RUTGERS
Molecular Biosciences
Graduate Student Organization

16th Annual MBGSO Research Symposium

Friday March 24, 2023

9:30 AM-4:30 PM

Life Sciences Building Atrium

Busch Campus

Rutgers University

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A Letter from the Organizers

Welcome to the 16th Annual Graduate Student Symposium hosted by the Rutgers University Molecular Biosciences Graduate Student Organization (MBGSO). We are delighted to have you join us today to support the outstanding research produced by graduate students in the Molecular Biosciences Graduate Program and related graduate programs. We are fortunate to be able to foster a scientific community here at Rutgers.

As a student-led organization, the goal of MBGSO is to facilitate the professional development of graduate students and provide opportunities for continued growth, learning, and networking. By hosting the MBGSO Annual Symposium we hope to not only showcase graduate student research but also to encourage collaboration and interdisciplinary discourse. With support from the university community our presenters receive valuable feedback and input on their work as well as an opportunity to disseminate their research to a broader audience. With these goals in mind, we organize the annual symposium and look to the university community to make it a success.

We would like to express our gratitude to our faculty advisor, **Dr. Janet Alder**, for her tireless work on behalf of the graduate students and for providing valued guidance and support. We would also like to thank **the administrative personnel supporting the Rutgers School of Graduate Studies** for their role in coordinating this and several other events throughout the year. We gratefully acknowledge the **Molecular Biosciences faculty** for their ongoing support and commitment to our graduate program and for volunteering their time to participate in the symposium to judge student presentations and offer valuable feedback and insight. We are also grateful to our keynote speaker, **Dr. Liping Zhao**. And finally, this event would not be possible without the participation of the graduate student presenters, and so **we offer a hearty thanks to our peers** for helping create and nurture the vibrant intellectual and social community within the Molecular Biosciences Graduate Program. We are proud and honored to be at the service of the Molecular Biosciences community.

Sincerely,
MBGSO Executive Board 2022-2023

President: **Nora Jaber**

Vice President: **Siwen Wu**

Treasurer: **Elena Forzisi**

Secretary: **Nikita Amin**

Program Coordinator: **Nathalie Groot**

Development Officer: **Derek Cavallo**

Symposium Schedule

9:30AM - 10:00AM……**Registration and Welcome**

10:00AM - 11:00AM……**Oral Presentations I**

11:00AM - 12:00PM……**Poster Presentations I**

12:00PM - 1:00PM……**Keynote Address**

1:00PM - 2:00PM……**Lunch**

2:00PM - 3:00PM……**Poster Presentations II**

3:00PM - 4:00PM……**Oral Presentations II**

4:00PM - 4:30PM……**Award Ceremony**

Keynote Address

“Ecology-based discovery of microbiome biomarkers for human health management”

Dr. Liping Zhao, PhD

Dr. Liping Zhao is a Professor in the Department of Biochemistry and Microbiology in the Rutgers School of Environmental and Biological Sciences with an extensive academic and professional resume. He received a B.S. in Plant Protection from Shanxi Agricultural University, and earned a Ph.D. in Molecular Plant Pathology from Nanjing Agricultural University in China. Since then, he has held positions as a professor, director and dean in esteemed academic institutions. In 2017, Dr. Zhao was appointed the Eveleigh-Fenton Chair of Applied Microbiology at Rutgers.

In the professional sphere, Dr. Zhao directs the Center for Nutrition, Microbiome, and Health at Rutgers University. He has also served as a board member of the International Society for Microbial Ecology (ISME), editor of various journals, including ISME and FEMS Microbiology Ecology, and Chair of the Big Idea Committee for the International Human Microbiome Consortium (IHMC). He is also a fellow of both the American Academy of Microbiology and Canadian Institute of Advanced Research (CIFAR), and a member of the scientific advisory board for the Center for Microbiome Research and Education, American Gastroenterological Association (AGA). Dr. Zhao holds an impressive 10 patents and has served on the Nutritional Advisory Board of Barilla Company.

Dr. Zhao’s research focuses on the connection between human health and the gut microbiome. His lab hopes to develop “health-centric” strategies that improve long-term health outcomes by restoring a healthy gut ecosystem to patients with chronic metabolic diseases. The goal is to formulate nutritional interventions that rectify the host-microbiome relationship to achieve preventative health benefits.

ORAL PRESENTATIONS

AM presentations

10 AM-10:15 AM: HBV integrations into KMT2B Drive Liver Carcinogenesis
Presenter: Gregory Marshall
Liver Cancer is the second-leading cause of cancer related deaths worldwide. Of all liver cancers, hepatocellular carcinoma (HCC) comprises 80% of all cases. Of these HCC cases hepatitis B virus (HBV) infection is known to be the cause of about half of all the cases, making HBV-induced HCC a necessary field of research. Our lab has previously performed genomic analysis on HBV positive HCC samples, and recurrent HBV integrations were found to localize in two major loci, TERT and KMT2B (MLL4), where in the case of KMT2B all integrations are localized between exon 3 and 6. Though TERT is a well-established oncogene, the oncogenic function of HBV integration into KMT2B remains largely unknown. Our preliminary data suggests that these HBV integrations into KMT2B result in a C-terminal truncated version of KMT2B (KMT2B-T) and that KMT2B-T is oncogenic in vivo. Furthermore, our data also shows that KMT2B-T binds to tumor suppressor MENIN (Men1) and its binding partner LEDGF. The overexpression of KMT2B-T decreases the binding of MENIN to the endogenous KMT2A/B histone methyltransferase complex. Our data also shows that the differential length of truncated KMT2B influences tumorigenesis. Therefore, we hypothesize that C-terminal truncated KMT2B produced by HBV integrations between exons 3 and 6 does induces HCC by sequestering MENIN from the endogenous KMT2A/B complex. These studies provide the basis for targeting dysregulated KMT2B as a potential therapeutic approach in HBV caused liver cancer.

10:15 AM-10:30 AM: A Kcnb1 Mutation Causes Early Onset Epileptic Encephalopathies
Presenter: Alessandro Bortolami
Potassium (K+) channels are robustly expressed throughout the cortex since the first stages of neurodevelopment, but their function is poorly understood. Here, we investigate the role of a voltage-gated K+ channel KCNB1 (Kv2.1) in the context of neocortical development. To determine whether defective KCNB1 can give rise to developmental channelopathy, we constructed Knock In (KI) mice, harboring the gene variant Kcnb1R312H (R312H mice) found in children with developmental and epileptic encephalopathies (DEEs). Neuronal migration of glutamatergic neurons was impaired in the neocortices of R312H mice. Migratory defects persisted into the adult brains, along with disrupted morphology and synaptic connectivity. Wild type and R312H KCNB1 channels made complexes with integrins α5β5 (Integrin_K+ channel_Complexes, IKCs), whose biochemical signaling was impaired in R312H brains. In vitro treatment with Angiotensin II, an agonist of Focal Adhesion kinase, a key component of IKC signaling machinery, corrected the neuronal abnormalities. This underscores a previously unknown role of IKCs as key players in neuronal development and implicate developmental channelopathies in the etiology of DEEs.

10:30 AM-10:45 AM: Linking Autophagy to Non-Small Cell Lung Cancer Metastasis
Presenter: Kyle Nunn
Autophagy is a conserved self-degradative process that has a key role in cellular stress responses and survival. Tumor cells rely on autophagy to tolerate microenvironment stress for survival while supporting their characteristic increase to growth and metabolic rate. Recent studies using GEMMs demonstrated that autophagy supports several types of tumor growth with distinct mechanisms. Lung cancer alone accounts for nearly 25% of all cancer related deaths in the United States, typically with a 5-year survival rate of only 22%. However, once metastasis has begun, these rates plummet to as low as 7%. While the role of autophagy in cancer has been intensively studied, the role of autophagy in lung tumor metastasis is still elusive and often controversial. Autophagy has been tied to several cellular functions important to both cell migration and invasion, with research detailing direct roles in migration machinery, cellular secretions, ECM remodeling and adhesion. Recent studies have focused on the effect of autophagic ablation, though "knowledge gap" surrounding host-specific effects still exists. It is imperative to explore the effect autophagic loss on the microenvironments of both tumors, and typical sites of metastasis. In preliminary studies, a KL tumor derived cell line (TDCL) was modified using a lentivirus to generate a model for luciferase expression. These cells were utilized in early disseminated tumor cell colonization (DTCC) in vivo trials, via tail vein injection into the systemic autophagy inducible GEMM under both normal and ablated autophagy conditions. Most interestingly, we also noticed a difference in both preferred tumor microenvironments (TME) and overall lung morphology between autophagy intact and ablated groups. To better understand the viability of autophagy inhibition as a method of treatment in lung cancers, it is critical to understand how autophagy influences these observed results.

10:45 AM-11:00 AM: Best practices for massively parallel perturbation techniques – a comprehensive assessment
Presenter: Jiayi Liu
Perturbation-based massively parallel reporter assays (MPRAs) have enabled the en masse identification of functional elements within non-coding genomic regions. However, the gold standard of perturbation sequence design methods remains scant. Here, we utilize a publicly available dataset we recently generated of both wild type (WT) and perturbed (PERT) sequences. The perturbed sequences were carefully designed to target transcription factor (TF) binding motifs in regulatory regions, using three different perturbation approaches. The first two perturbation approaches replace the motif with a constant 'non-motif' sequence, while the third approach randomly scrambles the motif's nucleotides. The MPRA readout of each perturbation sequence consists of a) functional regulatory sites (FRS) identification: a binary multiclass label indicating whether is a functional or non-functional site; b) regulatory effect (Log2FC): a numeric variable. We first define metrics to assess and compare the effect of the perturbation in each method. We next fit machine-learning models to predict MPRA outputs. In detail, we extract sequence-based features of WT and PERT sequences, and subtract the corresponding WT features from each of the PERT features. We use these feature differences as input for linear and non-linearseveral prediction models with FRS identity labels and Log2FC as target values in classification and regression models, respectively. We find that the third perturbation approach has a higher specificity for targeting the motif and the lowest number of net motif changes. Moreover, we find that the non-linear models exhibit high robustness for classification and regression in the three perturbation approaches, with the third perturbation outperforming the first two. Overall, our results suggest that modeling perturbation-based MPRA output is robust and that the random nucleotide shuffling model can serve as a framework for such assessments, leading to improvements in MPRA experimental design.

PM presentations

3 PM-3:15 PM: mTORC1 Activity Oscillates in a Cell Cycle-Dependent Manner
Presenter: Jay Joshi
Mechanistic Target of Rapamycin Complex 1 (mTORC1) is a master metabolic regulator that integrates nutrient and growth factor/hormone signals to control cellular anabolism. mTORC1 dysregulation contributes to the development and progression of many diseases, including most human cancers. mTORC1 is activated in most, if not all, proliferating eukaryotic cells, but whether mTORC1 is regulated in a cell cycle-phase specific manner is unknown. We hypothesized that mTORC1 activity changes throughout the cell cycle to meet the unique metabolic and biosynthetic demands of each cell cycle phase. Using multiple independent methods to track mTORC1 activity throughout a complete cell cycle, we find that mTORC1 activity oscillates from lowest in mitosis/G1 to highest in S-phase and G2. These changes correlate with the induction of Cyclin E and Cyclin A in S and G2 respectively, suggesting Cyclin E and A could promote mTORC1 activation during this time. Cell cycle regulation of mTORC1 appears to be mediated through changes in subcellular localization of an essential negative regulator of mTORC1 called the TSC complex. Inhibiting mTORC1 in G2 delays mitotic induction, revealing a novel role for mTORC1 in potentially driving cell growth to satisfy the G2/M checkpoint. Taken together, our data uncover cell cycle-dependent oscillations in mTORC1 activity and suggest that mTORC1 could have phase-specific functions in promoting cell growth and proliferation.

3:15-3:30 PM: Dietary fat source induces gut microbiota alterations and impacts graft-versus-host disease severity in mice
Presenter: Danielle Millick
It has been observed that a constriction of the gut microbiome occurs with high fat diet, and likewise decreased microbiome diversity is directly correlated with more severe graft-versus-host disease (GvHD) following allogeneic hematopoietic stem cell transplantation. Taken together it is plausible that not only fat content, but source of dietary fat could influence gut microbiota and GvHD outcome. We sought to address this question and developed experimental rodent diets that have the same fat content (25% kcal fat, a moderate increase in fat content from normal chow) which differ only by fat source: animal- versus plant-derived fat and found that these diets drive alterations in gut microbiota diversity. Both diets yielded similar outcomes in an acute model of GvHD, however we found that mice fed the animal-derived fat diet exhibited worse survival and outcomes in a model of chronic GvHD, than mice fed the plant-derived fat diet. These findings correlated with increased inflammation and alterations in intestinal immune cell populations following chronic GvHD induction. Collectively, our data show that diets that are equal in macronutrient content but different in fat source can promote different gut microbiomes and in turn alter intestinal homeostasis and chronic GvHD outcome.

3:30 PM-3:45 PM: Two RNA binding proteins, ADAD2 and RNF17, interact to form novel meiotic germ cell granules required for male fertility

Presenter: Lauren Chukrallah

Mammalian male germ cell differentiation relies on complex RNA biogenesis events, many of which occur in non-membrane bound organelles termed RNA germ cell granules that are rich in RNA binding proteins (RBPs). Though known to be required for male germ cell differentiation, we understand little of the relationships between and functions of the numerous granule subtypes. ADAD2, a testis specific RBP, is required for normal male fertility and forms a poorly characterized granule in meiotic germ cells. This work aimed to define ADAD2 granules in male germ cell differentiation and their relationship to other granules. Biochemical analyses identified RNF17, a testis specific RBP that forms meiotic male germ cell granules, as an ADAD2-interacting protein. Phenotypic analysis of mutants defined a shared and rare post-meiotic chromatin defect, suggesting shared biological roles. We further demonstrated ADAD2 and RNF17 depend on one another for granularization and together form a previously unstudied set of granules. Based on co-localization studies with well-characterized granule RBPs, a subset of the ADAD2-RNF17 granules are likely components of the piRNA pathway in meiotic cells. A second, morphologically distinct population of ADAD2-RNF17 co-localize with the translation regulators NANOS1 PUM1 and form a unique cup-shaped structure with distinct protein subdomains. This cup shape appears to be driven, in part, by association with the endoplasmic reticulum. This work identified a set of novel germ cell granules required for male fertility. These findings shed light on the relationship between germ cell granule pools and define new genetic approaches to their study.

3:30 PM-3:45 PM: Whole Transcriptome Screening for Novel Genes Involved in Meiosis and Fertility in Drosophila melanogaster

Presenter: Siqi Sun

The accurate segregation of chromosomes during meiosis is vital for reproductive success. Failure to segregate chromosomes properly can lead to infertility in women and various birth defects such as Down's Syndrome. Proper chromosome segregation is dictated by a variety of contributing factors, including protein complex involved in spindle assembly and sister chromatid cohesion. However, many proteins required for meiosis are undiscovered and thus unstudied. In this study we aimed to identify and characterize novel meiotic genes within the genome of Drosophila melanogaster to further elucidate the process of meiosis and contribute to the understanding of human reproduction. To accomplish this, genes showing upregulation within meiotically-active tissues were identified. About 200 genes with no known function were then targeted and silenced using RNA interference (RNAi) and the effects of their silencing was assessed. We identified 66 genes that when silenced, showed signs of meiotic errors, including infertility and/or high level of chromosomal nondisjunction. With this process, we identified novel genes, such as CG3430, that are important for meiosis. These genes are strong candidates for future studies to characterize their meiotic functions and their importance in the successful completion of meiosis.

POSTER PRESENTATIONS
11:00AM – 12:00PM Session

Presenter: Nathalie Groot
Poster: *Decoding Calcium Oscillations in Dendritic Cells in vitro and in vivo during Bacterial Stimulation*
Authors: Groot, N., Sathuvalli, P., Sung, C., Barbet, G.
Dendritic cells (DCs) play a major role in orchestrating immune responses to the various threats an organism faces. Disseminated throughout the organism, DCs serve as sentinels, the first line of the immune system's defense. Manipulation of DCs ex or in vivo is part of the strategy of most vaccines, and DC-based immunotherapy has been tested in clinical trials for anti-cancer therapy, as well as for infectious diseases like Influenza or HIV. In DCs, the ubiquitous secondary messenger Ca2+ regulates many different key functions like motility, migration, phagocytosis, cytokine production and antigen presentation. Despite its crucial role in orchestrating the immune response, the regulation and mobilization of Ca2+ in DCs remains poorly understood. Here, we observed and characterized in vitro Ca2+ signals in bone-marrow-derived Dendritic cells, BMDCs, upon bacterial stimulation. Our observations show specific Ca2+ oscillations elicited by different strains of bacteria. Furthermore, we observed drastic differences in the Ca2+ signals induced by live, heat-killed, and gram-negative but not gram-positive bacteria.
Using a newly designed reporter mouse, we also aim to study Ca2+ signals in DCs in situ, using two-photon microscopy. Like for the bone marrow-derived DCs, we are currently characterizing the Ca2+ signals in the dendritic cells within the skin of the ear and the spleen in response to bacteria.
Thanks to its central role to many immune functions, we aim to better understand the characteristics of calcium signals in the biology of DC to better tailor immune responses in autoimmune disorders, infections or vaccinations.

Presenter: Michelle Bilotti
Poster: *Peripubertal BPA exposure accelerates the onset of puberty and is associated with reduced motivated behavior in early adulthood: A role for the orexin (hypocretin) system*
Authors: Bilotti, M., Isskandar, V., Anthony, S., Bello, N., Roepke, T., James, M.
Perinatal exposure to estrogenic endocrine disrupting compounds such as bisphenol-A (BPA) is associated with precocious puberty, which itself is a strong predictor of poor mental health outcomes (e.g., depression) in early adulthood. Neurons in hypothalamus that produce orexin, a neuropeptide involved in motivated behavior, are important for the expression of depression-like outcomes in adulthood, including anhedonia (reduced reward seeking). However, no studies have explored how BPA exposure, at doses that promote early puberty, affects orexin system functioning and the implications for depression-like behaviors in early adulthood. Here, we exposed female Long-Evans rats to BPA (0, 25, 250µg/kg/day) via their drinking water between postnatal days (PND) 29-56. Animals were monitored for vaginal opening (pubertal onset) and then lavaged daily to track their estrous cycles. One cohort of females was tested for binge-like intake of palatable food. A separate cohort was trained to lever press for sucrose pellets at increasing fixed ratio (FR) schedules to measure economic demand (motivation) for a hedonic (rewarding) stimulus, followed by responding on a FR1 schedule for grain pellets, and a saccharin preference test. On PND 97, brains were collected for immunohistochemistry and qPCR analyses. Peripubertal BPA (25 and 250µg/kg) exposure was associated with irregular cycling and earlier vaginal opening. At the highest dose, BPA was associated with reduced binge-like eating, reduced motivation for sucrose and preferred intake of sucrose at null cost, and reduced preference for a saccharin solution. No difference was found in FR1 responding for grain pellets. Furthermore, BPA dose-dependently decreased orexin gene expression in the hypothalamus, and reduced reactivity of orexin neurons to food-associated stimuli.

Collectively, these findings suggest that peripubertal BPA exposure is associated with advanced puberty onset and reduced motivation for palatable food (i.e., anhedonia) in early adulthood, and that the hypothalamic orexin system may be a potential mediator of these outcomes.

Presenter: Rukia Henry
Poster: *Interrogating the Role of the Lysine Methyltransferase, EHMT2, in promoting responses to DNA Damage and Repair.*
Authors: Henry, R, Rodriguez, L, Ginjala, V, Kulkarni, A, Yao, M, Ganesan, S.
Cancer cells reprogram various cellular processes to support their malignant and tumorigenic phenotypes. In many stages of cancer cell reprogramming, the epigenetic landscape is usually hijacked to support the silencing of tumor suppressor genes through the aberrant activity of chromatin modifiers such as DNA methyltransferases and histone deacetylases. Our lab has recently published work on the lysine methyltransferases EHMT2 and EHMT1 (G9a and GLP1 respectively), and their role in supporting genetic stability. G9a amplification and its role in the silencing of tumor suppressor genes have been observed in a vast array of cancers, including breast cancer, and these amplificatory features exhibit the need to elucidate a clearer understanding of this lysine methyltransferase's role in maintaining genomic integrity. A catalytic inhibitor of G9a, UNC0638, has been developed and widely used to investigate its effects towards inhibiting the cellular activity of G9a. Recent studies in our lab have shown that there is altered signaling of the ATM substrates yH2AX and MDC1 upon G9a catalytic inhibition. Biochemical analysis showed that G9a is found in complex with HDAC 1, and this interaction is dependent on G9a's catalytic activity. Collectively, these data suggests that G9a interacts with and methylates HDAC 1 during the DNA damage response (DDR). We hypothesize that the regulation of HDAC 1 by G9a plays a critical role in the regulation of ATM signaling during the DDR. These findings are critical, as this pathway may serve as a pharmacodynamic marker in clinical settings, especially in cancers where G9a is aberrantly expressed.

Presenter: Yicong Le
Poster: *Comparison of different muscle-specific promoters in AAV vector*
Authors: Le, Y; Jiang, Z; Choi, S, Zhou, R
Gene therapy is a promising strategy for treating a large variety of diseases. An important consideration in the eventual application of many of the gene therapies in patients is restricting expression to particular cells and tissues involved in disease pathogenesis. Tissue specific (or selective) expression would likely reduce off-target or side effects, minimize immunological attack of transduced cells and might enable use of lower doses/titer of the therapeutic vectors, among other advantages. In this study, we compared 10 different muscle-specific promoters (Pmus) in Adeno-associated Virus (AAV) vector based on previous publications and constructions designed in our own lab to identify the strongest and most compact vector promoter for muscle gene expression. The muscle-specific promoters and regulatory elements were cloned into the AAV9 vector which express reporter gene luciferase. In vitro assay showed that all Pmus vectors have moderate to strong expression in differentiated C2C12 muscle cells but little to no expression in HEK293T cells. Among all 10 muscle-specific expression vectors, Pmus7 has the highest expression level in differentiated C2C12 cells. Brain-derived neurotrophic factor (BDNF) was cloned into this vector (AAV9-Pmus7-BDNF) and in vitro expression/secretion of exogenous BDNF was confirmed. This vector is now being assessed for therapeutic effects in the Amyotrophic Lateral Sclerosis (ALS) disease model mice. Muscle promoters we tested here show different strength in driving gene expression in muscle cells and could also be used as a platform for developing gene therapy approaches for neuromuscular diseases in which different expression levels are needed in muscle cells and tissues.

Presenter: Margot Shumaker
Poster: *The Impact of Gestational Antibiotics on Offspring Health*
Authors: Shumaker, M and Blaser, M
The increasing prevalence of obesity, diabetes, and autoimmune diseases in Western societies has been well documented. The cause of these health implications, however, remains unclear. One hypothesis for increasingly poor health is microbiome alterations caused by excessive antibiotic usage. Pregnant women in particular form a population that is subject to high antibiotic exposure, with 20-40% of them receiving antibiotics. Little is known about how the gestational antibiotics affect both the health of the mother and her offspring. To answer this question, we investigate the impact of gestational amoxicillin—a commonly used antibiotic during pregnancy in humans—on offspring immune function and metabolism. Using a mouse model (C57BL6/NTac), we gavaged pregnant dams with a therapeutic dose of amoxicillin (25mg/kg) for up to four days. Offspring metabolic and immune health was then monitored using body composition analysis, blood draws, fecal samples, as well as glucose and insulin tolerance testing. Our findings indicate that alterations to the maternal gut microbiome during late pregnancy resulted in phenotypic differences in the offspring, with pups of amoxicillin-treated dams gaining more weight and having higher fat composition than control pups. To determine the transgenerational impact of gestational antibiotics, this experiment will be repeated over four generations. This will reveal if the phenotypic differences caused by gestational antibiotics compound over generations, which will then help elucidate the role of gestational antibiotics in the increasing prevalence of poor metabolic and immune health.

Presenter: Mary Lally
Poster: *Investigating the local and systemic effects of orally administered vancomycin with a parabiotic model*
Authors: Lally, M and Blaser, M
Vancomycin is an antibiotic prescribed to treat infections caused by Gram-positive bacteria, including Clostridioides difficile and methicillin-resistant Staphylococcus aureus. Although vancomycin can combat life-threatening illnesses, the extent of its effects on the host-microbiome relationship remains unclear. When taken orally, vancomycin is non-absorbable into the systemic circulation, meaning that it cannot cross the gut-epithelial barrier, but it is bactericidal to susceptible commensal microbes within the gut lumen. By affecting commensal bacteria that play roles in host immunity, vancomycin can alter the host-microbiota relationship. To better address this question following vancomycin use, we established a parabiotic mouse model (surgical joining of two mice to create a shared circulatory system). We hypothesized that orally administering vancomycin to one mouse will alter the microbiota of only the recipient mouse; but through the shared circulation will alter the composition and distribution of circulating immune cells in both mice. To date, we have completed 13 survival surgeries. Our data support that chimerism is established approximately two weeks after the parabiosis surgery and that a single, oral dose of vancomycin (100 mg/kg) may alter the ratio of circulating immune cells. By investigating the local and systemic effects of vancomycin, we aim to better understand the systemic effects of local changes in the microbiome, and potentially develop new options for patients taking the antibiotic.

Presenter: Kyle Flannery
Poster: *Improving Genetic Diagnosis Yield In Congenital Muscular Dystrophy through Cryptic Splicing Analysis*
Authors: Flannery KP, Safwat S, Hoffman EP, Manzini MC
Whole exome sequencing (WES) has been a remarkable tool in identification of disease-causing variants in Mendelian disorders. However, intronic and synonymous variants are often overlooked in standard exome analyses. In this study, we maintained a database of 114 individuals with severe congenital muscular dystrophies (CMDs) such as Walker Warburg syndrome (WWS; N=65), Muscle Eye Brain disease (MEB; N=27) and merosin-deficient CMD (MDC1A; N=22). Through a combination of computational and functional analyses

of missense, frameshift, and nonsense mutations, we achieved a genetic diagnosis rate of 69.23% of WWS cases, 66.67% of MEB cases, and 86.36% of MDC1A cases. In the remaining unsolved cases, WES data was annotated with SpliceAI and SQUIRLS and filtered for intronic and synonymous variants that were confidently predicted to disrupt splicing. Through this strategy, we identified six additional variants. Three patients with MDC1A were found to harbor intronic variants in LAMA2 (c.3556-13T>A; c.4960-17C>A; c.5234+9A>G) in heterozygosity with another pathogenic variant. A homozygous deep intronic variant in POMT2 (c.817-394A>G) was also identified in one patient with WWS. In addition, two synonymous variants were identified in our analyses: one in POMGNT1 (c.570C>T) in a patient with MEB in heterozygosity with a splice donor variant, and one homozygous variant in LAMA2 (c.1884G>A) in a patient diagnosed with MDC1A. We are currently leveraging RT-PCR and minigene splicing assays to experimentally validate these variants. Future studies will include strategies such as RNA-seq and whole genome sequencing, both of which have the potential to improve the genetic diagnosis yield of severe CMDs.

Presenter: Jordan Levy
Poster: *General Control Non-Derepressible 2 is Required for Cold Induced Hepatic FGF21 Secretion in Male Mice*
Authors: Levy j, Mirek E, Rodriguez E, Anthony T
The integrated stress response (ISR), a core signaling pathway used to respond to a wide variety of cellular stresses, is implicated in playing a key role in maintaining core body temperature when challenged with an acute cold stress. Recent publications demonstrate that amongst the evolutionarily conserved kinases responsible for activating the ISR, general control non-derepressible 2 (GCN2) is required for survival at cold temperatures in plants. To understand if the function of GCN2 extends to thermal regulation in a mammalian system, wild type (WT) and whole body Gcn2 knockout (GCN2 KO) male mice between 11 and 13 weeks of age were challenged with 4°C cold exposure for 9-hours with both body temperature and indirect calorimetry measures taken over the study duration. Following the 9-hour period, mice were killed, and their brown adipose tissue (BAT) was collected. Differential gene expression of their BAT and ISR activity was analyzed using RT-qPCR and western blotting . Compared to their WT counterparts GCN2 KO mice were unable to maintain their core body temperature after 6-hours of cold exposure. In conjunction, GCN2 KO mice displayed significantly reduced energy expenditure and respiratory exchange ratio values over the 9-hour study period. Furthermore, following the 9-hour exposure period, GCN2 deletion significantly impeded cold induced hepatic FGF21 production in male mice. Overall, GCN2 is required to maintain core body temperature when challenged with an acute cold stress via impaired energy metabolism and energy substrate utilization that corresponds with reduced circulating FGF21.

Presenter: Nora Jaber
Poster: *Protein-Membrane Interactions and its Effects on Phase Separation Dynamics of Transmissive Huntingtin*
Authors: Jaber N., Lee Y., Jiang J., Favetta B., Barai M., Schuster B., Dai W.
The biological mechanism of neurodegenerative diseases remains an enigma. Many proteins associated with neurodegeneration are intrinsically disordered proteins (IDPs) and exhibit the phenomena of phase separation. There is currently little knowledge of the molecular factors that contribute to the formation of these phase-separating assemblies, as well as how organization of their microenvironment controls IDP function. Evidence shows the presence of lipid membranes modulate the formation of IDP inclusions, suggesting an IDP-membrane interaction in modulation of IDP phase separation dynamics and functions. The goal of this research is to characterize the protein-membrane interactions and phase-separation dynamics of IDPs to understand the functional regulation of intrinsically disordered proteins, using mutant huntingtin (mHTT) as a model protein. When huntingtin's intrinsically disordered poly-glutamine (polyQ) tract is expanded, one is at risk for Huntington's Disease. Little is known about this disease pathology, including how mHTT transmits from

cell-to-cell. We will take an interdisciplinary approach by combining cryo-electron tomography (cryoET) to elucidate the architecture of the polyQ inclusions, biophysical analyses to study their phase separating properties, as well as biochemical assays to better understand huntingtin transmission.

Presenter: Leelabati Biswas
Poster: *Decoding Pregnancy Loss: Validating a Novel Genetic Biomarker of Poor Egg Quality*
Authors: Biswas, L.; Aboelenain, M.; Tyc, K.; Sun, S.; Xing, J.; Schindler, L.
A quarter of all pregnancies end in miscarriage; half of miscarriages are caused by aneuploidy, a chromosome number imbalance. Maternal age at ovulation is the only biomarker for an individual's risk of ovulating an aneuploid egg. However, for a subset of infertility patients, egg aneuploidy occurs more often and earlier than is predicted by maternal age. For these patients, no predictive biomarker of egg aneuploidy exists.
We hypothesized that certain genetic variants increase egg aneuploidy relative to maternal age and could thus serve as predictive biomarkers for this trait. Therefore, we sought to identify and validate aneuploidy-causing variants as potential genetic biomarkers of increased aneuploidy relative to maternal age.
Using a biobank of whole-exome sequence data from 178 infertility patients with statistically extreme rates of aneuploid conception relative to maternal age, we identified 9 genetic variants enriched in individuals with disproportionately high rates of aneuploidy. Based on screening of these variants in mouse oocytes, we selected a promising candidate genetic biomarker, Kif18aMotorDomainMutant (Kif18aMDM), for in vivo validation. We generated a novel knock-in Kif18aMDM mouse line. Eggs from Kif18aMDM mice are more frequently aneuploid than those from wild-type counterparts and have morphological abnormalities, including micronucleation and fragmentation. Importantly, Kif18aMDM mice are significantly less fertile than wild-type counterparts. Therefore, Kif18aMDM, which was identified in infertility patients with extreme rates of aneuploidy, may increase egg aneuploidy and decrease fertility. These findings lay the groundwork for the establishment of Kif18aMDM as the first predictive genetic biomarker of increased egg aneuploidy relative to maternal age.

Presenter: Brian Zalma
Poster: *Autophagy regulates whole-body lipid metabolism in mice exposed to PEG-asparaginase*
Authors: Zalma, B., Rodriguez, E., Mirek, E., Anthony, T.
Hepatotoxicity and steatosis are common adverse events of treatment with asparaginase, a chemotherapeutic which degrades serum asparagine and is used to treat acute lymphoblastic leukemia. The mechanisms of these side effects are not well understood. Autophagy is upregulated during amino acid insufficiencies, but the role it plays in response to asparaginase is not known. To elucidate the role of autophagy during exposure to asparaginase, we subjected mice with autophagy intact (Atg7-intact) and absent (Atg7-KO) mice to 5 days of pegylated asparaginase (PEG-asparaginase) or phosphate-buffered saline (PBS) control. PEG-asparaginase induced significant loss of weight, lean mass, and fat mass in Atg7-intact mice. In contrast, Atg7-KO mice were partially protected from these effects. Atg7-intact mice also developed hepatic steatosis during exposure to PEG-asparaginase while Atg7-KO mice did not. Indirect calorimetry showed PEG-asparaginase increased energy expenditure over the 5d exposure in both genotypes, yet Atg7-KO mice moved substantially less. These results indicate that autophagy contributes to PEG-asparaginase-induced hepatic steatosis.

Presenter: Jiayu Shen
Poster: *Mitochondrial transcription factor TFAM dynamically bends DNA in a sequence-dependent manner*
Authors: J.Shen, H.Huh, A.Ramachandran, Y.Ajjugal, S.Lee and S.Patel
Mitochondrial transcription factor A (TFAM) belongs to the high-mobility-group-box (HMG-box) family that binds and bends the mtDNA in specific and non-specific ways, correlating with its two distinct roles as a

transcription initiation factor and a DNA packaging protein. TFAM binds to the upstream promoter (-39 to -16) region to recruit mitochondrial RNA polymerase (POLRMT) at the specific promoter sites (LSP and HSP) and form a pre-initiation complex (PIC). On the other hand, TFAM covers the whole genome and highly compacts it. How TFAM regulates its transcription and packaging functions still needs to be better understood.
In this study, we measured the TFAM DNA binding affinity, DNA bending angles, and TFAM-POLRMT complex on different DNA substrates (promoter: LSP/HSP, or non-promoter DNA). We observed: 1) TFAM binds promoter and non-promoter DNA at comparable affinity. 2) TFAM bends all DNAs but dynamically transits between two bending states corresponding to partially and fully bent (~180º) DNA. The bending/unbending transition rates and the bending stability depends on the DNA sequence, with the fully bent state most stable for LSP > HSP > non-promoter DNA. 3) The TFAM-POLRMT complexes are only formed in the presence of DNA and most efficiently when TFAM is bound to LSP and the least to non-promoter sequence. 4) The C-tail deleted TFAM mutant, Δ26 TFAM, which lacks the ability to bend DNA fully, barely forms the TFAM-POLRMT complex. Based on these results, we propose that the TFAM-POLRMT complex can only be formed when TFAM binds to DNA and fully bends it. Our data shows that the differential conformational dynamics and stability of the fully bent state of TFAM on promoter and non-promoter DNAs contribute to the specific recruitment of POLRMT to the transcription start site. This work provides new insights into the multifunctional role of TFAM and how it uses its degree of DNA bending to regulate its role as a transcription factor and packaging protein.

Presenter: Danny Wan
Poster: *Predicting Structures and Functions of Chlamydia trachomatis Plasmid-Encoded Proteins*
Authors: Wan, D., Fan, H.
Chlamydia trachomatis is the leading cause of bacterial sexually transmitted infections and curable blindness in the world. C. trachomatis is an obligate intracellular bacterium that infects epithelial cells of the urogenital tract and conjunctiva. Common among all C. trachomatis serotypes is a 7.5-kilobase plasmid that serves as a virulence determinant. The plasmid encodes 8 proteins termed Pgp1-8, whose molecular functions remain undefined. We used AlphaFold to predict the three-dimensional structures of the Pgps and we then searched for structural homologs for the modeled Pgp structures on DALI. Our analyses revealed strong homology at the three-dimensional structure level between the AlphaFold models and experimentally-resolved protein structures. Specifically, Pgp1 and Pgp2's structures matched protein structures involved with DNA replication, Pgp5 and Pgp6 matched structures involved with plasmid partitioning, and Pgp7 and Pgp8 matched integrases that mediate integron recombination. Our findings suggest that Pgps contribute to virulence by regulating plasmid and chromosome replication and segregation.

Presenter: Adesewa Adesina
Poster: *Functional Annotation of Chlamydial Hypothetical Protein through Structural Modeling and Homology Search*
Authors: Adesina, A., Wan, Danny., Fan H
Chlamydia is an obligate intracellular bacterium with a unique developmental cycle. The chlamydial genome encodes numerous hypothetical proteins. While their functions remain unknown, they are probably the most important proteins since most of them are unique to Chlamydia. To better understand chlamydial biology, our lab performed structure modeling using AlphaFold for all Chlamydia trachomatis hypothetical proteins and 3-dimensional structural homology search for the AlphaFold models on DALI. Our results suggest that CT274 serves as a potential molecular chaperone and may assist protein folding in normal physiological processes and during stress responses.

2:00PM – 3:00PM Session

Presenter: Karen Mae A. Bacalia
Poster: *Cannabidiol decreases intestinal inflammation in estrogen-deficient but not estrogen-sufficient mice*
Authors: Bacalia K, Tveter K, Palmer H, Douyere J, Martinez S, Sui K, and Roopchand D
Cannabidiol (CBD) (25 mg/kg peroral) treatment was shown to improve meta-bolic outcomes in ovariectomized (OVX) mice deficient in 17β-estradiol (E2). Herein, we investigated CBD effects on intestinal and hepatic bile acids (BAs) and inflammation. Following RNA sequencing of colon tissues, differentially ex-pressed genes (DEGs) were sorted in ShinyGO and inflammatory response and bile secretion pathways were further analyzed. Colon content and hepatic BAs were quantified by LC-MS. The direct effect of CBD (100, 200, 500 μM) on inflammation was assessed in gut organoids. Expression of 78 out of 114 inflammatory response pathway genes were reduced in CBD-treated OVX mice relative to vehicle (VEH)-treated OVX mice. In contrast, 63 of 111 inflammatory response pathway genes were increased in CBD-treated sham surgery (SS) mice compared to VEH-treated SS group and 71 of 121 genes were increased due to ovariectomy. CBD did not alter BA profiles in colon content or liver. CBD re-pressed TNFα and Nos2 expression in intestinal organoids in a dose-dependent manner. CBD suppressed colonic inflammatory gene expression in E2-deficient mice but was pro-inflammatory in E2-sufficient mice suggesting CBD activity in the intestine is E2-dependent.

Presenter: Nydia Chang
Poster: *Roles for astrocytic RIPK3 in Parkinson's disease pathogenesis*
Authors: Chang, N., Nissenbaum, M., Atkins, C., Kusnecov, A., Daniels, B.
Parkinson's disease (PD) is the second most common neurodegenerative disorder, imposing an estimated cost of $52 billion per year in the United States alone. PD pathology is driven by the progressive degeneration of dopaminergic neurons in the midbrain substantia nigra pars compacta (SNpc) and the reduction of dopamine release in the striatum. Emerging evidence suggests that neuroinflammation is a key player in the pathophysiology of this degenerative process. Astrocytes are the most abundant glial cells in the central nervous system (CNS), where they can mediate neuroinflammation following stimulation. Degenerative neurons can also facilitate a noxious feed-forward loop of neuroinflammation, aggravating the process of astrocyte activation. Reactive astrocytes can exert neurotoxic activity, resulting in neuronal cell death. Numerous studies have revealed that reactive astrocytes can contribute to clinical neurodegenerative diseases. However, the mechanism through which homeostatic astrocytes become activated requires further investigation. We have identified receptor-interacting protein kinase-3 (RIPK3) activation as a driver of inflammatory astrocyte activation, resulting in neurotoxic effects and neuron loss in the midbrain. Additionally, we have identified damage-associated molecular patterns (DAMPs) released by neurons undergoing cell death as potential drivers of inflammatory astrocyte activation. Our findings suggest that DAMPs induced by neuronal death activate RIPK3 signaling in astrocytes, resulting in inflammatory activation followed by neurotoxic effects. This astrocyte-mediated activation contributes to neuronal death and neurodegeneration. These findings highlight the complex interplay of cell death signaling and neuroinflammation, providing new insights into the roles for astrocytes in the pathogenesis of Parkinson's disease.

Presenter: Abby Heller
Poster: *Assessing a CC2D1A Hypomorph Mouse Model of Autism Spectrum Disorder*
Authors: Heller, A., Bhattacharya, A., Manzini, M.C.
Cc2d1a loss of function (LOF) mutations have been implicated in the development of Autism Spectrum Disorder (ASD), intellectual disability, and aggressive behavior, particularly in males. Knockout (KO) mice for Cc2d1a die at birth and behavioral impairments can only be studied in conditional models removing Cc2d1a expression in

the brain. In mice the homozygous insertion of the V5/HA epitope tag into the C-terminus end of the transcript leads to >84% protein degradation early in development, resulting in a novel hypomorph model of Cc2d1a LOF. So far, we found that 1a-V5/HA homozygous mice are viable and fertile and have no sensory-motor deficits. However, 1a-V5/HA homozygous males show a trend for increased anxiety in the open field and reduced exploration in the novel object recognition test.

Presenter: Kiranmayi Vemuri
Poster: *RNA polymerase II dynamics during intestinal differentiation in vivo*
Authors: Vemuri, K; Kumar, S; Verzi, MP
The mammalian intestinal epithelium is comprised of a series of finger-like projections called villi, and invaginations into the mesenchyme called crypts. The villus is a compartment of terminally differentiated cells, such as enterocytes, goblet cells, and enteroendocrine cells. Conversely, the crypt consists of adult stem cells and transit amplifying cells and is the site of proliferation. Differentiation of the intestinal epithelium underlies nearly all aspects of intestinal health including processing of dietary lipids. Hence, mechanisms of differentiation must be carefully balanced. A critical barrier to progress in the field of intestinal epithelial biology is that we do not have a complete understanding of transcriptional regulatory mechanisms driving differentiation in vivo. Gene expression analyses from our lab has revealed a dynamic transcriptome as cells differentiate from crypts to villi with nearly 4000 genes differentially regulated. Little is known about how RNA polymerase II (pol II) is regulated during intestinal differentiation, but it has potential to reveal new mechanisms through which differentiation is accomplished. We applied pol II ChIP-Seq in isolated intestinal crypt and villus cells to measure pol II occupancy at a gene. We identified nearly 1200 genes with significant pol II occupancy differences in the crypt and villus. Functional annotation revealed these genes-controlled compartment specific functions, including brush border and lipid metabolism in the villus and proliferation-specific terms like translation and ribosome in the crypts. Further, these same pol II enriched genes correlated strongly with compartment-specific enhancer-promoter interactions from a H3K4Me3 HiChIP and gene expression profiles. We also aimed to understand how HNF4, an important intestinal differentiation factor, impacted pol II dynamics in the intestine. HNF4 paralogs work at distal enhancers and play a critical role in villus differentiation. For this analysis, we leveraged a Villin-Cre recombination model for double mutant of Hnf4a and Hnf4g paralogs specific to mouse intestinal epithelium. Our results show a significant reduction in pol II recruitment upon loss of HNF4 at its target genes, and this impact is most felt at genes which control essential intestinal processes. In summary, differential pol II recruitment appears to be a major regulatory mechanism orchestrating functional changes along the crypt-villus axis, and this process is influenced by HNF4. We hope this work will enable subsequent discovery of other new intestinal gene regulatory mechanisms, such as promoter-proximal pausing of pol II or post-transcriptional mechanisms.

Presenter: Elizabeth Rosenzweig
Poster: *Improved Statistical Procedure for Evaluating New Cancer Drugs*
Authors: Rosenzweig, E.; Gordon, D.; Axelrod, D.
Background: In preclinical studies, candidate cancer drugs are screened for their ability to inhibit the growth of human tumors implanted in mice. Several mice bearing a patient-derived tumor xenograft (PDX) are treated with a drug for 21 days or more and the tumor growth trajectories are compared with untreated controls.
Problem: Improvements in statistical methods to increase sensitivity and specificity for these preclinical studies are necessary for more accurate determination of drug inhibition.
Method: Five different measures of tumor growth have been reported for multiple laboratory sites. These measures include area under the complete growth curve (AUC), AUC truncated at 21 days, relative change in tumor volume from initial to final measurement (Delta Tumor Volume, or DTV), Progression-Free Survival analysis (PFS), and Tumor Growth Inhibition (TGI). We produce diagnostic tests by adding meta-analysis using

Fisher's combined p-value test and false discovery rate correction. We apply sensitivity and specificity testing to the diagnostic tests.
Result: Multiple measures at multiple sites is optimal for sensitivity and specificity. Comparison of individual measures of tumor growth indicates that a regression-based TGI analysis is most predictive of tumor inhibition.
Conclusion: To determine tumor inhibition in PDX trials, we recommend using multiple measures of tumor growth from multiple sites. Meta-analysis via Fisher's combined p-value test and Bonferroni correction for multiple hypotheses are important components. If data from multiple sites are not available, independent replication at a single site is appropriate; also, TGI by itself is appropriate as a single measure of tumor inhibition.

Presenter: Uyen Nguyen
Poster: *Knockout of histone acetyltransferases KAT2A and KAT2B in the intestinal epithelium triggers intrinsic interferon signaling likely from self-derived double stranded RNA.*
Authors: Nguyen, MU; Verzi, M
Epigenomic regulatory mechanisms controlling intestinal homeostasis are incompletely understood. An in silico screen for epigenomic regulators revealed that KAT2A and KAT2B are highly expressed in the intestinal epithelium. However, the functions of KAT2A and KAT2B in the intestine is unknown.
VillinCre-ERT2 inducible double knockout (DKO) of Kat2a and Kat2b from the intestinal epithelium in mice triggered rapid weight loss and death, but also induced cell-intrinsic interferon signaling. Stem cell expression and proliferation were drastically diminished by immunostaining; additionally, the histone post-translational modification (PTM) H3K9ac was nearly abolished. Both RNA-seq and proteomic analysis revealed robust induction of interferon-stimulated genes (ISGs) and corresponding proteins. I hypothesize that interferon signaling is triggered by self-derived double stranded RNA (dsRNA), rather than changes in histone PTMs, disruptions in non-histone protein acetylation, or reactivation of endogenous retroviruses. Time course experiments suggest that dsRNA is released and stimulates ISGs prior to complete KAT2 and H3K9AC loss. dsRNA immunoprecipitation sequencing (dsRIP-seq) is currently underway to determine the dsRNA source, which preliminarily shows DKOs with an abundance of smaller dsRNA species. These observations are not found in either KAT2 single knockout model, suggesting the redundant functions of KAT2 paralogs in maintaining intestine integrity and function.
Next steps will be to substantiate the findings above and link them to the mechanisms behind how intestinal KAT2A and KAT2B modulate interferon signaling. A leading hypothesis is that KAT2 functions to prevent defects in transcription that would generate dsRNA and activate the interferon response.

Presenter: Elaheh S Hosseini
Poster: *The potential role of UHRF1BP1 as an antitumor immune regulator in ovarian cancer*
Authors: Hosseini, E; Payne, K
Despite all advances in onco-immunology, ovarian cancer patients' responses to the immunotherapy approaches are limited which drives us to understand the immunobiology of the ovarian tumor microenvironment (TME). Our laboratory has identified a single nucleotide polymorphism in the gene UHRF1BP1 that is associated with better overall survival for ovarian cancer patients. While the role of UHRF1BP1 in cancer is not clear, preliminary research by our laboratory has revealed that UHRF1BP1 is transcriptionally responsive to mitochondrial stress through the integrated stress response (ISR) in p-eIF2α dependent mechanism. According to our chromatin immunoprecipitation (ChIP)-PCR data in human ovarian cancer cells, ATF4 binds to two sites within the promoter regions of the UHRF1BP1 gene, which is amplified under mitochondrial stress. Additionally, RNA-sequencing pathway analysis in murine ovarian cancer cells reveals that type I interferon (IFN) pathways are upregulated in the absence of Uhrf1bp1. Critically, the extended survival of mice bearing ovarian tumors with dysfunctional Uhrf1bp1 was found to be dependent on

CD8+ T cells; suggesting UHRF1BP1 is a novel immunoregulator. These data support our hypothesis that dysfunctional cancer cell-intrinsic UHRF1BP1 promotes unmitigated stress responses in the TME which drives antitumor immunity through dysregulated type I IFN activity. Mechanistically, we postulate that dysfunctional UHRF1BP1 promotes the loss of mitochondrial membrane integrity, which drives type I IFN activity through cytoplasmic sensing of mitochondrial DNA. Collectively, our goal is to establish a new paradigm in our understanding of the immunobiology of ovarian cancer considering cellular stress responses as antitumor immunity regulators.

Presenter: Yanira Gonzalez
Poster: *Regulation of the G2/M Cell Cycle Checkpoint by BRCA1*
Authors: Gonzalez, Y.; Bunting, S.
The G2/M cell cycle checkpoint reduces the risk of chromosome mutations that drive the onset of cancer, by preventing cells with unrepaired DNA damage from entering mitosis. The tumor suppressor protein, BRCA1, is required for normal induction of the G2/M checkpoint, but it is not clear how BRCA1 activates the checkpoint. To address how BRCA1 regulates the G2/M checkpoint, we used mouse models expressing mutant forms of BRCA1 in B lymphocytes. Our results show that Aurora-A kinase, a key protein that promotes the transition into mitosis, remains active after DNA damage in BRCA1-mutant cells. In contrast, Aurora-A is inactivated by dephosphorylation in control cells that express normal BRCA1. Measurement of mitotic cell populations revealed that Aurora-A hyperactivity in BRCA1-mutant cells correlated with a failure of the G2/M cell cycle checkpoint, allowing cells to enter mitosis even after DNA damage. We find that treating BRCA1-mutant cells with an Aurora-A inhibitor can rescue the G2/M checkpoint, indicating that it may be possible to restore normal responses to DNA damage in cells that lack functional BRCA1. In addition, we found that BRCA1/53BP1 double-deficient cells, which have normal rates of homologous recombination but have a defective G2/M checkpoint, also show increased Aurora-A activity. These results suggest that BRCA1 has separable roles in HR and regulation of the G2/M cell cycle checkpoint. Overall, our results demonstrate a novel mechanism for the regulation of cell division by BRCA1 and offer a potential opportunity for future clinical interventions to reduce cancer susceptibility in patients with BRCA1 mutations.

Presenter: Justin Koesterich
Poster: *Decoding cell-type specific neuronal regulatory networks*
Authors: Koesterich, J., Kreimer, A.
Genome wide association studies (GWAS) have found that the majority of disease associated variants are located within the non-coding genome. It is still largely unknown how mutations in these non-coding sequences cause functional disruptions, even though these sequences constitute 98.5% of the human genome. One hypothesis is that they disrupt regulatory regions consisting of enhancer or promoter regions and disrupt the proper functioning of transcription. This hypothesis is challenging to explore since different cell types have different active enhancers, as well as different interactions between enhancers and nearby genes. To decipher the functional impact of non-coding mutations, we need to gain a better understanding of the mechanism by which they disrupt such regulatory regions.
To this end, we collected epigenetic and gene expression data from multiple neuronal cell types, including Neural Progenitor Cells (NPCs), Excitatory cells, Inhibitory cells, and Motor Neurons. Using this data, we generated enhancer-promoter interaction pairs using the Activity By Contact (ABC) model and grouped the interaction pairs based on the pattern of their scores within each cell type. We identify interaction pair groupings that are cell type specific and groupings that have similar interaction scores across closely related cells. For each of these groupings, we identify enrichments for cell type relevant biological processes.
Next we apply clustering techniques to the grouped interaction pairs to analyze the distribution of its different sub-structures. This analysis highlights different modes of gene regulation programs across cell-types. This was

done by identifying potential unique interactions and redundancies in enhancers and promoters.

Presenter: Jonathan Phan
Poster: *Recycling of Dissolved Organic Sulfur by Shewanella oneidensis*
Authors: Phan J., Macwan S., Gralnick J., Yee N.
Disulfide bonds play a crucial role in controlling the structure and reactivity of dissolved organic sulfur, yet little is known about the role of microbes in the redox cycling of disulfide compounds. In this study, we screened a Tn mutant library of Shewanella oneidensis MR1 to identify mutants impaired in disulfide reduction. One mutant, designated as D10, was unable to reduce 5,5-dithio-bis-(2-nitrobenzoic acid), and glutathione disulfide. Genome sequencing showed that the mutant harbored a transposon in the dsbD gene. These results indicate that disulfide bond oxidoreductases play an important role in recycling disulfide compounds. Future work aims to complement the mutant with the wild-type dsbD gene to restore disulfide reduction capabilities.

Presenter: Thushara Nethramangalath
Poster: *Functional Analysis of the CNNM3-TRPM7 Complex in Mouse Embryonic Stem Cells*
Authors: Nethramangalath, T. and Runnels, L.W.
TRPM7 was the first mammalian ion channel shown to play a critical role during early embryonic development. Recently, CNNMs were identified as TRPM7 interacting proteins and shown to be involved in channel regulation. However, the functional significance of the CNNM-TRPM7 complex and their tissue specific regulation in native systems remain poorly understood. Here we employed wildtype (WT) and TRPM7 knockout (KO) mouse embryonic stem cells (mESCs) to identify the composition of the native TRPM7 complex in stem cells. Mass spectrometry analysis identified CNNM3 as the major CNNM isoform interacting with the channel, along with known CNNM binding partners PTP4A and ARL15. To analyze the function of the CNNM3-TRPM7 complex we employed Crispr-Cas9 to knockout CNNM3 from mESCs. We then characterized WT, TRPM7 KO and CNNM3 KO mESCs and their capacity for self-renewal and differentiation into neuronal progenitors. TRPM7 KO mESCs required supplementation with 10 mM MgCl2 in the growth medium to survive, whereas knockout of CNNM3 from mESCs does not affect cell survival or growth. Expression of pluripotency markers Oct4 and Sox2, and alkaline phosphatase staining in all the clones confirmed that the cells retained their pluripotency. Our experiments also revealed a critical role for TRPM7 in the differentiation of mESCs into neuroectodermal cells, which represents one of the earliest stages in mammalian neurogenesis. In conclusion, our experiments identify components of the CNNM3-TRPM7 complex in mESCs and uncover a critical role for the TRPM7 channel in the maintenance and differentiation of embryonic stem cells during early embryonic development.

Presenter: Deimante Mikalauskaite
Poster: *Transcriptional co-repressor Atrophin regulates Hippo pathway target genes in D. melanogaster wing discs*
Authors: Mikalauskaite, D., Rauskolb, C., Lehan, T., Venkatramanan, S., Irvine, K.
The Hippo signaling pathway controls expression of growth-promoting target genes through its downstream effector, the transcriptional co-activator protein Yorkie (Yki). Hippo signaling removes Yki from the nucleus, under these conditions transcription of Hippo pathway target genes is repressed by the transcriptional co-repressor Tgi. Studies of Tgi suggest additional transcriptional repressors of Hippo pathway target genes exist. We have been investigating the transcriptional co-repressor Atrophin as a candidate co-repressor that contributes to Hippo signaling. Atrophin has multiple roles during Drosophila development. Using gene knock down and overexpression approaches, we have found that Atrophin regulates multiple Hippo pathway target genes in wing imaginal discs. Depletion of Atrophin results in elevated levels of Yki target genes in the middle of the wing pouch. Additionally, knockdown of Atrophin can partially suppress the reduction of target gene expression observed in Yki knockdown cells. In contrast, in the periphery of the wing pouch Atrophin

knockdown decreases the expression of Yki target genes. Overexpression of Atrophin increases expression of the same target genes in the proximal wing. Since Atrophin functions as a transcriptional co-repressor, these observations suggest that Atrophin could be directly repressing expression of Yki target genes in the distal wing, but indirectly activating them in the proximal wing. We are now identifying the mechanisms by which Atrophin exerts its effects on Hippo signaling and the regulation of Yki target genes.

Presenter: Alexander Logerfo
Poster: *Mechanisms of BMP signaling driving tissue pattern in the intestine*
Authors: Logerfo, A; Verzi, M
An appropriate balance of stem and differentiated cells must exist in the intestine to maintain homeostasis. Enterocytes, the major absorptive cell type, have recently been shown to switch cell states as they migrate up the villus in a phenomenon called zonation. Despite evidence that the bone morphogenetic protein (BMP) signaling pathway is involved in maintaining this zonation, the specific molecular mechanisms are not known. This work aims to define molecular mechanisms of enterocyte zonation and cell state switching. Canonical BMP signaling transduction involves DNA binding to target genes by three transcription factors, SMAD1, SMAD5, and SMAD4. Preliminary analysis of SMAD4 chromatin immunoprecipitation sequencing (ChIP-seq) data from mouse small intestine demonstrates SMAD4 binding in regulatory regions of enterocyte zonation genes. This indicates that canonical BMP signaling through SMAD1, SMAD5, and SMAD4 could be responsible for direct transcriptional regulation of genes responsible for zonation. Additional analyses of ChIP-seq and single cell RNA-sequencing (scRNA-seq) data suggest that this direct binding requires the recruitment of a secondary cofactor for specificity of activation and transcriptional regulation of enterocyte zonation genes in different functional zones. Additional experiments are ongoing to confirm direct regulation of enterocyte zonation genes by SMAD1, SMAD5, SMAD4 and to identify potential cofactors involved in transcriptional regulation of enterocyte zonation genes. This work will provide insights into the molecular mechanisms of appropriate maturation of functional cell types in the intestine which will lead to the development of better models of the intestine and smarter approaches for manipulation of the canonical BMP signaling pathway.

Presenter: Xiao Su
Poster: *Uncovering the cellular mechanism of Schizophrenia associated gene SETD1A loss-of-function in a human neural model*
Authors: Su X., Hong Y., Wang L., Zhang S., Zhang H., Song H., Duan J., Ming GL., Pang ZP.
Rare loss-of-function (LoF) mutations in SETD1A are strongly associated with schizophrenia (SZ), a debilitating mental disorder affecting 1% of the population, and other severe neurodevelopmental disorders. SETD1A encodes a component of the histone methyltransferase complex producing mono-, di, and trimethylated histone H3 at Lysine 4 (H3K4). H3K4 trimethylation (H3K4me3) and H3K4me1 are epigenomic marks of active gene transcriptional promoters and enhancers, respectively. Interestingly, histone methylation has also been suggested as one of the most enriched gene pathways in common variant-based genome-wide associations studies (GWAS) of major psychiatric disorders. However, the detailed molecular mechanism by which it causes neuronal dysfunction is still unclear. Recent advances in stem cell biology have allowed the efficient conversion of human stem cells into defined neuron subtypes allows to address this question. Using CRISPR/Cas9 gene editing, we have generated isogenic hiPSC lines carrying heterozygous LoF mutations on different genetic backgrounds of SETD1A. Preliminary data about morphological, electrophysiological and transcriptomic analyses of hiPSC neurons carrying SETD1A LoF mutation showed defective synaptic neurotransmission. Ongoing experiments are evaluating SETD1A LoF with functional, morphological, biochemical and genomic parameters to understand the cellular mechanisms that how SETD1A LoF contributes to the pathogenesis of SZ in a cell-type specific manner. The study enables us to perform a well-controlled assessment of the impact of SETD1A LoF mutations on the molecular and cellular mechanisms underlying deficits in early

neurodevelopment and synaptic properties.

Presenter: Elena Forzisi
Poster: *Antagonistic roles of Ras-MAPK and Akt signaling in integrin-K+ channel complex-mediated cellular apoptosis*
Authors: Jo, C, Forzisi, E, Yu, W, Sesti, F
Complexes formed with α5-integrins and the voltage-gated potassium (K+) channel KCNB1 (Kv2.1), known as IKCs, transduce the electrical activity at the plasma membrane into biochemical events that impinge on cytoskeletal remodeling, cell differentiation, and migration. However, when cells are subject to stress of oxidative nature IKCs turn toxic and cause inflammation and death. Here, biochemical, pharmacological, and cell viability evidence demonstrates that in response to oxidative insults, IKCs activate an apoptotic Mitogen-activated protein kinase/extracellular signal-regulated kinase (Ras-MAPK) signaling pathway. Simultaneously, wild-type (WT) KCNB1 channels sequester protein kinase B (Akt) causing dephosphorylation of BCL2-associated agonist of cell death (BAD), a major sentinel of apoptosis progression. In contrast, IKCs formed with C73A KCNB1 variant that does not induce apoptosis (IKCC73A), do not sequester Akt and thus are able to engage cell survival mechanisms. Taken together, these data suggest that apoptotic and survival forces co-exist in IKCs. Integrins send death signals through Ras-MAPK and KCNB1 channels simultaneously sabotage survival mechanisms. Thus, the combined action of integrins and KCNB1 channels advances life or death.