-PLS), although the function of ANO5 remains unknown. We propose that ANO5 regulates Ca^{2+}-PLS that regulates muscle repair. PLS is a process that exposes phosphatidylserine (PS), normally sequestered in the inner leaflet of the plasma membrane, to the extracellular environment where it is recognized as a cell-cell signaling ligand. PS exposure occurs coincidently with myoblast fusion and participates in this process required for muscle repair, which is defective in LGMD2L patients. Here we demonstrate that exogenous ANO5 expression elicits Ca^{2+}-PLS that is accompanied by a non-selective ionic current. *Ano5*$^{-/-}$ murine myoblasts lack Ca^{2+}-PLS and PS exposure in response to elevated intracellular Ca^{2+}. This defective Ca^{2+}-PLS is accompanied by perturbated fusion of *Ano5*$^{-/-}$ myoblasts, a necessary step in muscle maintenance and repair. To determine whether Ca^{2+}-PLS is disrupted in human LGMD2L, skin fibroblasts were obtained from a LGMD2L patient and unaffected controls. Sequencing demonstrated *ANO5* mutations in both patient alleles. The LGMD2L patient fibroblasts exhibited markedly reduced Ca^{2+}-PLS (>75% reduction). Our findings suggest that the loss of ANO5 Ca^{2+}-PLS leads to sustained decreases in the effectiveness of muscle repair in LGMD2L patients which compound over time and contribute to the development of later dystrophic symptoms.

Mwangala Akamandisa, CB **Poster #7**

SMALL MOLECULE INHIBITION OF *PPM1D* SUPPRESSES GROWTH OF PATIENT DERIVED DIPG

Mwangala Akamandisa[1,2], Jing Wen[2,3], Kai Nie[2,3], Briana Brown[1], Dolores Hambardzumyan[1,2,3], Robert C Castellino[2,3].

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Diffuse intrinsic pontine glioma (DIPG) is the most common brainstem tumor in children peaking in incidence in children aged between 5 and 10 years. Outcomes after diagnosis of DIPG are dismal with nearly all patients succumbing to disease within two years of diagnosis, a state that has remained unchanged over the last three decades despite advances in science. A recent increase in the availability of tumor tissue samples has fueled research into the molecular characteristics of DIPG development. Recent publications have shown that the protein phosphatase, *PPM1D*, a known inhibitor of tumor suppressor P53, is mutated in up to 25% of DIPGs. Our work shows that expression of mutant *PPM1D* cDNA suppresses activation of P53 and promotes proliferation of human DIPGs *in vitro*. We also show that inhibition of *PPM1D* in addition to radiation reduces proliferation of DIPGs *in vitro*, on organotypic tissue slices, and *in vivo.* This work contributes to our understanding of DIPG development, and provides a potential target for drug therapies in humans.

Briana Brown, CB **Poster #8**

DEFINING THE ROLE OF CO-MUTANT KRAS/LKB1 IN *DROSOPHILA MELANOGASTER* EPITHELIA

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Lung cancer is the leading cause of cancer-related deaths in the United States with a 5-year survival rate of 18%. Lung adenocarcinomas are marked by predominant oncogenic mutations in KRAS. Furthermore, *STK11*, the gene that encodes LKB1, is concurrently mutated with KRAS in one-third of patients. LKB1 is a serine/threonine kinase identified as a tumor suppressor and previous research shows that LKB1 loss is sufficient to drive tumor progression and metastasis. It is proposed that LKB1 is a key regulator of epithelial cell polarity, growth, and adhesion but which of these biologic functions promotes metastatic progression, especially in the context of oncogenic KRAS, is unknown. Our research aims to develop a co-mutant $Kras^{V12}$/Lkb1 *Drosophila* epithelium to define the mechanism and signaling pathways required for mutant cells to traverse the basement membrane and metastasize *in vivo.* Preliminary data show that co-mutant $Kras^{V12}$/Lkb1 larval wing discs result in increased actin filament disorganization and MMP1 activity. Additionally, co-mutations cause severely distorted wing structure and decreased survival. Our model will be used to define the role of existing and novel signaling pathways downstream of Kras/Lkb1 mutations in vivo, and may identify novel signaling components to target in this highly aggressive form of lung adenocarcinoma.

Alexander Chen, CB **Poster #9**

UNDERSTANDING THE MECHANISM OF RIOK2 FUNCTION IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM), a tumor derived from glia and glial progenitor cells, is the most aggressive and prevalent form of primary brain cancer and is incurable. Amplification, mutation, and/or overexpression of the EGFR receptor tyrosine kinase and activating mutations in components of the PI3K pathway are common in GBM tumors, although the pathways that act downstream of EGFR and PI3K to drive tumorigenesis remain poorly understood. To better understand the underlying biology of tumorigenesis, we use a *Drosophila melanogaster* GBM model in kinome-wide genetic screens that identified Right-Open-Reading-Frame-2 (RIOK2), an atypical serine-threonine kinase, as a possible driver of EGFR-PI3K-dependent GBM; however, little is understood about RIOK2 function or downstream targets. Preliminary experiments have identified several RIOK2 binding proteins and potential substrates, of particular note include RNA binding proteins, which are involved in stabilizing and promoting the translation of target mRNAs, several of which are known drivers of GBM that are overexpressed in response to oncogenic EGFR and PI3K signaling. Thus, we hypothesize that RIOK2 drives tumorigenesis by modulating the activity of RNA-binding proteins involved in stabilizing and promoting the translation of their target mRNAs, and that this promotes the translation of target mRNAs that drive tumor cell proliferation and survival.

Rachel Commander, CB **Poster #10**

DEFINING THE INVASIVE BIOLOGY OF CLINICALLY RELEVANT LKB1 MUTATIONS IN LUNG CANCER

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In 2016, approximately 224,390 new lung cancer cases were diagnosed. Among those patients diagnosed with metastatic lung cancer, 5-year survival is just 4.3%. A better understanding of the molecular mechanisms responsible for lung cancer metastasis is crucial for the treatment of this disease. The tumor suppressor and serine/threonine kinase LKB1 (STK11) is mutated in up to 30% of lung adenocarcinoma cases. LKB1 loss has been implicated in metastasis in a lung cancer genetically engineered mouse model, but it remains unclear how specific LKB1 mutations observed in patients enable cells to invade and eventually metastasize. Our analyses of differentially expressed genes in lung cancer patients revealed that subsets of patients with either LKB1 missense mutations or LKB1 truncating mutations have distinct expression profiles, suggesting that different LKB1 mutations have different biological consequences. We published that LKB1 has two roles in cell invasion, mediated either through its kinase domain or C-terminal domain (CTD). We show that LKB1-null cells invade in a collective manner, while LKB1 wildtype cells invade in a single-cell manner, and LKB1 represses collective invasion specifically through its CTD. These data support a model whereby loss of LKB1 CTD function leads to cellular escape through collective invasion of LKB1-compromised cells.

Nicholas Eyrich, CB **Poster #11**

REACTIVE OXYGEN SPECIES SIGNALING IN SHH DRIVEN MEDULLOBLASTOMA AND CEREBELLAR GRANULE NEURON PRECURSORS: THE NOX4-HIF1A AXIS

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Medulloblastoma is the most common solid pediatric malignancy. These tumors arise in the cerebellum and can be molecularly subdivided into 4 consensus subgroups, one of which is marked by amplification and activation of Sonic hedgehog (Shh) pathway components and downstream targets. This subclass is proposed to arise from oncogenic transformation of cerebellar granule neuron precursors (CGNPs), whose expansion during post-natal brain development is driven by and requires Shh pathway activation. CGNP cultures offer a model system for studying Shh driven medulloblastoma, given its similarities with normal cerebellar development. In addition to mitogens driving proliferation, it has been shown that low levels of intracellular reactive oxygen species (ROS) are required for proliferation through a myriad of mechanisms. This led us to investigate potential sources and targets of these ROS in NADPH Oxidase-4 (Nox4) and Hypoxia-Inducible Factor-1-Alpha (Hif1a) downstream of Shh. Hif1a, outside of its oxygen sensing role, has been implicated in the Warburg effect, which causes an increase in glycolytic activity and relative decrease in oxidative phosphorylation in oftentimes normoxic cancer cells. We've shown that Hif1a is dependent on Nox4 activity and a minimum level of ROS to remain stabilized in normoxic conditions, suggesting an axis mediated by Nox4 generated ROS.

Valentina Gonzalez-Pecchi and Lauren Rusnak, CB **Poster #12**

IDENTIFICATION OF NOVEL EPIGENETIC REGULATORS THAT BIND AND REGULATE MYC ONCOPROTEIN

Valentina Gonzalez-Pecchi[1,2]**,** ***Lauren Rusnak***[1,2]**,** Jinglin Xiong[2], Yuhong Du[2] and Haian Fu[2,3]

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The MYC transcription factor is one of the most commonly amplified oncogenes across tumor types. Previous studies have shown MYC is essential for many cancers, as loss or inactivation of this protein can cause tumor regression in animal models. However, it has been difficult to target MYC due to its unstructured protein conformation. One approach to address this issue is to identify MYC binding partners that are important for its function. Here we describe novel interactions between MYC and two epigenetic regulators, NSD3S and BRD4. NSD3S is a NSD lysine methyltransferase family member that lacks enzymatic activity, but can still bind chromatin. Using multiple binding assays, we demonstrated that NSD3S interacts with MYC under physiological conditions and enhances MYC protein stability and transcriptional activity. Interestingly, NSD3S is known to interact with BRD4, a bromodomain protein that has been functionally linked to MYC. This led us test and validate BRD4 as an additional MYC binding partner. Additionally, BRD4/MYC binding is enhanced by NSD3S, raising the possibility that these proteins form a complex to promote MYC activity and gene expression. Our discovery of the BRD4/NSD3S/MYC complex suggests a novel regulatory axis and potential protein-protein interaction target for inhibiting the MYC-driven oncogenic program.

Allyson Koyen, CB **Poster #13**

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

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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 SCLC patients develop cisplatin resistance. Targeting proteins critical to the repair of cisplatin DNA crosslinks is a strategy for overcoming acquired cisplatin resistance in SCLC, but many proteins that mediate crosslink repair have yet to be identified. To address this issue, we performed a synthetic lethal siRNA screen in cisplatin resistant SCLC cells, and identified EZH2 as one of the strongest mediators of cisplatin resistance. Additionally, we found EZH2 expression correlates with cisplatin resistance across SCLC cell lines. Mechanistically, EZH2 localizes to sites of DNA damage which are induced by UVA-crosslinking laser microirradiation and interacts in a complex with DDB1, which is involved in the initiation of the nucleotide excision repair (NER) pathway. Preliminarily, loss of EZH2 appears to enhance DDB1 localization to sites of UVA laser microirradiation, indicating that EZH2 may negatively regulate DDB1. Together, this data suggest that EZH2 may function upstream of DDB1 as a novel regulator of NER, and is a promising target for cisplatin resistant SCLC.

Kristin Limpose, CB **Poster #14**

OVEREXPRESSION OF THE NTHL1 GLYCOSYLASE RESULTS IN THE ACQUISITION OF CANCER PHENOTYPES AND IS INDEPENDENT OF NTHL1 CATALYTIC ACTIVITY

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Base excision repair (BER) is the frontline for repairing potentially mutagenic oxidative DNA damage, and is initiated by DNA *N*-glycosylase proteins. One glycosylase, NTHL1, excises a large proportion of DNA damage caused by reactive oxygen species, resulting in apurinic/apyrimidinic (AP) sites and DNA single strand breaks that must be further processed to avoid deleterious intermediates. Cancer genomic datasets indicate NTHL1 amplification in certain cancers. We find that NTHL1 protein is overexpressed in a panel of lung cancer cell lines. In order to determine the biological significance of overexpression, we overexpressed NTHL1 in the non-tumorigenic HBEC lung epithelial cell line. Surprisingly, wild type and catalytically inactive NTHL1 overexpression results in replication stress that leads to DNA double strand breaks and genomic instability, as measured by phosphorylation of ATR, colocalization of γH2Ax and 53BP1, and the micronucleus assay. To assess whether these molecular endpoints impact cellular phenotypes, cells were challenged to grow in soft agar and resulted in colony formation. Once isolated, these cells demonstrated loss of contact inhibition, and persistent genomic instability, all indicative of cancer hallmarks. We propose that overexpression of NTHL1 has potential oncogenic functions, and we propose a dual mechanism of transformation through deleterious BER intermediates and the induction of replication stress.

Emily Summerbell, CB **Poster #15**

CELL-CELL COOPERATIVITY MODULATES PROLIFERATION AND MITOTIC DEFECTS OF PHENOTYPICALLY HETEROGENEOUS INVADING CANCER CELLS

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Collective cell invasion is a major mode of cancer invasion and metastasis. In order to study how heterogeneous cell populations cooperate during collective cancer cell invasion, we have selected and maintained rare highly-invasive leader cell and poorly-invasive follower cell lines from H1299 lung cancer cells using a spatiotemporal optogenetic approach. Isolated follower cells are highly proliferative compared to leaders. The addition of follower conditioned media (CM) increases leader cell proliferation, whereas leader CM inhibits follower cell growth. Cell cycle analysis shows that only leader cells undergo a significant G1 arrest 20 hours post-serum starvation. Leader cells commonly display spindle pole defects and centrosome amplification, as well as mitotic defects that are rare in followers, such as cytokinetic instability or fusion of daughter cells. Overall 69.4% of leader cells display mitotic defects whereas only 6.1% of follower cells exhibit such defects. Co-culture of leader and follower cells abrogates leader cell mitotic failure from 33.1% to 2.7%; follower CM alone achieves a similar effect. Interestingly, leader cells conversely hinder follower cell mitotic success. Our data suggest a model wherein followers promote the proliferation and mitotic success of leaders but that leaders negatively impact follower proliferation, perhaps to increase follower cell invasive potential.

Katelyn Ponder, CB **Poster #16**

ROLE OF THE PRODOMAIN OF CASPASE-3 IN APOPTOTIC ACTIVITY

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Caspase-3 (C3) is a protease that is activated at the end of the apoptotic pathway. The apoptotic activity of C3 is well characterized, but the regulation of this process is not fully understood. Previous studies demonstrated removing of the prodomain enhances apoptotic activity. It is unknown whether this induction results in complete activation or lowers the activation threshold. We hypothesize there are prodomain regions that are required for activity regulation. We created deletion constructs that remove 10 (Δ10), 19 and up to 28 aa from the N-terminus. After stably expressing the constructs in $C3^{-/-}$ mouse embryonic fibroblasts (MEFs), we conducted apoptosis assays and cleavage analysis to determine which region regulates its activity. Preliminary results demonstrate that Δ10 have activity like that of $C3^{-/-}$. Therefore, the first 10 aa are required for activity. We discovered that $C3^{-/-}$ $C3^{D9A}$ results in loss of activity. Therefore, cleavage at D9 could be important. We compared the activation of $C3^{-/-}C3^{WT}$ and the mutant C3 MEFs by determining procaspase-3 cleavage. Our results demonstrate the first 10 aa of the prodomain are necessary to have proper removal of the prodomain. Elucidating the mechanism of C3 activation is important to better target C3 in cancer and other diseases.

Fadi Pulous, CB **Poster #17**

ENDOTHELIAL CELL TALIN1 IS REQUIRED FOR POSTNATAL ANGIOGENESIS AND THE MAINTENANCE OF ESTABLISHED BLOOD VESSELS

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Blood vessel development depends on integrin-dependent endothelial cell (EC) adhesion. Talin1 binding to β integrins functions to link integrins to the actin cytoskeleton and represents a key final step in integrin affinity modulation. To test the requirement of EC talin1 in postnatal angiogenesis, we administered tamoxifen to postnatal day 1 (P1) talin1(fl/fl),Cdh5CreERT2+ (talin1 EC-KO) and talin1(fl/fl),Cdh5CreERT2- (control) mice. Tamoxifen-treatment selectively deleted talin in ECs of talin1 EC-KO pups and led to death around P8 due to multi-organ vascular hypoplasia and hemorrhage. Analysis of retinal angiogenesis showed a 42% reduction in vascular radius and reduced blood vessel density in talin1 EC-KO mice. Empty matrix sleeves (collagen IV+/EC- structures) were more prevalent in talin1 EC-KO retinas suggesting that reduced vessel stability contributed to the reduced vascular density. To test the requirement of talin1 in the maintenance of established blood vessels, we treated adult (8-10 week old) mice with tamoxifen. Adult talin1 EC-KO mice died 16-20 days after tamoxifen treatment. Interestingly, gross pathology was most prominent in the intestines of talin1 EC-KO mice with hemorrhaging throughout the capillaries of the intestinal villi. Confocal microscopy of the intestinal vasculature revealed the formation of cyst-like structures comprised of several ECs. Together, these results indicate that EC talin plays an important role in postnatal angiogenesis and in the maintenance of established intestinal blood vessels.

Alessandra Richardson, CB **Poster #18**

BEYOND EMT: VIMENTIN FUNCTION IN LUNG CANCER INVASION AND METASTASIS

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Vimentin is used as a clinical biomarker for metastasis as vimentin expression correlates with increased metastatic potential. However, despite this abundance of correlative clinical data, it has never been shown whether vimentin plays a significant role in the metastatic cascade. In order to probe this question we developed a novel KrasG12D/LKB1fl/fl/Vim-/- model (KLV-/-) derived from the KrasG12D/LKB1fl/fl model (KLV+/+). By comparing the metastatic phenotype of these two mouse models we found that vimentin is a key player in metastasis. We show that loss of vimentin results in a significant reduction of metastasis to the lymph node. Upon probing for vimentin in our KLV+/+ model we found that vimentin is expressed not in the cancer cells, but in cancer-associated fibroblasts (CAFs) that surround collective invasive packs (CIPs) of epithelial tumor cells. This indicates that cancer cells in this model are not undergoing a complete epithelial-to-mesenchymal transition (EMT). This is consistent with vimentin staining in human lung adenocarcinoma samples where again vimentin is more highly expressed in the surrounding stromal tissue than in the cancer cells. Taken together, these results indicate that vimentin plays a key role in cancer invasion and metastasis through its role in the tumor microenvironment.

Hope Robinson, CB **Poster #19**

REGULATION OF HELLS AS A DOWNSTREAM EFFECTOR OF SONIC HEDGEHOG SIGNALING IN CEREBELLAR DEVELOPMENT AND MEDULLOBLASTOMA

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Aberrant hedgehog signaling has been implicated in a number of cancers, including medulloblastoma (MB), the most common malignant pediatric brain tumor. One of the molecular subgroups of MB is characterized by aberrant activity of the Sonic hedgehog (SHH) signaling pathway. While some of the signaling alterations in SHH MB are due to gene mutations or amplifications, others may have their roots in epigenetics. Therefore, we examined levels of expression of several candidate epigenetic regulators in Shh-treated cerebellar granule neuron precursors (CGNPs). We identified lymphoid specific helicase (Hells) as a gene whose expression was markedly induced by Shh. Hells is a member of the SNF2 family of chromatin remodelers with multiple reported epigenetic functions. Overexpression of Hells has been observed in many human cancers including medulloblastoma. We found that Hells mRNA expression and protein levels are increased in Shh-induced CGNPs and in medulloblastomas from a mouse model for Shh MB. Currently, we are determining SHH pathway regulation of Hells using inhibitors of Shh signaling activity and ascertaining Hells involvement in proliferation and survival by examining effects on these processes with knockdown of Hells.

Brian Pedro, CB **Poster #20**

IDENTIFYING GENOMIC SIGNATURES OF RARE CANCER CELLS WITHIN A HETEROGENEOUS COLLECTIVE INVASIVE PACK

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Tumor metastasis often proceeds via collective invasion, whereby cancer cells travel through the microenvironment as a cohesive pack. Experimental models have shown this pack to be phenotypically heterogeneous, containing both leader cells, which facilitate invasion into the microenvironment, and follower cells, which attach to and travel behind the leaders. To better elucidate the molecular mechanisms by which this phenotypic heterogeneity arises, we utilized the spatiotemporal genomic and cellular analysis (SaGA) technique to specifically isolate leader cells from 3-D H1299 lung cancer cell spheroids, allowing for the creation of leader and follower purified cell cultures. We hypothesized that leader and follower cells contain differential expression levels of certain genes, as well as unique gene mutations, that may contribute to their distinct phenotypes. RNA-seq analysis of purified H1299 leaders and followers revealed differential expression of genes involved in the VEGF signaling and focal adhesion pathways, among others, as well as mutations in genes involved in processes such as actin dynamics and protein ubiquitination. Overall, our data show that the SaGA technique can be utilized to reveal genomic and mutational signatures of rare but phenotypically important cells in a heterogeneous collective invasive pack, giving us new insights into the mechanisms underlying cancer metastasis.

David Weir, CB **Poster #21**

PROCASPASE-3 REGULATES THE APOPTOTIC THRESHOLD OF THE CELL THROUGH MODULATION OF FIBRONECTIN SECRETION

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The role of caspases in apoptosis is well defined. Mounting evidence indicates that caspases also have non-apoptotic functions. Our laboratory recently demonstrated that procaspase-3, the zymogen form of caspase-3, negatively regulates fibronectin secretion. This function is distinct and independent from the function of caspase-3 as an executioner caspase in apoptosis. Additionally, we have demonstrated that procaspase-3 regulates cell adhesion in the absence of a supplied extracellular matrix through control of fibronectin secretion. Furthermore, we have demonstrated that procaspase-3 regulates cell survival in an adhesion-dependent manner. However, it remains unclear whether fibronectin secretion is required for procaspase-3 to regulate cell survival. We hypothesize that procaspase-3 regulates cell survival through modulation of fibronectin secretion. To investigate this, we employed a doxycycline-inducible shRNA expression system to knockdown fibronectin thereby eliminating the ability of these cells to secrete fibronectin. Caspase-$3^{-/-}$ mouse embryonic fibroblasts subjected to doxycycline-induced fibronectin knockdown displayed increased death due to serum withdrawal compared to controls. Thus, fibronectin knockdown ablates the resistance of caspase-$3^{-/-}$ mouse embryonic fibroblasts to serum withdrawal. Furthermore, supplying exogenous fibronectin to caspase-$3^{-/-}$ mouse embryonic fibroblasts subjected to fibronectin knockdown restores this resistance. These data demonstrate procaspase-3 regulates the apoptotic threshold of the cell through modulation of fibronectin secretion.

Kameryn Butler, GMB **Poster #22**

DISEASE GENE AND VARIANT DISCOVERY FOR EPILEPSY UTILIZING PATIENTS REFERRED FOR GENETIC SCREENING

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Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures caused by neuronal synchrony and hyperexcitability. The Emory Genetics Laboratory (EGL) has developed a targeted sequencing library comprising 110 known epilepsy genes referred to as the 'epilepsy and seizure disorder panel' (ESDP). The ESDP is derived from a larger mendeliome library of approximately 5000 evidence-based disease genes, making this a valuable resource for identifying putative disease-causing alleles as well as new disease associations. Individuals referred for genetic testing are typically affected with severe childhood epilepsy. We are utilizing this rich source of available sequence data to identify new disease-causing variants. From the examination of 339 cases, we determined the diagnostic yield of the ESDP to be approximately 26% (87/339 had a positive finding). For the remaining 74% of individuals without a positive finding from the ESDP, we are examining variants from the larger mendeliome library to identify additional genes that can explain disease in these patients. From these individuals, we have identified several candidate variants in genes that could explain disease (*SLC6A1*, *CHD2*, *ANKRD11*, *SMC1A*, *PACS1*), as well as variants in genes not currently associated with epilepsy (*FZD9*, *SUPT16H*, *HUWE1*) that warrant further study.

Sarah Curtis, GMB **Poster #23**

EPIGENOME-WIDE ASSOCIATION STUDY IN A COHORT EXPOSED TO POLYBROMINATED BIPHENYL (PBB)

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In the 1970's, Michigan residents were exposed to polybrominated biphenyl (PBB), an endocrine disruptor, when it was accidentally added to farm animal feed. Exposed individuals and their children have numerous health problems, though the underlying mechanism remains unknown. Other endocrine-disrupting compounds have been linked to epigenetic differences, but no epigenetic studies have been done for PBB. Therefore, DNA from the blood of individuals with current (N = 671) or historic (N = 352) PBB levels was interrogated with the MethylationEPIC BeadChip. Associations between each of the ~850,000 CpG sites and serum PBB levels were tested with a regression that controlled for relevant covariates. After multiple test correction (FDR <0.05), 4736 CpG sites associate with current PBB levels, and 2300 CpG sites associate with historical blood PBB levels. Many of these CpG sites associate with both measures. These CpGs are in genes that are associated with immune, developmental, reproductive, and epigenetic regulation. For example, CpGs in *DNMT3A*, a gene essential for *de novo* methylation and mammalian development, associate with historic (p=1.98E-10) and current (p = 1.23E-05) PBB levels. Future work will determine whether these CpG sites associate with the development of health problems in exposed individuals.

Christine Doriono, GMB **Poster #24**

EPIGENETIC CONTRIBUTIONS TO HOMOLOGOUS CHROMOSOME RECOGNITION IN MEIOSIS

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During meiosis, homologs must correctly identify one another in order for proper alignment and recombination to occur. Improper pairing between chromosomes can lead to chromosomal rearrangements and aneuploidies causing sterility and embryonic lethality. Currently, little is known about homolog recognition and identification. During meiosis, distinct transcription patterns are produced on each chromosome and are associated with specific epigenetic modifications such as the methylation of Lysine 36 on Histone H3 (H3K36me). In humans, H3K36me is recognized by the chromodomain containing protein MRG15. Recently, homolog pairing defects were observed in *C. elegans* lacking normal function of the MRG15 homolog, *mrg-1*. The specific role of MRG-1 in homolog recognition is currently unknown. Our hypothesis is that histone modifications, like H3K36me, provide an "epigenetic barcode" used to distinguish chromosomes during homolog searching and is facilitated through MRG-1. We have knocked down and mutated *mes-4* and *met-1,* the histone methyltransferases responsible for H3K36me in the *C. elegans* germline. The resulting mutants lack H3K36me and exhibit decreases in brood size and signs of improper synapsis. Similar results were seen in *mrg-1* mutants. Our results suggest that, in addition to MRG-1, epigenetic modifications such as H3K36me may play an important role in homolog recognition during meiosis.

Salma Ferdous, GMB **Poster #25**

INVESTIGATION OF ZNF593 AS A REGULATOR OF DIFFERENTIAL EXON EXPRESSION WITHIN THE RETINA

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Abstract: RNA processing produces multiple mRNA isoforms from a single gene, ultimately promoting proteome diversity. Retinal tissue is particularly susceptible to aberrant RNA processing and multiple mutations causing Retinitis Pigmentosa (RP) are found in pre-mRNA processing genes [1]. RP is the most prevalent form of hereditary blindness and characterized by night blindness and loss of peripheral vision followed by central retinal degeneration [2]. Transcriptional master regulators are hypothesized to control tissue specific RNA processing and allow for rapid, coordinated shifts in gene expression. Previous work used Affymetrix microarrays to test retinal exon expression levels from 55 recombinant inbred mouse lines [3]. The two parental strains, C57BL6/J and DBA2/J, contained exons that displayed differential Mendelian inheritance expression patterns. From a trans-QTL on mouse chromosome 4, Znf593 is hypothesized to control differential exon expression [4]. The function of Znf593 is currently unknown. Molecular assays have determined relative protein levels and localization within the retinas of C57BL6/J and DBA2/J mice. Additional experiments will interrogate specific biochemical and molecular function such as identifying protein binding partners or direct target DNA sequences. Finally, mice with nonsense mutations in Znf593 will be tested for vision specific defects and aberrant mRNA transcriptomes.

Sarah Fielder, GMB **Poster #26**

SEX SPECIFIC DIFFERENCES IN C. ELEGANS MEIOSIS

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Meiotic synapsis is a conserved process that is required for proper pairing and segregation of homologous chromosomes in both oogenesis and spermatogenesis. My lab previously showed that male *C. elegans* (karyotype XO) target the unpaired X for heterochromatin assembly, becoming highly enriched in the repressive histone H3 modification H3K9me2. This process, called "meiotic silencing" has been observed in numerous organisms. In mutants that specifically eliminate alignment and synapsis of just one or two homologs (e.g., *zim* mutants, which coordinate pairing of one or two chromosomes), we observed that unpaired chromosomes that lack SYP proteins also show enrichment of H3K9me2, indicating that SC assembly blocks addition of H3K9me2 to chromatin. Unexpectedly, mutants that cannot initiate synapsis do not exhibit any H3K9me2 enrichment. This indicates that there is a previously undescribed checkpoint that activates H3K9me2 targeting during early meiosis that is either not reached or is bypassed in the complete absence of synapsis. This pattern is strikingly different in males lacking synapsis: all chromosomes accumulate H3K9me2. Additionally, females display large aggregates of synapsis proteins without the function of dynein light chain. However, males still synapse their chromosomes normally without the light chain. These results indicate that males regulate synapsis differently than females.

Crystal Grant, GMB **Poster #27**

A LONGITUDINAL STUDY OF DNA METHYLATION AS A MEDIATOR OF AGE-RELATED DIABETES RISK

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Background: Age is the main risk factor for many chronic diseases in humans. Aging is marked by widespread changes in biological processes and the epigenome. The most studied epigenetic mechanism, DNA methylation (DNAm), shows robust and widespread age-related changes. We tested whether DNAm-based predictions of age contribute to risk factors for diabetes, with the goal of identifying risk factors potentially mediated by DNAm. Methods: Participants were 43 women, aged 50-76 years. We obtained methylation data from participants at three timepoints. We employed the method and software of Horvath to calculate a DNAm-based estimate of chronological age. We then calculated the difference between participants' chronological age and DNAm age (termed epigenetic age acceleration, or Δ_{age}) at each timepoint. We fit linear mixed models to characterize how Δ_{age} contributed to a longitudinal model of diabetes risk factors. Results: Participants' Δ_{age} remained constant over the course of the follow-up period, indicating that age acceleration is generally stable over time. We found that Δ_{age} contributed significantly to models of body mass index ($p=0.0007$), waist circumference ($p=0.0200$), and fasting glucose ($p=0.0071$). Conclusions: Our results suggest DNAm has the potential to play a role in age-related risk for diabetes, and that further studies are warranted.

Robert Haines, GMB **Poster #28**

LSD1 IS REQUIRED FOR THE DIFFERENTIATION OF ACTIVATED B CELLS INTO PLASMA CELLS

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Differentiation of B cells into plasma cells is an essential process of the humoral immune response. It is now becoming clear that epigenetic remodeling occurs during B cell differentiation. How transcription factors that regulate B cell differentiation influence the epigenome is not well understood. Blimp-1, a key transcription factor, has been previously shown to directly interact with LSD1, thus we hypothesize that LSD1 plays a role in B cell differentiation. To measure the effect of LSD1 deletion on B cell differentiation, LSD1-deficient and -sufficient mouse B cells were subjected to ex vivo differentiation with LPS. After 3 days, quantification of B220+ CD138+ plasma cell populations with flow cytometry revealed a 50% reduction in plasma cells. To measure in vivo effects of LSD1 deletion, B cell conditional LSD1 knockout mice and control mice were immunized with LPS. After 3 days, quantification of splenic plasma cell populations with flow cytometry recapitulated ex vivo results. Analysis of B220+ GL7+ activated B cell populations showed no difference, suggesting a defect specifically in differentiation and not activation. Overall, these experiments confirm a role for LSD1 in B cell differentiation and suggest that it is required for the transition of activated B cells into plasma cells.

PamelaSara Head, GMB **Poster #29**

SIRT2 DIRECTS DNA-PKCS IN THE DNA DAMAGE RESPONSE

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DNA double strand breaks (DSBs) if left unrepaired lead to chromosomal abnormalities and cancer. There are two major DNA damage repair (DDR) pathways that repair DNA DSBs: homologous recombination, and non-homologous end joining (NHEJ). One well established NHEJ mediator is DNA-PKcs. It is able to self-regulate by autophosphorylation at serine 2056 to promote end ligation. Sirtuin 2 (SIRT2) is a deacetylase implicated in maintaining genomic stability and tumor suppression. It's necessary for the regulation of known DNA damage repair proteins CDK9 and ATRIP in the replication stress response. Preliminary data demonstrates a strong interaction between SIRT2 and DNA-PKcs following ionizing radiation in human cell lines. SIRT2 knockdown inhibits localization of DNA-PKcs to sites of microirradiation. Inhibition of localization leads to decreased self-activation of DNA-PKcs at serine 2056 in SIRT2 knockout lines in response to gamma irradiation. DNA-PKcs self-phosphorylation is rescued following damage with wild-type SIRT2 but not with deacetylase dead SIRT2 H187Y, indicating that SIRT2 enzymatic function is necessary for regulation. SIRT2 overexpression leads to increased DNA-PKcs self-phosphorylation before and after gamma irradiation. Our data suggests that SIRT2 regulates DNA-PKcs through deacetylation, promoting DNA-PKcs localization and activation in NHEJ, providing novel insight into the regulation of a well-established DDR kinase.

George Inglis, GMB **Poster #30**

IDENTIFYING NOVEL FUNCTIONAL GENETIC ELEMENTS IN THE VOLTAGE-GATED SODIUM CHANNELS *SCN1A* AND *SCN8A*

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Epilepsy is a common neurological disorder that is characterized by recurrent, unprovoked seizures. Reduced brain expression of the voltage-gated sodium channel (VGSC) α subunit gene *SCN1A* has been shown to directly cause some forms of epilepsy. In these cases, restoring *SCN1A* expression or reducing expression of the VGSC gene *SCN8A* has been demonstrated to improve seizure outcomes. Despite the important role of the VGSC genes in epilepsy, and the clinical potential of modulating *SCN1A* and *SCN8A* expression as a therapy for patients with epilepsy, we have limited knowledge about the genetic elements or factors responsible for the transcriptional regulation of these two genes. By analyzing genomic markers of open chromatin across multiple neuronal cell types, we have identified genetic elements in both *SCN1A* and *SCN8A* with putative transcriptional regulatory ability. We have cloned several of these elements into a vector upstream of a human *SCN1A* promoter driving luciferase reporter expression. A number of these constructs resulted in altered luciferase activity relative to empty vectors or constructs with just the *SCN1A* promoter, suggesting the cloned elements have functional significance. Additional analyses, including identification of proteins responsible for transcription, are being conducted on a subset of these elements.

Anna Knight, GMB **Poster #31**

METHYLATION OF SLC9B1 PREDICTS FETAL INTOLERANCE OF LABOR

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Fetal intolerance of labor is diagnosed when a category III fetal heart rate tracing, an abnormal heart rate pattern, occurs during labor and is an indication for Caesarean delivery. This study aimed to identify DNA methylation patterns prior to delivery that associate with fetal intolerance of labor. Pregnant African American women were recruited from Atlanta area hospitals during their prenatal visits, and provided blood samples for isolation of DNA and RNA. We performed an epigenome-wide association study for fetal intolerance of labor and found DNA methylation predicted fetal intolerance of labor at 4 CpG sites within *SLC9B1*, a Na+/H+ exchanger. The association between each CpG site and fetal intolerance of labor was replicated in an independent sample. These CpGs were in the same region, highly correlated, and associated with *SLC9B1* expression over pregnancy. Using Receiver Operator Characteristic curves, each CpG site had high positive (>.80) and negative (>.89) predictive values. This study suggests fetal intolerance of labor can be accurately predicted from methylation patterns in maternal blood before the onset of labor. Early identification of pregnant women at elevated risk for this condition would allow delivery at hospitals with the required resources to perform advanced monitoring and emergency Caesarean section.

Trenell Mosley, GMB **Poster #32**

SEARCH FOR CAUSAL VARIANT FOR MARTIN-PROBST SYNDROME

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Martin-Probst Syndrome (MPS) is a rare X-linked recessive disorder that affects multiple organ systems. MPS manifests with many symptoms, including sensorineural deafness, cognitive impairment, craniofacial dysmorphisms, and kidney malfunction. Previous work identified a 68 Mb haplotype shared between two related males diagnosed with MPS. Sequencing of the two males uncovered a missense mutation in *RAB40AL* (102,937,494-102,937,495AC →GA; p.D59G), which encodes a small Ras-like GTPase. *In vitro* studies revealed RNA expression in the brain, kidney, heart, liver, and skeletal muscle and subcellular mislocalization of RAB40AL-p.D59G. These findings led Bedoyan, et al. to classify p.D59G as the causal variant for MPS, via disruption of *RAB40AL*. Surprisingly, subsequent studies identified p.D59G in males with phenotypes uncharacteristic of MPS. Additionally, presence of p.D59G in the general population at low frequency suggests the variant is not causal for MPS. Thus, the specific lesion at *RAB40AL* remains unknown. To identify a causal variant, we performed whole genome sequencing (WGS) and variant analysis on a male case and female carrier. Successful identification of a causal variant will not only provide insight into the genetic etiology of MPS, but into the dynamics of rare variants in the context of rare Mendelian disorders.

Rebecca Pollak, GMB **Poster #33**

THE ROLE OF THE MICROBIOME AND METABOLOME IN 3Q29 DELETION SYNDROME

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3q29 deletion syndrome is a rare chromosomal disorder characterized by the deletion of a 1.6 Mb region of chromosome 3[1]. While all patients share the same genetic lesion, the phenotypes that patients present with are extremely variable, including neuropsychiatric phenotypes such as autism, intellectual disability, and schizophrenia; feeding problems; and reduced birth weight[1]. We seek to understand the factors accounting for this variation. There is mounting evidence that the gut microbiome can communicate directly with the brain via the gut-brain axis[2-3], as certain microbial taxa can produce and release neurotransmitters such as norepinephrine, serotonin, acetylcholine, and dopamine[4-6]. Additionally, evidence from human and animal studies suggests a link between gut microbiome dysbiosis and autism spectrum disorder[7-8]. Therefore, we hypothesize that there are microbiome differences between 3q29 patients and controls that are driving the patients' neuropsychiatric phenotypes. We have proposed a study of the oral and gut microbiomes and blood metabolome in the context of 3q29 deletion syndrome. We will be collecting samples from human patients and controls, as well as samples from a novel mouse model of the disorder developed at Emory. If we are successful, this will be the first study to conclusively link the microbiome to a neurodevelopmental disorder.

J. Christopher Rounds, GMB **Poster #34**

THE CONSERVED INTELLECTUAL DISABILITY RNA BINDING PROTEIN DNAB2 MAY REGULATE NEURONAL TRANSLATION IN CONJUNCTION WITH ATAXIN-2

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Intellectual disability refers to a common class of etiologically heterogeneous neurodevelopmental disorders. Recently, we have shown that one form intellectual disability is caused by loss-of-function mutations in *ZC3H14*, a gene encoding a ubiquitously expressed RNA binding protein whose molecular function, protein binding partners, and RNA targets are largely unknown. To better understand the function of human ZC3H14, we have begun to dissect the function of its *Drosophila* ortholog dNab2. We find that a loss-of-function allele of *Ataxin-2*, a gene encoding the neuronal translational regulator Atx2, ameliorates effects of dNab2 loss or overexpression on survival and neuromorphology, implying that dNab2 and Atx2 may interact in nuanced ways to regulate these processes. Moreover, we find that regional neuronal knockdown of dNab2 specifically elevates protein but not RNA levels of a fluorescent reporter corresponding to Ca^{2+}/calmodulin-dependent kinase II (CaMKII), an Atx2 target. In agreement, we show that neuronally expressed, FLAG-tagged dNab2 immunoprecipitates with endogenous CaMKII RNA. Finally, we show that loss of dNab2 does not alter steady-state levels of Atx2 protein during fly development. These data provide insight into ZC3H14-linked intellectual disability, supporting a role for ZC3H14 ortholog dNab2 in regulating translation of specific neuronal transcripts through, in part, molecular interactions with Atx2.

Alyssa Scott, GMB **Poster #35**

MATERNALLY PROVIDED LSD1 ENABLES THE MATERNAL-TO-ZYGOTIC TRANSITION AND PREVENTS DEFECTS THAT MANIFEST POSTNATALLY

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Somatic cell nuclear transfer has established that the oocyte contains maternal factors with epigenetic reprogramming capacity. Yet, the identity and function of these maternal factors during the gamete to embryo transition remains poorly understood. In *C. elegans*, LSD1/KDM1a enables this transition by removing H3K4me2 and preventing the transgenerational inheritance of transcription patterns. Here we show that loss of maternal LSD1 in mice results in embryonic arrest at the 1-2 cell stage, with arrested embryos failing to undergo the maternal-to-zygotic transition. This suggests that LSD1 maternal reprogramming is conserved. Moreover, partial loss of maternal LSD1 results in striking phenotypes weeks after fertilization; including perinatal lethality and abnormal behavior in surviving adults. These maternal effect hypomorphic phenotypes are associated with alterations in DNA methylation and expression at imprinted genes. These results establish a mammalian disease paradigm where defects in early epigenetic reprogramming can lead to defects that manifest later in development. To further characterize these heritable effects we are currently using CRISPR to engineer a maternal hypomorphic LSD1 mouse. In addition, we are further exploring the relationship between LSD1 and non-CpG methylation in the nervous system.

Sarah Suciu, GMB **Poster #36**

THE CILIARY PROTEIN ARL13B REGULATES AXON GUIDANCE IN THE MOUSE HINDBRAIN

Sarah Suciu[1,3], Laura Mariani[2,3], Julien Ferent[4], Jiami Guo[5], Eva Anton[5], Frederic Charron[4], Tamara Caspary[3]

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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 and failure of axons called superior cerebellar peduncles (SCPs) to cross the brain's midline. Mutations in cilia-associated genes including *ARL13B* cause JS. ARL13B regulates transcription-dependent Shh signaling, which requires cilia to regulate cell-fate specification and proliferation. *Arl13b* mutations in mice lead to constitutive, 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 abnormal Shh signaling might provide a common mechanism for the diverse symptoms of JS. To examine Arl13b's potential role in transcription-independent Shh signaling, we examined SCP crossing in mouse brains lacking either Arl13b or all Shh signaling in projection neurons. We observed significant midline crossing 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 JS phenotypes.

Shannon Torres, GMB **Poster #37**

UNDERSTANDING THE TRANSCRIPTIONAL REGULATORY MECHANISMS OF THE HISTONE VARIANT, H2A.Z, THROUGH ITS INTERACTIONS WITH CHROMATIN REMODELERS

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Differentiating cells acquire a stable transcriptional program necessary for proper development and homeostasis, which is facilitated by chromatin components, such as incorporation of histone variants into nucleosomes. The histone H2A variant, H2A.Z, is involved in many genomic processes, including transcriptional regulation. However, the mechanism through which it regulates transcription is currently unclear. H2A.Z is necessary for transcription of the *FLOWERING LOCUS C* (*FLC*) gene in *Arabidopsis*, but in the absence of transcriptional repressors, such as the chromatin-remodeling component Brahma, H2A.Z is no longer required for transcription of the gene. This project focuses on elucidating the mechanism by which Brahma antagonizes H2A.Z function to infer the role of H2A.Z in transcriptional regulation. We will determine whether Brahma antagonizes H2A.Z-mediated activation at *FLC* and similarly regulated loci through affecting nucleosome positioning or occupancy and whether H2A.Z localization across the gene is dependent on Brahma. Additionally, we are conducting a forward genetic suppressor screen to identify mutants that alleviate the need for H2A.Z incorporation into nucleosomes for *FLC* activation. Currently, one suppressor line has been identified and characterization is underway. Future work to identify and characterize these genes will provide a deeper understanding of H2A.Z function and transcriptional regulation through chromatin remodeling.

Morgan Barham, IMP **Poster #38**

PHENOTYPIC CHARACTERIZATION OF MYCOBACTERIUM TUBERCULOSIS-SPECIFIC CD4 T CELLS IN INDIVIDUALS WITH HIV CO-INFECTION

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Approximately 90% of immunocompetent individuals infected with *Mycobacterium tuberculosis* (*Mtb*) never develop symptoms of clinical disease and are considered to have latent *Mtb* infection (LTBI). However, co-infection with HIV greatly increases the risk of reactivation of LTBI and progression to TB disease. Although *Mtb*-specific T cell immunity is important in maintaining immune control of *Mtb*, the immune parameters perturbed by HIV infection and result in loss of control of LTBI have not been defined. We hypothesize that *Mtb*-specific T cell function is impaired in the setting of HIV co-infection, which contributes to increased risk of TB disease in co-infected individuals. One mechanism contributing to impaired T cell function is upregulation of immunoregulatory receptors, including PD-1, BTLA, and CTLA-4. Using blood samples from HIV-infected and uninfected adults with LTBI, we tested the hypothesis that immunoregulatory receptors are upregulated on *Mtb*-specific T cells in the setting of LTBI/HIV co-infection. PBMCs were stimulated with *Mtb* CFP-10 and ESAT-6 peptide pools, and analyzed by flow cytometry to evaluate expression of inhibitory receptors by *Mtb*-specific CD4 T cells producing IFN-γ and TNF-α. We have shown that CTLA-4 and PD-1 are upregulated by IFN-γ producing *Mtb*-specific CD8 T cells in HIV-infected individuals.

Lynette Chea, IMP **Poster #39**

DELAYING APOPTOSIS ENHANCES IMMUNOGENICITY OF MVA-BASED VACCINATIONS

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Developing an HIV vaccine to protect from infection is critical to prevent the spread of HIV/AIDS. Modified vaccinia Ankara (MVA) is an immunogenic, attenuated poxvirus being developed as a viral vector for various vaccines. However, MVA-infected cells undergo rapid apoptosis leading to faster clearance of recombinant antigens. This could be due to the fragmentation of the MVA anti-apoptotic gene B13R. Here, we replaced the fragmented B13R with a functional copy and tested its effects on the viability and immunogenicity of MVA. MVA-B13R infected Hela cells were protected from chemically induced apoptosis better than WT MVA confirming functionality of B13R. MVA-B13R infected mouse myoblast cells demonstrated similar effects. To determine immunogenicity, BALB/c mice were immunized twice with MVA or MVA-B13R expressing SIV Gag, Pol and HIV Env (SHIV). One week after the second immunization, MVA-B13R/SHIV immunized mice developed 2-fold higher Env-specific serum antibodies compared to MVA/SHIV mice. MVA-B13R/SHIV immunized mice also had 2.5-fold higher Env-specific memory B cells at four weeks post second immunization. These results demonstrate that restoring B13R functionality in MVA significantly delays MVA-induced apoptosis and is associated with augmented anti-Env antibody responses. Preclinical NHP studies will elucidate the potential of MVA-B13R vector as an HIV vaccine candidate.

Sarah Connolly, IMP **Poster #40**

FC-GAMMA RECEPTOR AFFINITY AS A HOST FACTOR FOR HIV TRANSMISSION

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Heterosexual HIV transmission is modulated by host and viral factors. Previous HIV vaccine trials suggest that the Fc-gamma receptor (FcγR) is one such host factor. The FcγRIIa and FcγRIIIa alleles are polymorphic, with SNPs resulting in different affinities for IgG antibodies, which affect immune cell effector function and HIV disease progression. This study aims to assess the role of FcγRs on HIV acquisition in a heterosexual transmission cohort. The 378 participants in this study, from the Zambia-Emory HIV Research Project (ZEHRP) study cohort, were genotyped at both FcγRIIa and FcγRIIIa loci to define homozygous high affinity (Hhi), heterozygous (Het), or homozygous low affinity (Hlo) genotypes at each locus. No significant difference in allele frequency for either receptor was observed between infected and uninfected groups ($p=0.97$; 0.59). The distribution of FcγRIIa alleles was 21% Hhi/49% Het/30% Hlo, while the FcγRIIIa allelic distribution was heavily skewed towards low affinity alleles, 8%/37%/55%. The genotypic distribution of FcγR alleles in the Zambian population was previously unknown, as is the role of FcγR affinities in HIV acquisition. Although no significant difference was observed based on HIV infection status, further analyses on the impact of FcγR genotypes on the frequency and time to transmission are warranted.

Elina El-Badry, IMP **Poster #41**

GENDER DIFFERENCES IN TRANSMISSION OF HIV-1 VIRAL VARIANTS AND THEIR IMPACT ON EARLY IMMUNE ACTIVATION

Elina El-Badry[1], **Daniel Claiborne**[1], Jessica Prince[1], Gladys Macharia[2], Eileen Scully[3], Jill Gilmour[2], Susan Allen[4], Eric Hunter[1]

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Elucidating the early viral and host interactions in HIV-1 infection is critical to understanding transmission and subsequent disease progression. In a previous analysis of gag, pol, and nef in 135 transmission pairs, we identified a bias for transmission of consensus amino acid residues, apparently related to the transmission of viruses with greater overall fitness. However, females were infected with viruses that had significantly more non-consensus amino acids than males. We hypothesized that transmission of viruses with lower fitness to females may impact early immune activation. We evaluated inflammatory cytokine profiles in a group of males and females using a multiplexed Luminex assay and compared CD4+ and CD8+ T cell activation by multiparameter flow cytometry. Early in infection (0-24 months), females exhibit lower VL and higher CD4+ T cell counts and decreased levels of cellular immune activation in the CD8+ T cell compartment. We also found that females express lower levels of CCL2 and I-FABP. Our results contrast data gathered from the chronic stages of infection, in which females were shown to exhibit exacerbated immune activation. This suggests that the immunological response and inflammatory profile in females may differ between the acute and chronic stages of HIV-1 infection and may be related to the transmission of viruses with lower overall fitness.

Osric Forrest, IMP **Poster #42**

RESISTIN, A NEUTROPHIL-DERIVED IMMUNOMETABOLIC MEDIATOR, IS A CELL-SURFACE ASSOCIATED AND SOLUBLE MARKER OF EARLY AND CHRONIC CF AIRWAY DISEASE

Osric Forrest[1,3], Daniel Chopyk[1,3], Sarah Ingersoll[1,2], Milton Brown[1,2], Bob Scholte[4], Hettie M. Janssens[4], Jessica Alvarez[5], Vin Tangpricha[5], and Rabindra Tirouvanziam[1,2]

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In mice, resistin was identified as an adipokine promoting insulin resistance and inflammation. However in humans resistin is made primarily by neutrophils (PMNs). Human resistin is found in abnormal levels in metabolic disorders and in disorders linked to PMN-driven inflammation. Consistently, resistin is elevated in the plasma and airway fluid of CF patients and correlate negatively with CF lung function. Blood and sputum PMNs from adult CF patients were analyzed by flow cytometry for surface expression of resistin and of its recently identified receptor, adenylyl-cyclase associated protein 1 (CAP1). In vivo, PMNs acquire significantly higher resistin and CAP1 surface expression upon migration from CF blood to airways, concomitant with previously observed high primary granule release. In vitro, primary granule exocytosis of PMNs leads to high surface expression of CD63, resistin, and CAP1. We measured significant resistin levels in CF infant BALF, suggesting that this mediator is not just present at the chronic stage of the disease, but also early in the process. Our results suggest that (i) live CF airway PMNs express both cell surface resistin and CAP1, which may mediate cell-cell-signaling and (ii) resistin may serve as a marker, not only of chronic, but also early CF airway disease, as evidenced by its presence in BALF from CF infants.

Mojibade Hassan, IMP **Poster #43**

OPTIMIZING PDC GRAFT SOURCE FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Mojibade Hassan[1], Kaifeng Lin[1], Jingxia Li[1], Wayne Harris[1], Edmund Waller[1]

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Plasmacytoid dendritic cells (pDC) have been described as facilitating cells and shown to reduce the incidence and severity of GvHD after allogeneic BM transplantation (BMT). Results of BMT CTN 0201 indicate that the incidence of chronic GvHD is increased in patients who receive GCSF peripheral blood (G-PB) grafts despite higher numbers of pDC. We compared the effect of G-PB pDC to BM pDC in the regulation of GvHD in murine allogeneic BM transplant models. BM pDC express CCR9 and CD62L at a higher frequency, which predict homing to the gut, thymus and lymph nodes. In contrast, G-PB pDC expressed maturation markers MHC II and CD86 at a higher frequency, which suggest increased effectiveness in activating T cells. IL-12, a GvHD protecting cytokine, was significantly higher in BM pDC compared to G-PB pDC. Allo-tranplsants containing purified HSC, T cells, and pDC from BM or G-PB we performed. Results showed improved survival among recipients of BM versus G-PB pDC, consistent with published clinical data. These data confirm that donor BM pDC protect against lethal GvHD, and suggest decreased protection using pDC from G-PB grafts. Discovery of mechanisms to recapitulate the beneficial properties of BM pDC in G-PB grafts are of great interest.

Andrew Jones, IMP **Poster #44**

TRIMERIC STABILIZED GP120 IMMUNOGEN PROMOTES ANTI-GP70-V1V2 ANTIBODIES AND NEUTRALIZATION BREADTH AGAINST HIV IN RABBITS AND RHESUS MACAQUES

Andrew T. Jones[1], Venkateswarlu Chamcha[1], Sannula Kesavardhana[2], Linda S. Wyatt[3], Celia C. LaBranche[4], David C. Montefiori[4], Bernard Moss[3], Raghavan Varadarajan[2], and Rama Rao Amara[1]*

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Trimeric HIV-1 Env immunogens are important due to their ability to express quaternary epitopes targeted by broadly neutralizing antibodies while obscuring unfavorable epitopes. Additionally, the RV144 trial results highlighted the importance of inducing antibodies directed against HIV-1 Env V1V2 loops. We recently reported that a trimeric JRFL gp120, stabilized via fusion of a trimerization domain to the de novo N-terminus of a cyclically permuted gp120 (cycP), induces neutralizing activity against HIV-1 including multiple cross-clade tier 2 viruses in guinea pigs in a DNA prime/protein boost approach. Here, we tested the immunogenicity of the cycP protein in a protein prime/protein boost approach in rabbits and as a booster immunization to clade B DNA/MVA vaccine expressing trimeric HIV Env on virus-like particles in rhesus macaques. In rabbits, two cycP protein immunizations induced very high titers of high avidity gp120- and gp70-V1V2-specific IgG that neutralized tier-1A and -1B HIV isolates. These responses were markedly higher than four monomeric gp120 immunizations. Similarly, in rhesus macaques, a single cycP booster immunization significantly expanded gp120- and gp70-V1V2-specific IgG and broadened the neutralization breadth to tier 1B HIV isolates demonstrating that cycP gp120 serves as a robust immunogen that promotes anti-V1V2 antibodies and neutralization breadth against HIV.

G. Michael La Muraglia II, IMP **Poster #45**

T_{FR}:T_{FH} CELL RATIO CORRELATES WITH ANTI-DONOR GERMINAL CENTER REACTIVITY AND DONOR-SPECIFIC ANTIBODY FORMATION FOLLOWING TRANSPLANTATION

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The critical role T follicular helper cells (T_{FH}) play in the development of protective antibody responses to vaccines and pathogens has been recently established, but little is known regarding their role in the generation of donor-specific antibody (DSA) following transplantation. To elucidate the role T_{FH} cells play in antibody-mediated allograft loss, we utilized a minor antigen (mOVA) mismatch T cell receptor transgenic murine transplant model to define both TCR transgenic and endogenous donor-specific T_{FH} responses to mOVA skin grafts. Flow cytometric analysis of graft-draining lymph nodes revealed a robust expansion in the frequency and absolute number of donor-specific TCR transgenic and endogenous $CXCR5^{+}PD\text{-}1^{hi}$ $Bcl6^{+}$ T_{FH} cells, and endogenous $CD95^{+}GL7^{+}$ GC B cells in response to an mOVA skin graft as compared to a syngeneic graft. The frequency of $CXCR5^{+}Bcl6^{+}Foxp3^{+}$ T follicular regulatory (T_{FR}) cells reciprocally decreased with an mOVA graft, along with the T_{FR}:T_{FH} ratio as compared to the response to a syngeneic graft. These donor-reactive GC changes correlated with the initiation of DSA (anti-OVA IgG) formation, and were abrogated with CD28 costimulation pathway blockade. These data serve as a platform to investigate the mechanisms of DSA formation for the development of therapeutic strategies to control humoral alloimmunitty following transplantation.

Sonia Laurie, IMP **Poster #46**

2B4-MEDIATED INHIBITION OF PROLIFERATION AND GLYCOLYTIC FUNCTION ATTENUATES T CELL ALLOREACTIVITY

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Current thinking holds that costimulation-blockade resistant rejection following solid organ transplantation may be mediated by $CD28^{null}$ T cells. To further investigate this possibility, we performed a retrospective immunophenotypic analysis of adult renal transplant recipients who experienced acute rejection on belatacept treatment compared to those that did not. We identified a subset of $CD28^{null}$ $CD4^{+}$ T_{EM} that are elevated at baseline in patients that did not go on to experience acute rejection ($p<0.0001$) and noted increased expression of the coinhibitory molecule 2B4 on this population ($p=0.05$). To investigate whether 2B4 confers exhaustion in transplantation, we retrogenically expressed 2B4 on murine $CD8^{+}$ OT-I T cells. 2B4 expression resulted in reduced accumulation of OT-I T cells in the spleen 10 days post-transplantation ($p=0.03$) due to reduced proliferation of 2B4rg cells ($p=0.002$). To investigate the mechanisms by which 2B4 expression results in decreased proliferation, we interrogated differences in the metabolism of 2B4-deficient T cells. 2B4KO cells exhibited increased glycolytic capacity and showed significantly enhanced uptake of 2-NBDG following *Listeria monocytogenes* infection ($p=0.02$). We conclude that 2B4 signals dampen glycolysis, limiting proliferation and accumulation of alloaggressive T cells, suggesting that manipulating metabolism may be a novel strategy to target donor-reactive T cells following transplantation.

Amanda Mener, IMP **Poster #47**

C3 NEGATIVELY REGULATES ANTIBODY RESPONSE TO RBC ANTIGEN BY MODIFYING TARGET ANTIGEN

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Although red blood cells (RBCs) provide a tool to study complement, the impact of complement on anti-RBC alloantibody development remains unknown. As previous studies demonstrate that C3 positively impacts antibody formation, we examined C3 as an adjuvant during RBC alloimmunization. We transferred RBCs that transgenically express human KEL into wild-type (WT) or C3KO recipients. While KEL RBCs induced robust anti-KEL antibody formation and C3 deposition in WT recipients, exposure to KEL RBCs in C3KO recipients showed unexpectedly increased anti-KEL antibodies compared to WT recipients. We next examined antibody engagement and complement fixation on masking the KEL antigen. Consistent with a potential role for complement in impacting KEL antigen availability to the immune system, KEL RBCs transferred into WT recipients experienced decreased detectable KEL antigen over time that paralleled the development of anti-KEL antibodies and C3 deposition. In contrast, C3KO recipients failed to experience the same degree of KEL antigen reduction. Western blot analysis of RBCs post-transfusion revealed complete removal of the KEL antigen, indicating that C3 may mediate the removal of KEL from the cell surface. In summary, these results suggest an unexpected role for C3 in negatively regulating antibody responses by specifically modulating the target antigen.

Maria White, IMP **Poster #48**

QUANTIFYING THE EFFECTS OF RNA PACKAGING SIGNAL DIVERGENCE ON INFLUENZA A VIRUS REASSORTMENT

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Influenza A virus (IAV) is an RNA virus with eight distinct genomic segments. These segments contain regions called RNA packaging signals, which direct the incorporation of segments into assembling virus particles. These packaging signals are segment- and strain-specific, and as such, they could potentially impact reassortment outcomes between different IAV strains. Our study aimed to quantify the importance of HA, NA, and NS packaging signal mismatch to IAV reassortment using viruses of the seasonal H3N2 and pandemic H1N1 lineages. We constructed pairs of viruses with identical open reading frames but differing packaging signals and genotyped emergent viruses from co-infected cells. Our results show a significant preference for incorporation of HA segments containing matched packaging signals relative to the background of the virus, but no preference for incorporation of NA or NS segments containing matched packaging signals. Our data suggest, based on packaging signals alone, that the NA and NS segments would move somewhat freely between H3N2 and H1N1 lineages, while movement of the HA segment would be constrained. These data imply that the HA segment and its inherent packaging signals could be an important factor in determining the likelihood that two IAV strains of public health interest will undergo reassortment.

Kelsie Brooks, MMG **Poster #49**

DIVERSITY OF HIV-1 PROVIRAL SEQUENCES DURING ANTIRETROVIRAL TREATMENT FROM TRANSMITTED VARIANTS

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Antiretroviral therapy (ART) can suppress HIV in infected individuals, but does not cure infection. ART does not target a population of latently infected cells, or the HIV reservoir, which serves as a source of viral resurgence if ART is discontinued. Enhanced understanding of latent proviruses may contribute to their elimination. Here we investigate four HIV-1 infections in Zambia-Emory HIV Research Project volunteers. These individuals were identified as HIV+ early in infection, and were also treated with ART. We amplified and sequenced HIV genomes from early infection to identify the transmitted virus, and also amplified and sequenced genomes during ART to assess the relationship between transmitted and latent HIV. Proviral sequences from during ART show an array of sequence diversity when compared to the transmitted virus in the same individual, indicating viral evolution prior to persistence of variants in the reservoir. Mutations observed in proviral sequences include deleterious frameshifts and nonsense mutations, but 60% of proviral sequences were free of obvious defects and inferred to be capable of replicating. Ongoing investigation into the diversity of pre-ART and during ART sequences will provide a clearer understanding of the source of reservoir proviruses, which are the major barrier to HIV cure.

Jessica Coates, MMG **Poster #50**

RANDOM POPULATION FLUCTUATIONS UNDER ANTIBIOTIC STRESS LEAD TO UNPREDICTED TREATMENT OUTCOME

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Increased antibiotic treatment failure is an alarming threat to public health. While many studies have focused on understanding large populations and the rise of genetic mutations, there is very little information available on the importance of small populations and transient phenotypic resistance. Here, we present a study demonstrating the stochastic nature of small bacterial populations nearing extinction. Using single cell microscopy, we characterize how small susceptible populations respond to antibiotics. We found that when exposed to bactericidal drugs, bacteria experience stochastic cell death causing fluctuations in population size. We also find that when exposed to bacteriostatic drugs, cell dynamics are less random and more uniformed. Using a coarse-grained model to explain these observations, we explore the likelihood of increasing population eradication using combination drug therapy. This study advances our understanding of population dynamics of bacteria exposed to antibiotics and has implications for the future design of antibiotic treatments to promote eradication.

Ashley Cross, MMG **Poster #51**

REGULATION OF LIPOPOLYSACCHARIDE O ANTIGEN IN MUCOID *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa isolated from the lungs of people with cystic fibrosis overproduces the exopolysaccharide alginate resulting in a mucoid phenotype that is associated with the establishment of a chronic infection. This phenotype correlates with decreased lung function and a worse prognosis of disease. Mucoid clinical isolates generally express a defective lipopolysaccharide lacking both long and very long (VL) O antigen while nonmucoid strains from initial infections express both O antigen lengths. An intermediate phenotype has been observed where mucoid strains express long O antigen but fail to express VL O antigen. Wzz proteins control the O antigen chain length with Wzz2 regulating VL O antigen. We have determined that *wzz2* mRNA levels are decreased in mucoid strains and that regulation is at the level of transcription initiation. Additionally, we have discovered two putative *wzz2* promoters and confirmed a *wzz2* regulator. Additional experiments are ongoing to unravel the network that mucoid strains use to alter their O antigen profile. Understanding the important processes involved in establishing a chronic infection will provide opportunities for targeted intervention at early stages of infection.

Kara Phipps, MMG **Poster #52**

REASSORTMENT POTENTIAL OF H1N1 AND H3N2 HUMAN INFLUENZA A VIRUSES

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Reassortment events, which occur when multiple influenza viruses co-infect the same cell and exchange gene segments, facilitate viral diversification and have led to pandemics as well as seasonal outbreaks. Since 1977, human influenza viruses of H1N1 and H3N2 subtypes have co-circulated with relatively few documented cases of co-infection and intersubtype reassortment and little sustained spread. To assess reassortment potential between these subtypes, we performed homologous co-infections using a pair of phenotypically identical of H3N2 viruses and heterologous co-infections between H3N2 and pandemic H1N1 (pH1N1) viruses. Homologous and heterologous co-infections produced similar levels of reassortment and genotype diversity, while analysis of genotype patterns revealed only heterologous co-infections resulted in biased reassortment. Detected gene segment linkages were assessed for functional differences by measuring chimeric polymerase activity and growth in primary human tracheobronchial epithelial cells which revealed fitness differences. *In vivo* guinea pig experiments demonstrated that while parental viruses dominated infection, reassortant genotypes also successfully infected and transmitted to other animals. These results indicate that diverse reassortant genotypes can arise from co-infection of H3N2 and pH1N1 viruses, and while some genotypes formed may exhibit fitness defects, some reassortant viruses may be sufficiently fit to spread in the human population.

Lalita Priyamvada, MMG **Poster #53**

PRE-EXISTING DENGUE IMMUNITY MAY ALTER THE COURSE OF ZIKA VIRUS INFECTIONS

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Zika virus (ZIKV) is a mosquito-borne flavivirus of significant public health concern. Due to its association with Guillain-Barre Syndrome in adults, and neurological and ocular complications in neonates, it has become increasingly important to understand the immunology and pathobiology of ZIKV infections. ZIKV shares a high degree of sequence and structural homology with other flaviviruses, including dengue virus (DENV), resulting in immunological cross-reactivity. In this study, we addressed the issue of cross-reactivity between DENV and ZIKV by testing sera and plasmablast-derived monoclonal antibodies from acutely infected secondary dengue patients against ZIKV. We observed that both acute and convalescent dengue sera could potently bind and neutralize ZIKV. A majority of the dengue plasmablast-derived mAbs we tested bound to whole virus, and a small subset was also able to neutralize virus in vitro. In addition, we also demonstrated antibody dependent enhancement of ZIKV infection in the presence of dengue antibodies. Taken together, these findings suggest that preexisting immunity to DENV may impact protective immune responses against ZIKV. In addition, the extensive cross-reactivity may have implications for ZIKV virulence and disease severity in DENV-experienced populations.

Roxana Rodríguez Stewart, MMG **Poster #54**

DEVELOPMENT OF REOVIRUS AS AN ONCOLYTIC THERAPEUTIC AGAINST TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer constitutes approximately 15% of all breast cancer, has a higher rate of relapse, and shorter overall survival after metastasis than other subtypes of breast cancer. Treatment for this type of cancer is largely limited to cytotoxic chemotherapy. We aim to make an improved therapeutic against triple-negative breast cancer using mammalian orthoreovirus (reovirus), a virus that infects most humans during childhood, but is mostly asymptomatic. Reovirus preferentially kills transformed cells and is currently in Phase I-III clinical trials to assess its efficacy as an oncolytic agent against a variety of cancers. We adapted reovirus to replicate in MDA-MB-231, a triple-negative breast cancer cell line, by co-infecting these cells with parental reoviruses and serially passaging them. A reassortant reovirus (r2Reovirus) was isolated and has shown to have genomic segments from two different reovirus serotypes. Although r2Reovirus attachment to MDA-MB-231 cells is not altered, it has greater infectivity and induces more cell death with faster kinetics than parental reoviruses. These data suggest that r2Reovirus encodes genomic changes that enhance its ability to infect and kill triple-negative breast cancer cells. Future work will characterize the contribution of genetic modifications on r2Reovirus replication and cell death pathways induced by virus infection.

Riley Perszyk, MSP **Poster #55**

CHANNEL OPEN PROBABILITY CONTROLS ALLOSTERIC MODULATION OF POTENCY AND EFFICACY

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Kinetic models of NMDA receptors (NMDARs) that separate binding and gating into two distinct steps predict that the enhancement of open probability will shift the equilibrium to activated states and therefore enhance agonist EC50. CIQ is a positive allosteric modulator (PAM) of GluN2C- and GluN2D-containing NMDARs, increasing the current response to maximal effective concentrations of agonist by 3-fold. Surprisingly, CIQ did not detectably alter glutamate potency. We identified a CIQ analogue, (-)1180-55, that potentiates GluN2B-containing NMDARs. (-)1180-55 enhances GluN2D-containing NMDARs by 3-fold but has no detectable effect on glutamate EC50, which was 0.5±0.07μM in control and 0.4±0.01μM in 20μM (-)1180-55. By contrast, (-)1180-55 increased the response of GluN2B-containing NMDARs to maximally concentrations of agonist by 2-fold and increased glutamate potency by 2-fold, 2.0±0.16μM in control to 1.2±0.07μM in 20μM (-)1180-55. To explore why, we modelled the potentiation of efficacy for a de Castillo and Katz model of channel function. For low open probability, doubling open probability from 0.01 to 0.02 does not detectably alter the EC50. The relationship between open probability and allosteric modulation is thus critical to understanding whether PAMs will alter agonist potency by shifting the gating/binding equilibria, or whether their binding pose impacts the ligand binding domain.

Brandon Stauffer, MSP **Poster #56**

BACTERIAL SPHINGOMYELINASE IS A STATE-DEPENDENT INHIBITOR OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR)

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Bacterial sphingomyelinase C (SMase) inhibits CFTR chloride channel activity in multiple cell systems, an effect that could exacerbate disease in CF and COPD patients. The mechanism by which sphingomyelin catalysis inhibits CFTR is not known but evidence suggests that it occurs through a mechanism independent of CFTR's regulatory "R" domain. In this study we performed experiments in the *Xenopus* oocyte expression system to shed light on the molecular basis of inhibition. We found that the pathway leading to inhibition is not membrane delimited and that inhibited CFTR channels remain at the cell membrane, indicative of a novel silencing mechanism. Consistent with an effect on CFTR gating behavior, we found that altering gating kinetics influenced the sensitivity to inhibition by SMase. Specifically, increasing channel activity by introducing the mutation K1250A or pretreating with the CFTR potentiator VX-770 (Ivacaftor) imparted resistance to inhibition. Some mutations that impede CFTR gating led to an increase in sensitivity suggesting that SMase targets a subset of closed states. Finally, we found that SMase-inhibited currents could not be restored by VX-770. Taken together, these data suggest that SMase inhibits CFTR currents by stimulating a cytosolic pathway that locks channels into a closed state at the cell membrane.

Matt Tillman, MSP **Poster #57**

ELUCIDATING THE MECHANISM OF LBP-8 SHUTTLING OF LYSOSOMAL LIPIDS INTO THE NUCLEUS IN *C. ELEGANS*

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Lipids not only play a vital role as an energy source and structural component in the cell, but also serve as signaling molecules. Many lipids have been identified as ligands for nuclear receptors, such as PPARs, to regulate transcription, however, it isn't understood how insoluble lipids derived from membranes and organelles are transported into the nucleus. Lipid binding proteins (LBPs) were discovered to solubilize lipids and transport them to the nucleus, serving an integral role in lipid-signaling pathways. Lipid binding protein 8, LBP-8, which is highly expressed in the fat storage tissue of *Caenorhabditis elegans,* was recently discovered to bind to lipids derived from lysosomes and shuttle them into the nucleus to prolong the lifespan of worms. Oleyolethanolamide was identified as a lysosomal lipid that bound to LBP-8 and activated transcriptional activity of NHR-49 and NHR-80 proteins, orthologs of human PPARs, to mediate the life extending effects. We have determined the first 1.38 Å high-resolution structure of LBP-8, which has allowed us to identify a possible nuclear localization signal that is conserved in other LBPs. We now plan to use this structure for directed mutational studies to elucidate the mechanism of lysosomal lipid signaling to the nucleus.

Laura Butkovich, NS **Poster #58**

A NOVEL TRANSGENIC MOUSE MODEL TO INVESTIGATE PARKINSON'S DISEASE-LIKE α-SYNUCLEIN PATHOLOGY IN NORADRENERGIC NEURONS

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While cell loss and α-synuclein (αsyn) aggregates in the substantia nigra pars compacta (SNpc) are a major hallmark of Parkinson's disease PD, pathology in the locus coeruleus (LC) is commonly more severe, and may even precede that found in the SNpc and contribute to nigrostriatal loss. While most PD research has focused on the SNpc, we have lacked suitable models to understand how αsyn pathology specifically affects noradrenergic neurons in PD, and whether noradrenergic neurons are vulnerable to αsyn pathology. To examine this question, we have developed a BAC-transgenic mouse model overexpressing wild-type human αsyn under the control of the noradrenergic-specific dopamine β-hydroxylase promoter. These animals overexpress human αsyn in LC neurons, and enteric neurons derived from the neural crest. Preliminary analysis revealed human αsyn immunoreactivity and mRNA expression in noradrenergic neurons of the LC in transgenic mice. In the transgenic gastrointestinal system there is a significant increase in αsyn expression in enteric neurons at 14 months, raising the possibility that the model may also prove useful for examining peripheral pathologies commonly seen in PD in the gastrointestinal tract related to αsyn accumulation.

Mary Herrick, NS **Poster #59**

ROLE OF SOLUBLE TNF AND PERIPHERAL INFLAMMATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Obesity and hypertension contribute to increased risk for neurodegenerative diseases, including Alzheimer's disease (AD). Peripheral inflammation, induced by poor diet and lack of exercise, influences normal brain function and increases blood brain barrier (BBB) permeability and brain inflammation, both of which contribute to an increased risk for neurodegenerative disease. However, the exact mechanism by which chronic peripheral inflammation impacts AD-like pathology is unknown. Elevated levels of TNF in the CSF and plasma have been reported in AD patients. TNF promotes brain inflammation, which can result in increased BBB permeability, and abnormal access to the brain. Here we tested the hypothesis that soluble TNF mediates diet impacted AD-like pathology and facilitates peripheral immune cell trafficking across the BBB. To test this hypothesis, we used an AD mouse model with 5 point mutations (5xFAD mouse) fed a high-sugar high-fat diet to induce low grade-chronic peripheral inflammation. We inhibited peripheral and central soluble TNF signaling with the BBB permeable peptide XPro®1595, and assessed brain immune cell populations via flow cytometry. Here we demonstrate that after induction of peripheral inflammation from diet, immune cell populations are changed in the brain. Experiments are ongoing to test the hypothesis that XPro®1595 modulates these population changes.

Carlie Hoffman, NS **Poster #60**

DEVELOPMENT OF A HIGH-THROUGHPUT ASSAY TO MEASURE VMAT2-MEDIATED VESICULAR DOPAMINE TRANSPORT

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Cytosolic dopamine is packaged into vesicles by the vesicular monoamine transporter 2 (VMAT2; SLC18A2). Perturbations in VMAT2 function have been found to result in altered vesicular integrity within the nigrostriatal dopamine system, modified neuronal vulnerability to toxic insult, and increased risk for neurodevelopmental and neurodegenerative diseases such as Parkinson's disease. Recent studies indicate that overexpression of VMAT2 enhanced dopamine release and conferred protection to toxicants (Lohr et al., 2014). The recent development of the false fluorescent neurotransmitter, FFN206, by Sames and Sulzer (Rodriguez et al., 2013) allowed us to optimize a cell-based high-throughput assay using FFN206 and HEK293 cells stably transfected with VMAT2 in 96-well plates. This assay has a dynamic range that allows detection of compounds that inhibit or enhance vesicular uptake. We have demonstrated that the assay yields appropriate values for dopaminergic drugs such as tetrabenazine (TBZ) and environmental toxicants such as polychlorinated biphenyls (PCBs) when compared to values obtained from vesicular dopamine uptake in isolated synaptic vesicles. This assay should provide a robust initial screening of compounds that alter VMAT2 function that can then be verified using dopamine uptake in isolated vesicles and fast scan cyclic voltammetry.

Elizabeth Kline, NS **Poster #61**

THE ROLE OF MYELOID MHC-II IN A-SYNUCLEIN-INDUCED DEGENERATION AND RISK FOR PD

Elizabeth M. Kline, George T. Kannarkat, Jianjun Chang, Jeremy M. Boss, and Malu G. Tansey

Antigen presentation via major histocompatibility complex class II (MHC-II) controls the immune response to specific targets. In Parkinson's disease (PD), substantia nigra dopamine neurons are burdened with α-synuclein aggregates. Cells expressing MHC-II have been identified within post-mortem substantia nigra (SN) of PD patients, often near dopamine (DA) neurons and α-synuclein aggregates. Microglia and peripheral myeloid cells may contribute to neuroinflammation by presenting a neuron-derived antigen (possibly α-synuclein) to CD4+ T cells via MHC-II. *rs3129882,* a common single nucleotide polymorphism (SNP) in the *HLA-DRA* gene in the MHC-II loci is associated with PD risk. Our group found that peripheral blood monocytes of healthy control (HC) human subjects with the high-risk genotype at *rs3129882* (GG) display 30 times more MHC-II protein relative to HC (AA); and PD (GG) patients exhibit a 300-fold greater increase in MHC-II mRNA compared to PD (AA), suggesting that myeloid MHC-II levels may contribute to progression. In order to better understand the contributions of myeloid MHC-II to α-synuclein-induced degeneration, mice with deletion of the MHC-II gene *I-Ab* in peripheral myeloid cells received a stereotaxic injection of rAAV2-human WT α-synuclein to the SN. Unbiased stereology was used to estimate loss of DA neurons in the SN.

Stephanie Pollitt, NS **Poster #62**

NOVEL ROLE FOR ACTIN BINDING PROTEIN LASP1 IN THE AXON GROWTH CONE

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During neural development, axons must extend rapidly and precisely to form the specific synaptic connections that underlie mature circuits in the brain. This process relies on directional responses of the motile growth cone at the tip of the axon to a wide range of extracellular cues. How extracellular cues are translated into distinct motile behaviors remains to be fully understood. LIM and SH3 Protein 1 (LASP1) is a unique actin-binding protein that contains several protein interacting domains for signal transduction and actin regulation. To explore the role of LASP1 in growth cone dynamics and axon guidance, we first examined its expression and subcellular distribution in the mammalian neuronal CAD cell line as well as in mammalian primary neurons. Interestingly, we found that LASP1 is localized to the leading edge of both lamellipodia and filopodia. This unique localization in motile actin structures requires both the LIM and Nebulin domains. We are currently performing knockdown experiments to examine the functional role of LASP1 in growth cone motility and guidance.

Ashley Swain, NS **Poster #63**

ULTRASTRUCTURAL FEATURES OF PALLIDAL GABAERGIC TERMINALS IN THE VENTRAL MOTOR AND CAUDAL INTRALAMINAR THALAMIC NUCLEI IN NORMAL AND MPTP-TREATED PARKINSONIAN MONKEYS

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The internal segment of the globus pallidus (GPi) is an output nucleus of the basal ganglia. In primates, single GPi neurons send GABAergic projections to the parvocellular part of the ventral anterior nucleus (VApc) and the centromedian nucleus (CM) of the thalamus. In the parkinsonian state, the activity of GPi neurons and their axonal projections to the thalamus is altered. In this study, we used anatomical and electron microscopic methods to determine potential changes in neurotransmitter content and morphology of GPi terminals in the VApc and CM in control and MPTP-treated parkinsonian monkeys. Light microscopic quantification of various presynaptic GABA markers did not reveal any significant difference between control and parkinsonian animals. Similarly, the pattern of synaptic connectivity of GPi terminals was not altered, such that they contacted preferentially proximal dendrites of thalamic neurons in both thalamic nuclei in control and parkinsonian monkeys. Three-dimensional reconstruction of single pallidothalamic terminals is in progress to assess potential changes in the total number of synapses formed by individual GPi terminals in parkinsonian monkeys. These findings will provide a solid foundation for potential changes in structure-function relationships that may underlie the pathophysiology of the pallidothalamic system in Parkinson's disease.

Mfon Umoh, NS **Poster #64**

PROTEOMIC PROFILING OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMETIA DISEASE OVERLAP

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are progressive neurodegenerative diseases with substantial clinical, pathological, and genetic overlap. A hexanucleotide repeat expansion in the C9orf72 gene is the most frequent reported genetic cause of ALS and FTD. The objective of this study was to identify pathways involved in disease pathogenesis that are unique and similar to each clinical phenotype across this spectrum. We performed an unbiased, quantitative proteomic screen using post-mortem brain tissue from patients clinically diagnosed with ALS (n=19), FTD (n=12), and patients who had both ALS and FTD (n=10), compared to normal (without neurological disease) controls (n=10). Patient tissue included those with and without the C9orf72 expansion mutation. Our data identified several pathways that differentiated these clinically defined groups using weighted correlation network analysis. These included RNA binding proteins, inflammatory markers, proteins involved in nucleocytoplasmic transport, and synaptic proteins amongst other pathways. Using principal component analysis, we found that the proteomic signatures segregated out by clinical diagnosis. The presence of a C9orf72 expansion mutation was not an independent variable associated with proteomic differences. Nevertheless, we found protein changes that correlated with the expansion status in the ALS cases. These data provide new protein pathway targets for experimental research and therapeutic development.

EXPERIMENTAL PERTURBATIONS OF *CULEX RESTUANS* MOSQUITO POPULATIONS AND THEIR EFFECT ON THE TRANSMISSION OF WEST NILE VIRUS BY THE PRIMARY MOSQUITO VECTORS, *CULEX QUINQUEFASCIATUS*

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West Nile virus (WNv) is a zoonotic vector-borne pathogen transmitted among song birds by *Culex spp.* mosquitoes. Currently, the ecological mechanisms behind the seasonal re-emergence of the WNv transmission cycle are unknown. In the eastern United States, one possible source of viral re-emergence is the survival within and amplification by the early season mosquito species *Culex restuans;* as the season progresses WNv transmission then transitions to the primary epidemic vector *Culex quinquefasciatus*. To test the hypothesis that WNv transmission is ecologically linked between these two species, we conducted a field experiment in which we suppressed *Cx. restuans* breeding populations in two urban parks in Atlanta, GA with the larvicide Altosid (8.6% methoprene). We then monitored WNv activity in the enzootic cylec by testing blood samples obtained from birds for WNv antibodies and testing adult female mosquitoes for active WNv infections. These surveillance methods were paired with collections in two untreated parks. Estimates of sero-prevalence of WNv in birds were similar across parks (range 50 – 70%); the detection of antibodies in hatch year birds indicate active WNv transmission during the transition period between *Cx restuans* and *Cx. quinquefasciatus* populations. There was no detectable effect of *Cx. restuans* control on WNv transmission.

Amanda Vicente-Santos, PBEE **Poster #66**

NEOTROPICAL BATS THAT CO-HABIT WITH HUMANS FUNCTION AS DEAD-END HOST FOR DENGUE VIRUS

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Studies suggest that Neotropical bats may be susceptible to Dengue Virus (DENV) infection. We aim to elucidate the role of house-roosting bats in DENV transmission cycle. Households were sampled from high and low dengue incidence regions during rainy and dry seasons in Costa Rica. We captured 318 bats from 12 species in 29 households. Necropsies were performed to analyze virus presence in organs and histopathology studies showed no manifestation of disease or infection. Sera were analyzed by $PRNT_{90}$ for a seroprevalence of 22% (53/241), and by PCR for 8.8% (28/318) positive bats for DENV RNA. Viral isolation from positive samples was unsuccessful. Positive blood samples showed virus concentrations under the minimal dose required for mosquito infection (qRT-PCR). Simultaneously, 651 mosquitoes were collected and analyzed for DENV and feeding preferences (cytochrome b). Three mosquitoes were DENV-positive and none was positive for bat cytochrome b. Our results suggest an accidental presence of DENV in bats probably caused from oral ingestion of infected mosquitoes. Phylogenetic analyses suggest also a spillover event from humans to bats. We conclude that bats in these urban environments do not sustain DENV amplification; not having a role as reservoirs, but function as an epidemiological dead-end host for DENV.

Signe White, PBEE **Poster #67**

DAUER ALTERS BACTERIA PREFERENCE IN CAENORHABDITIS ELEGANS

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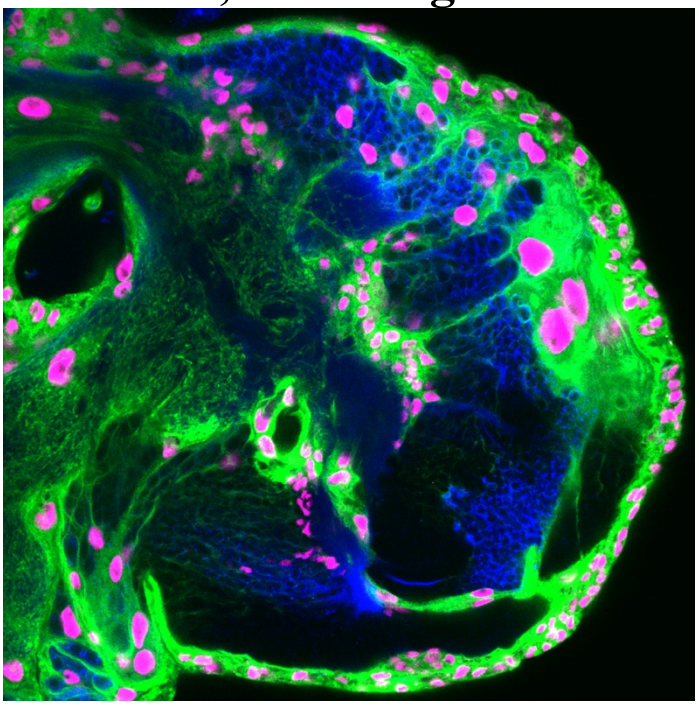
Pathogenic microbes are ubiquitous in nature and hosts must be able to mitigate the negative effects of these pathogens. Hosts often use avoidance as the first line of defense against pathogenic bacteria in order to prevent infection. *Caenorhabditis elegans*, a commonly used nematode in experimental biology, exists with a large diversity of bacterial species in nature, many of which serve as the worm's primary food source. While previous studies have shown that adult worms are unexpectedly attracted the pathogenic bacteria *Serratia marcescens*, these studies do not take into account an important nematode life stage known as "dauer." Dauer is known to be an important phenotype in nature as most natural isolates are found and collected in this stage. Dauer serves as a developmental arrest when external conditions become stressful. In our experiments, we found that dauer larvae do not choose *S. marcescens* as seen in adult worms and instead prefer *Escherichia coli*, a benign food source. Our experiments suggest that behavioral differences may allow dauer worms to better discriminate between bacterial species, thus providing them with a potential fitness advantage relative to non-dauer worms.

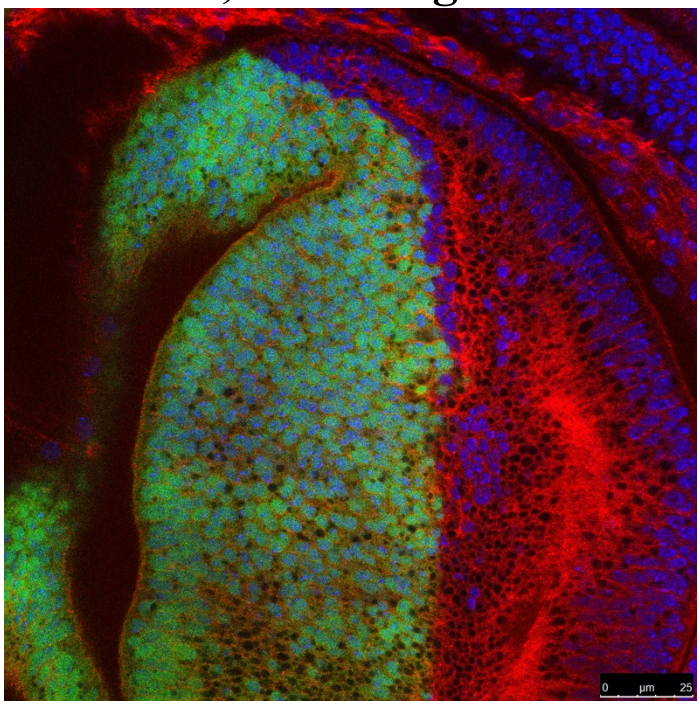
4th Place, ICI Image Contest



Alex Chen, Cancer Biology

These are Drosophila melanogaster 3rd instar larval brains used as a model organism to study Glioblastoma. They have constitutively active EGFR and PI3K and stained with morphological stains. Neuronal projections are stained blue, glial cell bodies are stained green, and glial cell nuclei are stained magenta.

5th Place, ICI Image Contest



Briana Brown, Cancer Biology

DAPI and Phalloidin stain in the Apterous expression domain of D. Melanogaster 3rd instar larvae wild type wing imaginal disc.