20TH ANNUAL GDBBS DSAC STUDENT RESEARCH SYMPOSIUM

Wednesday, March 22nd, 2023

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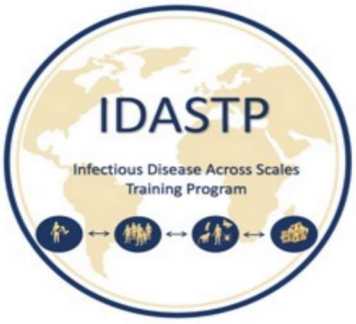

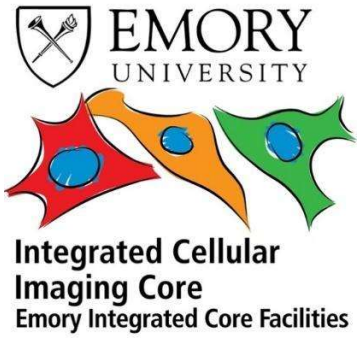

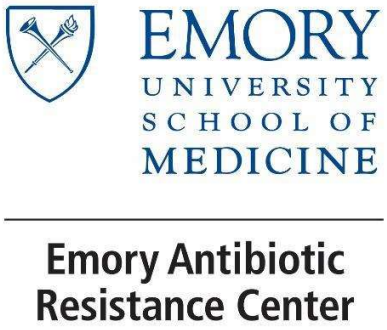

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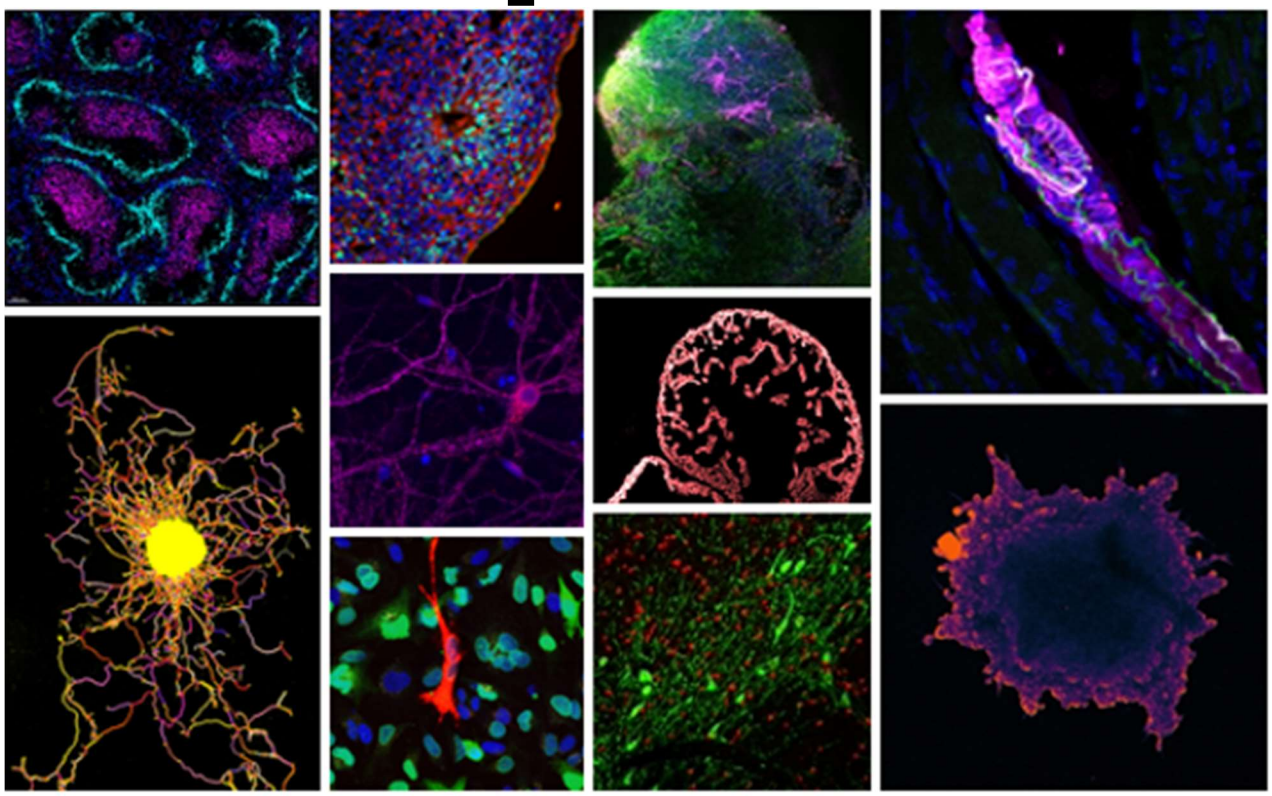
ICI Image Competition



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Oral Presentation Abstracts

Session 1:

Human Development and Aging

8:30AM

Samantha Lanjewar, GMB **8:30AM**

Identifying drivers of human astrocyte development

Samantha Lanjewar[1], Caitlin Sojka[1], Alexia King[1], Steven Sloan[1]

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

Astrocytes are among the most abundant non-neuronal cells in the central nervous system and play active roles as choreographers of synapse formation and neural circuit development. Neurons and astrocytes are derived from the same progenitor cells called radial glia, which sequentially produce neurons and then astrocytes. The timing of this transition, termed the “gliogenic switch”, is critical for proper brain development. Our goal is to uncover novel factors that control astrocyte development using human cortical organoids. This system recapitulates the timing of the gliogenic switch seen in human fetal development, thus serving as an ideal reductionist model. Transcription factors are key modulators in cell fate determination. We aimed to determine which transcription factors drive human astrocyte development by performing paired RNA-sequencing and ATAC-sequencing on human cortical organoids at timepoints surrounding the gliogenic switch. We utilized a Time Course Regulatory Analysis pipeline that pairs gene expression and chromatin accessibility data at multiple timepoints to identify driver transcription factors regulating astrocytic gene expression. Using this pipeline, we identified seven candidate transcription factors: EMX1, EOMES, FOXG1, POU3F3, RFX4, SOX21, and TBR1. Many of these transcription factors are well known regulators of neuronal development, indicating a potential repurposing of their function from promoting neuronal to astrocytic cell fates. We will next perform functional analyses to test the role of these candidates in driving astrocyte development. Ultimately, understanding the key drivers of the gliogenic switch will provide insight into how perturbations to the timing and production of astrocytes contribute to the pathogenesis of neurodevelopmental disorders.

Rachel Bear, NS **8:45AM**

Primary cilia regulate the rate of astrocyte proliferation in the mouse cortex

Rachel Bear[1,2], Claire Wei[2], Tamara Caspary[2]

[1]Neuroscience Graduate Program, Emory University, Atlanta, GA
[2]Department of Human Genetics, Emory University, Atlanta, GA

Astrocytes serve an indispensable role in the developing brain, yet our knowledge of how astrocytes develop is incomplete. Astrocyte dysfunction is implicated in several neurodevelopmental disorders, highlighting the importance to resolve mechanisms of astrocyte development. Primary cilia are singular, microtubule-based projections that function as specialized signaling centers. Critically, cilia dysfunction results in ciliopathies that present with a spectrum of neurological abnormalities. Astrocytes possess a primary cilium, yet the function of cilia in astrocyte development remains unexplored. The goal of my research is to determine how primary cilia regulate astrocyte development. First, we characterized astrocyte cilia at several developmental timepoints and found that the expression of ciliary proteins changes. To determine which developmental process are linked to cilia, we used a genetic mouse model to ablate cilia specifically in developing astrocytes *in vivo.* We found that astrocytes lacking cilia have reduced proliferation. Cilia assembly and disassembly are intricately linked to the cell cycle. Next, we are exploring the relationship between cilia and cell cycle in astrocytes by examining the impact loss of astrocyte cilia has on cell cycle. These results indicate that cilia are critical in regulating astrocyte proliferation. We have evidence that astrocytes lacking cilia show altered expression of target developmental genes. This suggests that cilia may regulate astrocyte differentiation, which we are now addressing with transcriptomic analysis. Our work establishes a foundation for understanding the functions of primary cilia in developing astrocytes. This is significant in broadening our knowledge of astrocyte development in relation to cilia.

Alex Nazzari, IMP **9:00AM**

Human prenatal- versus adult-derived B cells have biased specificities against self and may differentially contribute to autoimmune diseases

Alexandra F. Nazzari,[1] Fathma Abdulkhader,[1] Junkai Yang,[1] Astrid Kosters,[1] Weiwen Zhang,[1] Benjamin R. Babcock,[1] Eliver E. B. Ghosn[1,2]

[1]Department of Medicine, Division of Immunology, Lowance Center for Human Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA
[2]Emory Vaccine Center, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, GA 30322, USA

B cells are a key component of an immune response and produce protective antibodies against pathogens. However, B cells also produce autoreactive antibodies against self-antigens. Consequently, when dysregulated, autoreactive B cells can initiate autoimmune diseases. While the production of autoreactive B cells in prenatal mice is well-established, their generation, function, and persistence in humans are understudied.
Here, we apply multi-omics single-cell technologies to reveal the prenatal origins and functional characteristics of human autoreactive B cells, shedding new light on mechanisms of tolerance and autoimmunity. Using Hi-D flow cytometry to map B-cell development from early to late gestation, we identified a population of autoreactive B cells, using the *IGHV4-34* gene, in the fetal liver as early as seven weeks post-conception (wpc). Autoreactive VH4-34+ B cells rapidly increased to approximately four percent of the total B-cell pool in the fetal liver by 21 wpc and subsequently matured in the fetal spleen.

Surprisingly, the autoreactive VH4-34+ B cells that emerge in prenatal life are transcriptionally, phenotypically, and functionally distinct from their adult counterparts. Strikingly, prenatal autoreactive B cells express a unique and biased B-cell receptor specificity that is shared across individuals, suggesting an evolutionarily conserved mechanism in prenatal life to tolerize against self. This is further supported by an increase in B-cell signaling associated with antigen recognition and activation that is absent in autoreactive B cells generated in adult life. Defining the origins, longevity, and function of prenatal-derived autoreactive B cells is critical to understanding their role in homeostasis and autoimmune disease.

Colby Schweibenz, BCDB **9:15AM**

The coactivator Taiman modulates cell competition via glypican-dependent diffusion and availability of the Wg morphogen in *Drosophila*

Colby K. Schweibenz[1]**,** Ken H. Moberg[1]

[1]Department of Cell Biology, Emory University School of Medicine, Atlanta, Georgia

Cell competition ensures that the fittest cells populate developing primordia but also may contribute to pathogenesis: cancer cells expressing 'super-competitor' genes can eliminate slow growing neighbors and take over an epithelial tissue. Our prior work demonstrated cells overexpressing the *Drosophila* protein Taiman (Tai), a transcriptional co-activator of the Ecdysone receptor, can kill wildtype neighbors within the larval wing epithelium. We used both wing and eye epithelia to show that cells with reduced Tai expression (Tai^{low}) are competitive 'losers.' A genetic screen identified mechanisms in which wildtype neighbors eliminate these loser cells. 'Hits' included pro-apoptotic genes acting as dominant suppressors of the Tai^{low} phenotype, confirming a dependance on apoptosis. Intriguingly, we recovered the two *Drosophila* Adenomatous polyposis coli (APC) tumor suppressor homologs, Apc1 and Apc2, which are conserved elements of the Wg/Wnt pathway and inhibit competition in the fly midgut. Apc1/Apc2 loss rescues elimination of Tai^{low} cells in eye and wing epithelia, arguing that Tai loss may reduce Wg signaling. We find that Tai is required for expression of Wg gene targets in larval wing cells, and that Tai promotes expression of the glypicans Dally and Dally-like protein (Dlp), which respectively enable short or long-range Wg signaling. Thus, we hypothesize Tai^{low} cells become losers due to decreased Dally/Dlp, which in turn leads to reduced capture of Wg by Tai^{low} cells relative to adjacent wildtype cells. Ongoing experiments seek to establish mechanistic links between Tai and Dally/Dally-like, with the goal of defining how Tai modulates winner/loser status by remodeling the extracellular matrix.

Kedamawit Tilahun, GMB **9:30AM**

Elucidating the role of TMEM106B fibrils in brain health and diseases

Kedamawit Tilahun[1,3]**,** Tatiana Bolds[1], Morgan Kuchar[1], Fuying Ma[1], Janani Parmeswaran[1], Devesh Pant[1], Daniel Pun[1], Ganesh Chilurkuri[1], Cici Zhang[1], Jonathan D. Glass[2], Gary Bassell[1,3], Jie Jiang[1,3]

[1]Department of Cell Biology, Emory University, Atlanta, GA
[2]Department of Neurology, Emory University, Atlanta, GA
[3]Graduate Program in Genetics and Molecular Biology, Laney Graduate School, Emory University, Atlanta, GA

Neurodegenerative disorders are conditions resulting from progressive damage to cells in the nervous system and affect millions of people world-wide, and with an increase in the aging population, the incidences of these disorders are expected to rise. A hallmark of neurodegenerative disorders is misfolding and aggregation of proteins in neurons and glia which will ultimately lead to cytotoxicity and cell death resulting in brain atrophy. Recently, several independent groups have reported amyloid fibrils in brain tissue of a diverse set of neurodegenerative disorders as well as older individuals to comprise the C-terminal domain of a transmembrane protein 106B (TMEM106B). This protein has previously been shown to modulate disease risk in neurodegeneration and has also been implicated in healthy aging. To discover whether TMEM106 fibrils are pathogenic or just a byproduct of neurodegeneration or aging, we have cloned full length TMEM106B as well as the c-terminal fragment that makes up the fibril. We have evidence that TMEM106B-fibril is neurotoxic and perturbs the localization of nuclear RNA binding proteins (RBPs) like TDP43 and fused in sarcoma (FUS). The toxicity seen in primary cortical neurons and the mislocalization of RBPs points to a nucleocytoplasmic transport (NCT) deficit, a phenotype seen in other neurodegenerative diseases with protein aggregation. Here, we use classical GFP-NES-NLS and GFP-NLS constructs to test whether TMEM106B fibrils play an important role in neurodegeneration and disease pathogenesis through disruption of the NCT. TMEM106B fibril constructs have also been generated to test in vivo toxicity in Drosophila.

Session 2:

Genomics and Gene Regulation

10:00AM

Carly Lancaster, BCDB **10:00AM**

A conserved RNA binding protein regulates RNAs critical for neurodevelopment

Carly L. Lancaster[1,2,3], Pranav Yalamanchili, Anita H. Corbett[1], Kenneth H. Moberg[2]

[1]Department of Biology, Emory University, Atlanta, GA
[2]Department of Cell Biology, Emory University, Atlanta, GA
[3]Graduate Program in Biochemistry, Cell, and Developmental Biology, Emory University, Atlanta, GA

Intellectual disabilities (ID) are common in the general population and are linked to lesions in >700 genes. Emerging evidence suggests that this diverse group of genes converge on a limited set of neurodevelopmental pathways, including those that rely on RNA binding proteins (RBPs) to guide spatiotemporal patterns of neuronal mRNA expression. Our labs co-discovered a monogenic form of ID caused by loss-of-function mutations in the ubiquitously expressed RBP ZC3H14. Functional analysis of the conserved ZC3H14 ortholog in *Drosophila*, Nab2, illustrates that Nab2 localizes to neuronal nuclei and cytoplasmic ribonucleoprotein granules and is required specifically within brain neurons for olfactory memory and proper axonal patterning. However, neuronal signaling pathways regulated by Nab2, as well as mechanisms that elevate ZC3H14/Nab2 function in neurons, remain elusive. We will present evidence that Nab2 controls neuronal expression of a well-conserved guanine-nucleotide exchange factor (GEF), Trio that mediates growth cone guidance and axon projection. Nab2 controls Trio levels by modulating an intron-retention event within the 5' UTR of *trio* mRNA isoforms, and this mechanism appears to be dependent on N^6-methyladenosine (m^6A) deposition on the *trio* pre-mRNA. Data will be presented on the role of m^6A and Nab2 in controlling Trio splicing and expression, along with Nab2-Trio coregulation of axonal development in the CNS. Given that human TRIO is mutated in a dominant form of ID, this link between Nab2 and Trio in *Drosophila* could suggest that Nab2/ZC3H14 and Trio/TRIO act in a conserved ID pathway required to pattern neuronal processes in the developing nervous system.

Tala Khatib, BCDB **10:15AM**

IL13ra2 defines a tumor subpopulation within non-small cell lung carcinoma critical for efficacious metastasis

Tala O. Khatib[1,2,3], Janna K. Mouw[1,2], Veronika Matsuk[1,2], Adam I. Marcus[1,2,3]

[1]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia, USA
[2]Winship Cancer Institute of Emory University, Atlanta, Georgia, USA
[3]Graduate Program in Biochemistry, Cell, and Developmental Biology, Emory University, Atlanta, Georgia, USA

Metastatic disease accounts for 90% of all cancer-related deaths. One primary mode of metastasis is collective invasion, where cellular packs invade into the adjacent stroma while maintaining cell–cell contacts. Cells within the pack are heterogenous and harbor distinct genetic and phenotypic subpopulations that cooperate to drive invasion. To deconstruct how this phenotypic heterogeneity facilitates distinct molecular profiles within a single tumor, we established the technique, Spatiotemporal Cellular and Genomic Analysis (SaGA). SaGA is an image-guided approach that optically highlights phenotypically defined cell(s) for live fluorescence-activated cell sorting and analysis. We utilized SaGA to isolate leaders, cells along the leading edge of the collective invasion pack, and followers, cells trailing behind. Long-term cellular propagation revealed stable leader and follower phenotypes and RNA sequencing indicated distinct transcriptomic profiles. We identified significant leader and follower surface markers for broader applicability across a panel of NSCLC samples and discovered that followers binarily express the surface marker, Interleukin13 receptor alpha 2 (IL13Rα2). IL13Rα2 is highly upregulated in many cancers and can be used to mark distinct populations within tumor cells. Flow cytometry demonstrated that NSCLC includes IL13Rα2 heterogeneous (containing positive and negative IL13Rα2 populations) and homogenous (100% IL13Rα2 negative population) samples and single cell sequencing indicated IL13Rα2 heterogenous samples upregulate fatty acid response compared to homogeneous samples. Further, *in vivo* mouse modeling led to increased metastasis in IL13Rα2 heterogeneous samples, and low to no metastasis in IL13Rα2 homogenous samples. These data suggest IL13Rα2 positive and negative subpopulations cooperate to promote metastatic disease in NSCLC.

Juan D Rodriguez, GMB **10:30AM**

Ectopic transcription due to inappropriately inherited histone methylation may interfere with the ongoing function of terminally differentiated cells

***Juan D. Rodriguez*[1]**, David J. Katz[1]

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

C. elegans larvae that are double mutants for the H3K4 demethylase *spr-5* (LSD1/KDM1A in mammals) and the H3K9 methyltransferase *met-2* (SETDB1 in mammals) exhibit L1 developmental delay, chemotaxis defects, muscle defects and a failure to elongate the gonad. Some of these phenotypes, such as developmental delay and behavior abnormalities, broadly overlap with those observed in human Kabuki Syndrome-like patients, caused by mutations in *Lsd1*. In *C. elegans,* we observed that *spr-5; met-2* phenotypes are associated with the ectopic somatic expression of germline genes. In addition, we found that both the ectopic germline expression and phenotypes are dependent upon the H3K36 methyltransferase, MES-4. This suggests that the larval phenotypes observed in *spr-5; met-2* mutants are due to the ectopic expression of MES-4 dependent germline genes in somatic tissues. Since we first observe the ectopic expression of germline genes in *spr-5; met-2* mutant embryos, it provided the unique opportunity to determine how ectopic expression, due to inappropriate histone methylation, interferes with the invariant *C. elegans* embryonic lineage. We proposed to perform single cell RNA-seq to study this ectopic expression at the single-cell level. The results from the automated lineage tracing, has shown that *spr-5; met-2* mutants have no embryonic lineage defects through the 200-cell stage, despite the ectopic germline expression and the major phenotypes we observe one cell division later. This raises the intriguing possibility that the defects caused by mutations in these enzymes are not confined to development and may be due to ongoing functional defects in terminally differentiated cells.

Hannah Hrncir, BCDB **10:45AM**

SOX9 regulates ductule morphogenesis in mouse liver by inhibiting Activin A

Hrncir HR[1,2], Hogan CB[1], Bombin S[1,3], Gracz AD[1,2]

[1]Division of Digestive Diseases, Emory University, Atlanta, GA
[2]Graduate Program in Biochemistry, Cell, and Development Biology, Emory University, Atlanta, GA
[3]Emory Integrated Computational Core, Emory University, Atlanta, GA

Liver homeostasis relies on efficient bile removal by intrahepatic bile ducts (IHBDs). Cholestasis can result from improper development or loss of ducts, resulting in liver damage. The IHBD network is a hierarchical structure with smaller ducts ("ductules") draining into larger ducts. Region-specific regulators of IHBD morphogenesis are not well defined. The transcription factor *Sox9* is an early and ubiquitous marker of biliary epithelial cells (BECs, or cholangiocytes). Here, we show that *Sox9* is required for proper biliary maturation and IHBD morphogenesis in mice. We identified ductal paucity in Sox9cKO mice by conventional histology. To further explore the role *Sox9* plays in IHBD morphogenesis, we performed tissue clearing and whole lobe imaging using light sheet microscopy. We found that Sox9cKO mice maintain ducts but have broad ductule loss. To determine if loss of ductules is associated with subpopulation-specific changes in BEC gene expression, we performed scRNA-seq and found differential clustering in some Sox9cKO BEC subpopulations compared to controls. Functional assays of mouse IHBD organoid formation revealed that a subset of Sox9cKO organoids have impaired morphology with loss of lumen. We found that *Inhba*, a regulator of branching morphogenesis in other tissues and an activator of TGF- β signaling, is upregulated in Sox9cKO mice. Treating WT BECs with Activin A, a protein product of *Inhba*, impaired mICO morphology consistent with Sox9cKO mICO phenotypes. Together, our work demonstrates the critical role of *Sox9* in promoting proper duct specification and morphogenesis, providing insight into the differential regulation of duct and ductule formation.

Kelsey Robinson, GMB **10:55AM**

Enrichment of *de novo* variants in 491 cleft palate trios

Kelsey Robinson[1], Sarah Curtis[1], Terri H. Beaty[2], Azeez Butali[3], David J. Cutler[1], Michael P. Epstein[1], Carmen J. Buxó-Martinez[4], Gary M. Shaw[5], Jacqueline T. Hecht[6], Lina Moreno Uribe[6], Jeffrey C. Murray[7], Harrison Brand[8], Seth M. Weinberg[9], Mary L. Marazita[9], Elizabeth J. Leslie[1]

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[2]Department of Epidemiology, Johns Hopkins University, Baltimore, MD
[3]Department of Oral Biology, Radiology, and Medicine, University of Iowa, Iowa City, IA
[4]School of Dental Medicine, University of Puerto Rico, San Juan, Puerto Rico
[5]Department of Pediatrics, Stanford University, Stanford, CA
[6]Department of Pediatrics, University of Texas Medical School at Houston, Houston, TX
[7]Department of Pediatrics, University of Iowa, Iowa City, IA
[8]Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA
[9]Department of Oral and Craniofacial Sciences, University of Pittsburgh, Pittsburgh, PA

Cleft palate (CP) is a common craniofacial birth defect, affecting 1 in 1700 live births. Despite heritability estimates of 80-90%, there remains a relative dearth of known risk variants associated with CP even with well-powered genome-wide association studies. Many other structural birth defects are enriched for protein-altering *de novo* variants (DNs), but no large-scale studies of DNs in CP have been performed. We therefore hypothesized that CP cases would be enriched for protein-altering DNs, revealing novel genes associated with CP. Using whole genome sequencing data from 491 case-parent CP trios, we found that 332 probands harbored at least one DN in their exome with a total of 549 DNs in 524 genes. We tested for DN enrichment in probands using denovolyzeR, which compares the observed and expected number of variants based on context-dependent mutational models. We found exome-wide enrichment for loss-of-function (1.54, $p=6.5\times10^{-4}$) and protein-altering (1.17, $p=1.2\times10^{-3}$) DNs. On a per-gene basis, we identified two genes (*COL2A1* and *IRF6*) reaching exome-wide significance ($p=1.3\times10^{-6}$), and an additional three genes (*PRKCI*, *SATB2*, and *SLC25A41*) that were significant based on correction for 524 genes ($p=9.5\times10^{-5}$). *COL2A1*, *IRF6*, and *SAT2B* have been previously associated with CP, thereby demonstrating the effectiveness of this approach and providing confidence that *SLC25A41* and *PRKCI* play roles not yet reported. We show that CP probands are enriched for protein-altering DNs, and identified two novel genes of interest. Ongoing characterization of these DNs is expected to further our understanding of CP etiology, improving prevention and treatment strategies in the future.

Session 3:

Therapeutics

11:30AM

James Ackley, CB **11:30AM**

The impact of the stromal microenvironment on BCMA targeted CAR-T therapy in multiple myeloma

James C Ackley[1], Samuel McCachren[1,2], Sagar Lonial[1, 2, 3], Damian J. Green[4], Stanley R. Riddell[4], Geoffrey R. Hill[4], Madhav V. Dhodapkar[1, 2, 3], Lawrence H. Boise[1, 2, 3]

[1]Emory University, Atlanta, GA
[2]Emory University School of Medicine, Atlanta, GA
[3]Winship Cancer Institute, Atlanta, GA
[4]Fred Hutchinson Cancer Research Center, Seattle, WA

Multiple myeloma is a disease of malignant plasma cells. Clinical trials have demonstrated that BCMA CAR-T therapy induces disease remission in MM patients with a cumulative ORR of 85.2%. However, the duration of response is disappointing with median PFS being 14.5 months. Myeloma is dependent on the bone marrow microenvironment (BMM) which promotes myeloma survival and drug resistance. To examine the impact of the BMM on CAR-T therapy, we performed in-vitro cytotoxicity assays where anti-BCMA CAR-T cells were cocultured with myeloma cell lines in the presence or absence of HS5 stromal cells. Coculture protected all three cell lines tested from CAR-T-induced cell death. CAR-T cells kill through CD95L, therefore myeloma cell lines were cultured with HS5 cells and exposed to rCD95L. Coculture protected three of four myeloma cell lines from rCD95L with the EC50 increasing 2.4 - 3.85 fold. HS5 conditioned medium (CM) similarly protected myeloma cell lines from rCD95L indicating the protection is due to a soluble factor. Loss of intrinsic apoptosis protected KMS18 and RPMI8226 from rCD95L but not KMS12PE or OCIMY5 indicating that CD95 induces type 2 cell death and type 1 cell death respectively. When KMS12PE and OCIMY5 cells deficient in intrinsic apoptosis were treated with CM then exposed to rCD95L, the loss of intrinsic apoptosis significantly enhanced the protection of CM alone suggesting that stromal factors in the BMM can convert cells from type I to type II death receptor signaling. This suggests that inhibiting BMM signals may enhance activity of immunotherapy in myeloma.

Ana Cole, CB **11:45AM**

Th17 cells cooperate with B cells to mediate tumor immunity

Anna Cole[1], Hannah M. Knochelmann[2], Amalia M. Rivera-Reyes[1], Aubrey S. Smith[2], Megan M. Wyatt[1], Guillermo O. Rangel Rivera[2], Megen Wittling[1], Jeremy M. Boss[3], Gregory B. Lesinski[4], Chrystal M. Paulos[1]

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[2]Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, United States
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Adoptive T cell transfer therapy mediates potent immunity in some patients with aggressive malignancies, but many individuals do not respond, or may relapse. One reason for therapy failure is due to lack of T cell persistence. To overcome this problem, we aimed to generate a cell product that will lead to long term antitumor immunity in aggressive models. Accordingly, our team reported that a subset of $CD4^+$ T cells termed Th17 cells, persist long term and can eradicate solid tumors when infused into mice. To understand how Th17 cells elicit robust antitumor activity, we performed an unbiased analysis of RNA transcripts on tumor-draining lymph nodes of mice treated with Th17 cells. Surprisingly, we found that mice infused with anti-tumor Th17 cells have increased transcripts associated with B cells, and factors that trigger B cell maturation, antibody-secretion, and enhanced antigen presentation. Furthermore, host B cells, but not $CD8^+$ T cells, were surprisingly critical in sustaining long-term immunity, as their depletion significantly impaired survival. B cells enhance Th17 cell persistence and promote their differentiation into IFN-γ producers and away from regulatory IL-10 production. Th17 cells induce B cell activation and maturation, causing the production of class switched tumor specific antibodies which can alone partially protect against tumor challenge. Altogether, this suggests a cooperative relationship between transferred Th17 cells and host B cells in mediating long term tumor immunity. Our novel findings highlight Th17 cell therapy as a way to harness both T and B cell responses against cancer.

Garrett Cooper, GMB **12:00PM**

Deep mutational scanning of *SMARCB1* reveals a role of DPF2 in SMARCB1-deficient cancers

***Garrett W. Cooper*[1,2]**, Benjamin P. Lee[1,2], Victor Z. Chen[1,2], Andrew L. Hong[1,2,3]

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Malignant rhabdoid tumors are one of the most aggressive and lethal cancers in pediatric oncology with overall survival rates of 20-25% despite intensive multi-modal therapies. Loss of *SMARCB1* is the primary recurrent genetic alteration found in over 90% of cases. SMARCB1 is a critical component of the BAF chromatin remodeling complex, a complex which controls gene transcription by positioning nucleosomes at gene regulatory regions. Recent advances have implicated *SMARCB1* loss in several other cancers broadly referred to as SMARCB1-deficient cancers. More generally, alterations in *SMARCB1* have been identified in 1% of all cancers. Due to the limited number of patients presenting with SMARCB1-deficient cancers, our understanding of mutational spectrum of *SMARCB1* remains limited. We have previously performed deep mutational scanning to elucidate the functional consequences of all possible single amino acid substitutions in *SMARCB1*. We have since identified a cluster of four residues within the RPT2 domain of SMARCB1 that are particularly intolerant to mutations. Structural rendering of the assembled BAF complex reveals that this cluster of residues directly interacts with a neighboring histone reader subunit, DPF2. Further, we have observed a decrease in DPF2 association with the BAF complex upon mutation of one of these residues. These data suggest that SMARCB1 exerts its tumor suppressor function at least partially through its interaction with DPF2, and that patients presenting with missense mutations in these residues may have similarly aggressive cancers as *SMARCB1*-deficient cancers.

Delaney K. Geitgey, CB **12:15PM**

Adipocyte-secreted purines inhibit T-cell function and alter *in vitro* leukemia cell cycle dynamics in B-cell acute lymphoblastic leukemia

Delaney K. Geitgey[1,2,3]**,** Uma Obalapuram[1,2,4], Miyoung Lee[1,2], Joshua D. Chandler[1,5], Curtis J. Henry[1,2,5,6]

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood cancer and is a leading cause of pediatric illness-related death. Among children with B-ALL, the survival of patients with obesity is about 30% lower than similar lean patients. Two-thirds of the United States population is overweight or obese with numbers steadily increasing; oncology researchers need to understand how obesity impacts B-ALL pathogenesis in order to improve these outcomes. Mass spectrometry revealed that adipocytes secrete the purine nucleobases adenine and guanine at high levels relative to cell-inexperienced unconditioned media (UCM) or bone marrow stromal cell-conditioned media (SCM). Treating cultured B-ALL cells with exogenous adenine and guanine altered cell cycle dynamics as indicated by DNA incorporation of the fluorescent thymidine analog EdU; guanine increased the number of cells in S-phase and decreased the number of cells in growth phases or mitosis relative to controls. These results provide a tentative mechanism for our previous findings: adipocyte-conditioned media (ACM) induced B-ALL senescence and resistance to cell cycle-targeting chemotherapies. Furthermore, adding exogenous purines to primary human T-cells during activation decreased costimulation receptor expression (CD3 and CD28), decreased effector cytokine production (IFNγ and TNFα), and increased oxidative stress (via mitochondrial superoxide) over three days. These findings demonstrate that adipocyte-secreted purines can modulate both the anti-tumor immune response and the growth dynamics of leukemia *in vitro*. Findings from these studies will be applied in murine models to validate the potential of purinergic signaling as a therapeutic target in the context of B-ALL and obesity.

Session 4:

Metabolic Pathways and Immune Function

2:45PM

Qiao Jiao, CB **2:45PM**

Proteolytic regulation of CD73 by TRIM21 orchestrates tumor immunogenicity

Ziyi Fu[1,2]**, Siqi Chen**[3]**, Yueming Zhu**[4,5]**,** Donghong Zhang[4], Ping Xie[3], ***Qiao Jiao***[4], Junlong Chi[1], Shipeng Xu[1], Yifan Xue[6], Xinhua Lu[6], Xinxin Song[7], Massimo Cristofanilli[8], William J Gradishar[3], Kevin Kalinsky[5,9], Yongmei Yin[2], Bin Zhang[3] and Yong Wan[4,5,9]

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Despite the rapid utilization of immunotherapy, emerging challenges to the current immune checkpoint blockade need to be resolved. Here, we report that uncontrolled elevation of CD73 levels due to its aberrant turnover is tightly correlated with poor prognosis in immune-cold triple-negative breast cancers (TNBCs), which impedes the efficacy of chemotherapy and immunotherapy. We have identified TRIM21 as an E3 ligase that governs CD73 destruction. Disruption of TRIM21 stabilizes CD73 that in turn enhances CD73-catalyzed production of adenosine, resulting in the suppression of CD8+ T cell function. The immunostaining demonstrated the cytosolic colocalization between TRIM21 and CD73. Molecular mapping further identified the amino acid stretches from 340-476 on TRIM21 and residues from 176–224 on CD73 mediated the interaction between TRIM21 and CD73. Replacement of lysine 133, 208, 262, and 321 by arginine on CD73 attenuated CD73 ubiquitylation and degradation. Moreover, TRIM21 is upregulated but CD73 is downregulated in response to IFN-g secreted from activated CD8+ T cells in a feedback manner. Importantly, in preclinical animal models, diminishing CD73 ubiquitylation remarkably promotes tumor growth and impedes antitumor immunity. In addition, a $TRIM21^{high}/CD73^{low}$ signature in a subgroup of human breast malignancies was associated with a favorable immune profile. Collectively, our findings uncover a novel mechanism that governs CD73 proteolysis and point to a new therapeutic strategy by modulating CD73 ubiquitylation.

Jessica Root, NS **3:00PM**

Investigating the ability of granulin subunits to rescue lysosome dysfunction, inflammation, and disease-like pathology in a mouse model of Frontotemporal Dementia

Jessica Root[1,2], Anna Mendsaikhan[1,2], Srijita Nandy[1,2], Ludmilla Troiano Araujo[1,2], Paola Merino[1,2], Minzheng Wang[1,2], Georgia Taylor[1,2], Thomas Kukar[1,2,3]

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Frontotemporal dementia (FTD) the most common cause of dementia before the age of 60. Mutations in the granulin gene cause FTD by reducing the production of the encoded progranulin (PGRN) protein, yet the molecular function of progranulin remains unknown. The Kukar lab discovered that PGRN is trafficked to the lysosome and rapidly processed into stable proteins called granulins. Based on these data we hypothesize that lysosomal granulins are the functional subunits of PGRN.
This study tests our hypothesis by asking if delivery of a single granulin protein to the brains of *Grn*$^{-/-}$ mice is sufficient to ameliorate disease-like phenotypes. We used *Grn*$^{-/-}$ mice because they accurately replicate many pathological features observed in human *GRN*-FTD cases. We performed intracerebroventricular injections of adeno-associated virus (rAAV2/1) encoding granulin-2, granulin-4, PGRN, or GFP in neonatal *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mice. Mice aged for 12 months, before tissues were collected and analyzed. Quantitative proteomics of the thalamus identified lysosomal function and neuroinflammation as functions ameliorated by the expression of granulins. Biochemical markers of lysosomal dysfunction, neuroinflammation, and disease-like pathology were assessed via immunoblot and immunohistochemistry. We find that both granulin-2 and granulin-4 rescue markers of lysosomal dysfunction (galectin-3, cathepsin Z) and neuroinflammation (Cd68) in cortical, hippocampal, and thalamic tissue. This study is the first of its kind to demonstrate that an individual granulin subunit can rescue disease-like phenotypes in a mouse model of PGRN deficiency. We conclude that granulins are beneficial, not harmful, to neuronal health, and that granulins hold promise as therapeutics to treat FTD.

Ewelina Sobierajska, IMP **3:15PM**

Isolating human RCC tumor cells details evolutionary relationships between cancer cell subsets and a role of OXPHOS in disease progression.

Ewelina Sobierajska[1], Christopher Medina[3,4], Rachel Greenwald[1], Caroline Jansen[1], Viraj Master[5], Christian Larsen[2], Haydn Kissick[1,2,3,5]

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Approximately 50% of late-stage clear cell renal cell carcinoma (ccRCC) patients develop disease recurrence after surgery with only a 10% 5-year survival rate. Currently within the cancer field, whole tumor biopsies are examined for the development of novel therapies. Although useful, the cellular heterogeneity of tumor tissue complicates the analysis of cancer-cell intrinsic features that may dictate tumor progression. Therefore, to directly investigate cancer cells in human RCC, we employed a previously unused approach to isolate cancer cells from the tumor. First, using gene expression information gained from scRNA-seq of whole tumors, we developed a FACS sorting method to isolate two distinct populations of cancer cells: $CD24^{hi}ENPP3^{hi}$ and $CD24^{lo}ENPP3^{lo}$. Whole genome sequencing of our populations from over 100 patients confirmed >85% cancer cell purity. Genomic lineage analysis revealed a progressive relationship between $CD24^{lo}ENPP3^{lo}$ precursors and $CD24^{hi}ENPP3^{hi}$ differentiated cells. $CD24^{hi}ENPP3^{hi}$ had an increased proliferative state as measured by Ki67 and associated with advanced stages of ccRCC, suggestive of their malignant potential. Furthermore, RNA-seq and flow cytometry analysis showed that $CD24^{hi}ENPP3^{hi}$ cells were enriched in oxidative phosphorylation pathways, which significantly correlated with disease progression and a poor response to immunotherapy. Overall, this work presents a novel human cancer cell isolation approach which identified an aggressive cancer cell population. Additionally, this study discovers a cancer cell oxidative metabolic shift that can be leveraged for unexplored treatment avenues in ccRCC.

Keenan Wiggins, GMB **3:30PM**

Class switched memory B cell development and transcriptional programming is regulated by EZH2

Keenan J. Wiggins[1], James Rose[1], Jeremy M. Boss[1], and Christopher D. Scharer[1]

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For an effective vaccine, the development of antigen-specific memory B cells (MBCs) is crucial. MBCs are a diverse population derived from germinal center (GC) dependent or independent processes, resulting in class-switched and non-class switched MBCs. Class-switched IgG MBCs become plasma cells, while non-class switched MBCs seed secondary germinal centers after reinfection. The epigenetic factors influencing MBC differentiation and programming are not well understood. EZH2 is a histone methyltransferase that catalyzes H3K27me3, repressing gene expression, and regulates aspects of B cell differentiation. However, it is unclear whether EZH2 regulates MBC development. To study EZH2, multiple knockout models were established. In an influenza (PR8) model, the kinetics of antigen-specific MBC differentiation in cEZH2-KO mice were determined. The results showed that EZH2 is required for the development of certain MBC subsets, including IgG+, CD73+, and DP (PDL2+CD80+) MBCs. Extrafollicular/non-GC derived MBCs differentiate in an EZH2-independent manner, which reveals a distinct gene profile. EZH2 represses key gene regulators, determining the fate of class-switched and non-switched MBCs. Overall, this model highlights the role of EZH2-dependent programming in MBC development and function.

Supported by R01 AI148471 from NIH/NIAID to CDS

Katie Alexander, IMP	**3:45PM**

CD154:CD11b blockade increases MPEC differentiation of virus-specific CD8$^+$ T cells

***Katie Alexander*[1]**, Danya Liu[1], and Mandy Ford[1]

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CD154 pathway blockade has been shown to significantly improve graft survival in transplantation. Recently, CD11b was identified as an alternate receptor for CD154. CD154:CD11b interactions promote rejection by increasing the recruitment of innate and adaptive immune cells into the allograft. However, the effects of CD154:CD11b blockade on the protective immune response to infection during immunosuppression has not been elucidated. To address this, MHV68, a murine homolog of EBV, was used to assess the antigen-specific CD8$^+$ T cell response. Naïve B6 mice were infected with MHV68 and treated on days 0, 2, 4, and 6 with a peptide inhibitor of CD154:CD11b binding (cM7). cM7 treatment resulted in an increase in tetramer$^+$ antigen-specific CD8$^+$ T cells 10 days post-infection that exhibited an increase in gene expression associated with activation and antigen processing. To determine if this increase was a result of enhanced proliferation, CTV-labeled OT-I T cells were stimulated in vitro. Results indicated that CD154:CD11b blockade did not impact proliferation. Instead, cM7 treatment in vitro resulted in an increase in the expression of CD69, CD25, and CD44, and in vivo resulted in an increase in CD127^{hi}KLRG1^{lo} MPECs. In conclusion, these data demonstrate that CD154:CD11b blockade enhances the quantity and quality of virus-specific CD8$^+$ T cells during protective immunity. We speculate that this could be due to the inhibition of terminal differentiation of antigen-specific T cells resulting in enhanced differentiation of high quality memory precursors, or could be due to altered balance of CD154:CD40 vs CD154:CD11b binding, promoting CD40-driven costimulation.

Session 5:

Biochemistry of Microbes and Pathogens

4:15PM

Carter Brzezinski, MSP **4:15PM**

Total synthesis and biological characterization of natural-product inspired *Pseudomonas aeruginosa* specific antibiotics

Carter Brzezinski[1], Martina Golden[1]

[1]Department of Chemistry, Emory University

Promysalin is a natural product originally isolated from the roots of rice plants in Sri Lanka that exhibits unique species-specific antibacterial activity (IC50 =64nM) against Pseudomonas aeruginosa (PA), a highly pathogenic gram-negative bacteria implicated in cystic fibrosis, pneumonia, and other nosocomial infections. Our lab previously confirmed the exact stereochemical configuration of this natural product and used a diazirine photoaffinity probe to identify its target, succinate dehydrogenase (SdH). Based on a computational model of promysalin bound to PA Sdh (Fig 1A), we hypothesize that arylation of the extended alkyl side chain of promysalin could introduce a new π-π stacking interaction with a nearby tryptophan residue, potentially improving the potency of Sdh inhibition. To test this, I first synthesized a single brominated side chain intermediate via a 5-step synthetic sequence that could then be diversified into multiple different aryl substituents. Using a Nickel-catalyzed cross-coupling, I appended structurally and electronically diverse heterocycles to probe which aryl appendages engage in the desired π-π stacking interaction best. Each synthesized analog will be screened for antibacterial and anti-virulence via IC_{50}, MIC, and pyoverdine biosynthesis assays. This work will further explore the potential of PA SdH as a novel species-specific antibacterial target, and could lead to new tool compounds to study novel pharmacological approaches to combat PA infections.

Alexa Snyder, BCDB **4:30PM**

Mechanisms of HIV hypersensitivity to Islatravir

Snyder, A.A.[1], MacQuillian, J.[1], Kaufman, I.[1], Song, K.[1], Du, H., Kirby, K.A.[1,2], Michailidis, E.[1,2], Sarafianos, S.G[1,2].

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Highly active antiretroviral therapy (HAART) are highly effective at suppressing the Human Immunodeficiency Virus (HIV), allowing patients to have increased quality and length of life. Although HAART is very successful, drug resistance mutations can pose a major threat to the efficacy of these treatments. Islatravir, often referred to as EFdA (4'-ethynyl-2-fluoro-2'-deoxyadenosine), is a highly potent antiviral drug candidate currently in phase III clinical trials that has the potential to be a long-acting HIV-1 reverse transcriptase (RT)-targeting drug. EFdA inhibits HIV-1 by blocking RT translocation after its incorporation in the elongating viral DNA chain. Interestingly, the F227C mutation that emerges during therapy with some non-nucleoside reverse transcriptase inhibitors (NNRTI), was found to make HIV more susceptible to EFdA. Thus, pairing such an NNRTI with EFdA may be an optimal combination therapy. Here we explore the mechanism of F227C hypersensitivity to EFdA using structural, biochemical, and virological methods. Understanding this mechanism is important for future drug development utilizing hypersensitivity mutations to combat drug resistance.

Lisa Blackmer-Raynolds, NS	**4:45PM**

Alzheimer's disease-associated gut microbes uniquely shape microglia activation state

***Lisa Blackmer-Raynolds*[1]**, Maureen Sampson[2], Adam Hamilton[1], Aimee Yang[1], Anna Koslov[1], Jianjun Chang[1], Steven Sloan[2], and Timothy Sampson[1]

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Microglia have the capacity to play dual roles in Alzheimer's disease (AD), providing neurotrophic support and/or furthering neurotoxicity. Despite growing evidence of the importance of microglia responses for AD outcomes, extrinsic factors that modulate microglia activation remain poorly understood. The gut microbiome is one such factor, with the ability to modify both microglia state and AD outcomes. However, the link between specific AD-associated gut bacteria and microglia activation state remains unknown. Therefore, the present study mono-colonized wildtype germ-free mice with type strains of bacterial species of interest (*Escherichia coli, Bacteroides thetaiotaomicron, Clostridium celatum,* and *Lactobacillus johnsonii)* for 2 weeks before assessing microglia state. A bacterial dependent shift in overall inflammatory tone was observed with altered cytokine responses in the gut, serum, and brain. Further, RNA-seq analysis demonstrated sex and bacteria specific effects of mono-colonization on microglia, with *E. coli* mono-colonized mice, displaying a more active, phagocytic, and disease associated state. Thus, to determine whether *E. coli* can modify AD outcomes, conventionally raised 5xFAD mice were enriched with E*. coli* for one month. *E. coli* exposed 5xFAD mice displayed accelerated cognitive impairments compared to vehicle controls and increased neuroinflammatory markers. Together, these results suggest that carriage of non-pathogenic *E. coli* within the gut microbiome has the ability to modify both microglia state and cognitive outcomes, highlighting the potential importance of this microbe for AD.

Logan Kavanaugh, MMG **5:00PM**

Berberine dimers exhibit increased affinity for the *Pseudomonas aeruginosa* MexXY-OprM efflux pump compared to natural berberine

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An astounding 4.95 million deaths worldwide in 2019 were associated with failed antibiotic treatments, underscoring the urgent need to identify new targets and to revitalize current antibiotics. One potential resistance mechanism is through the active removal of drugs from the bacterial cell via efflux pumps. For example, the Resistance-Nodulation-Division (RND) efflux pump family are tripartite complexes that contribute extensively to clinical antibiotic resistance. The serious threat pathogen *Pseudomonas aeruginosa* encodes 12 RND efflux systems, including MexXY-OprM which uniquely effluxes aminoglycoside antibiotics. As aminoglycosides are the preferred treatments for cystic fibrosis patients with *P. aeruginosa* infections, molecular tools to study efflux pump function and efflux pump inhibitors (EPI) to block their action are both urgently needed. The natural alkaloid, berberine, was reported to be a specific, but weak, EPI for the MexXY-OprM system. To increase the efficacy of natural berberine, we used an *in-silico* high-throughput screen of berberine analogs against MexY, the inner membrane transporter. Through iterative rounds of computational validation (e.g., structure guided docking and molecular dynamics simulations) and microbiological testing (e.g., antimicrobial susceptibility assays and time-kills assays), we observed berberine dimers to have increased synergy with aminoglycosides. Particularly, a berberine dimer with a propane linker, Ber-C3 [64 µg/mL], reduced the minimum concentration of aminoglycosides required to kill wild-type *P. aeruginosa* strains PAO1, PA14, and PA7 and pan-aminoglycoside resistant clinical isolates 2- to 6-fold. These studies suggest the propane berberine dimer, Ber-C3, to be an effective and specific lead EPI for the MexXY-OprM efflux system in *P. aeruginosa*.

Poster Presentation Abstracts

Poster Presentations

Session 1: 1:15 – 2:00PM - Even-numbered posters

Session 2: 2:00 - 2:45PM - Odd-numbered posters

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1	Benjamin	Babcock	CB	**29**	Nicolas	Janto	GMB
2	Yu	Bai	NS	**30**	Xiyu	Li	MSP
3	Milon	Barmon	PBEE	**31**	Yonina	Loskove	GMB
4	Poulami	Basu Thakur	MMG	**32**	Manly	Lester	MSP
5	Julia	Bazzano	IMP	**33**	Veronika	Matsuk	CB
6	Kelsey	Bennion	CB	**34**	Jacob	Mattingly	BCDB
7	Bethany	Bogan	MSP	**35**	William	McFadden	BCDB
9	Maegan	Brockman	MSP	**37**	Olivia	Morrison	GMB
10	Joey	Buehler	BCDB	**38**	Roy	Mulpur	GMB
11	Jose	Castro	BCDB	**39**	Grace	Neilsen	BCDB
12	Diane	Choi	MSP	**40**	Tommy	O'Haren	GMB
13	Amber	Coats	MMG	**41**	Chiemela	Ohanele	BCDB
14	Ananya	Dash	IMP	**42**	Jonathan	Owen	MMG
15	Elizabeth	Feldman	GMB	**43**	Jordan	Owyoung	GMB
16	Tyshawn	Ferrell	BCDB	**44**	Cynthia	Perez	GMB
17	Saahj	Gosrani	NS	**45**	Tori	Placentra	GMB
18	Kyndal	Goss	IMP	**46**	Sayalee	Potdar	CB
19	Alexander	Gulka	GMB	**47**	Tana	Pottorf	NS
20	Lydia	Gutema	BCDB	**48**	Vedhika	Raghunathan	MMG
21	Melissa	Gutierrez	IMP	**49**	Monica	Reeves	GMB
22	Dominick	Hellen	GMB	**50**	Juliet	Santiago	NS
23	Megan	Hinrichsen	BCDB	**51**	Taylor	Shue	MMG
24	Heidi	Ulrichs	BCDB	**52**	Bri	Silver	GMB
25	Lauren	Hodkinson	GMB	**53**	Tina	Tian	NS
26	Claire	Holden	MSP	**54**	Elijah	Ullman	MSP
27	Yasmin	Ibrahim	BCDB	**55**	Sarah	Webster	BCDB
28	Kyeong Ran	Jang	MSP	**56**	Katherine	Westover	GMB
				57	Megen	Wittling	CB

Benjamin Babcock, CB **Poster #1**

FlowBEAT reveals an autoantibody signature in blood that predicts both vaccine response and effective anti-SARS-CoV-2 immunity in the nasal mucosa

Benjamin R. Babcock[1], Astrid Kosters[1], Nadia R. Roan[2,3], Sulggi Lee[4], Eliver E. B. Ghosn[1,5,*]

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Mounting an effective antibody response against SARS-CoV-2 is key to efficient recovery and lasting protection from severe disease. However, despite robust production of antibodies in blood after infection or vaccination, virus transmission of new variants of concern (VOC) through the nasal mucosa remains a threat to community health. Therefore, to determine whether repeated vaccination/boosting in infected-recovered and naïve individuals elicits effective antibody production in the nasal mucosa, we developed flowBEAT (*flow cytometry-based BEad assay to detect Antigen-specific antibody isoTypes*). A single flowBEAT reaction reveals all eight antibody isotypes/subclasses (IgG1-4/IgA1-2/IgM/IgE) against twenty-two SARS-CoV-2 structural and non-structural proteins – including VOC – along with a panel of host autoantibodies associated with disease severity.

We profiled greater than 500 samples collected from 100 donors over 18+ months and identified a flowBEAT signature which stratifies vaccine high-responders and non-responders. Surprisingly, vaccine high-responders show elevated levels of IgG1-switched autoreactive antibodies in blood. This autoreactive signature in blood predicts increased anti-SARS-CoV-2 responses in the nasal mucosa. Repeated vaccination in non-responders results in seroconversion to IgG4 but not IgG1. Most importantly, intramuscular vaccination after infection can induce antibody production distally in the nose, a mechanism which may help control transmission. Finally, flowBEAT reveals pronounced autoantibody profiles in severe disease pre-vaccination, suggesting that high autoreactive antibodies may be associated with disease severity in non-vaccinated individuals, but disease protection in the repeatedly vaccinated. These findings emphasize the importance of continued vaccination/boosting to both infected-recovered and naïve individuals and inform predictive blood markers of nasal immunity to help prevent community spread.

Yu Bai, NS **Poster #2**

The inhibition of LSD1 via sequestration contributes to Tau-mediated neurodegeneration

Yu Bai[1], Christopher Gadomski[1], Saahj Gosrani[1], Sonia Dalal[1], and David Katz[1,2]

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Recent data from our lab suggest that pathological neurofibrillary tau tangles (NFTs) may drive neurodegeneration in Alzheimer's disease (AD) by sequestering LSD1 in the cytoplasm of neurons and interfering with the continuous requirement for LSD1 to repress the inappropriate transcription of neurodegeneration pathways. However, the molecular mechanism through which NFTs inhibit LSD1 remain unknown. Tau is an intrinsically disordered protein that may function in a prion-like fashion, where the intrinsically disordered nature of the protein may seed the misfolding of normal copies of itself and other proteins. Based on this model, we considered the possibility that pathological tau may sequester LSD1 in the cytoplasm of neurons by interacting with an 180AA intrinsically disordered region (IDR) at the N-terminus of LSD1. To address this hypothesis, we utilized AAV virus to overexpress full length or N-terminal deleted LSD1 (LSD1ΔN) in the PS19 tau mouse model. In contrast to full length LSD1 which becomes sequestered in the cytoplasm by tau in PS19 mice, we find that LSD1ΔN properly localizes to the nucleus even in the presence of pathological tau. This suggests that the N-terminal IDR of LSD1 is required for pathological tau to sequester LSD1 in the cytoplasm of degenerating neurons. To determine whether pathological tau physically interacts with LSD1, we utilized a HEK 293 cell model, in which we have recapitulated the sequestration of LSD1 by pathological tau. Our preliminary data suggest that pathological tau can be immunoprecipitated with LSD1, indicating a physical interaction between them.

Milon Barmon, PBEE	**Poster #3**

Cost-effective measurement of nitrogen content in corn using Nix Pro color sensor

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Nitrogen (N) is one of the most essential but expensive inputs in agricultural production. Thus, if not managed properly, it can be lost through ecological (volatilization, denitrification and leaching) processes. Traditional laboratory measurement of plant leaf N requires access to expensive instruments like inductively coupled plasma (ICP). Turnaround times for processing samples and laboratory measurements can delay real-time decision-making for N management in the field. Inexpensive tools like the Nix Pro color sensor can be promising in the early detection of N deficiency symptoms, thus this rapid assessment of N can help sustainable management while mitigating crop demand. We developed N prediction models with the Nix Pro color sensor using a greenhouse experiment conducted at Emory University. Thirty-six measurements were collected at different growth stages of corn grown with a sterile soilless potting medium. Prediction models were developed using Nix Pro-measured color spaces (RGB, CMYK, LABC, and HXYZ) and compared with ICP-analyzed plant leaf N content. We found the best prediction model by combining different color parameters across multiple Nix Pro-measured color spaces followed by a stepwise regression analysis. Model efficiency evaluations for calibration (R^2 = 0.87, RMSE = 0.32, AIC = -17.43, and KGE = 0.9; n=18) and validation (R^2 = 0.63, MSPE = 0.26, and KGE = 0.6; n=18) indicated that the Nix Pro color sensor can be utilized to estimate plant N content in real-time. Expanding our findings requires evaluating Plant N in field conditions using different soil and crop types.

Poulami Basu Thakur, MMG **Poster #4**

Assessing the role of bacterial surface polysaccharides in influenza A virus infections.

Poulami Basu Thakur[1,2], Taronna R Maines[1], and Jessica A. Belser[1]

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Influenza A viruses (IAV) have a broad host range, and infect both humans and numerous zoonotic species. IAV are primarily transmitted between mammals by the respiratory route, and encounter commensal bacteria in the respiratory tract prior to binding to and infecting susceptible epithelial cells. Since IAV that circulate in humans and birds are well adapted to replicating in the human respiratory and avian gastrointestinal tracts, respectively, we hypothesized that IAV utilize surface components of commensal bacteria in their preferred host niche to enhance environmental stability prior to infection. Here, we examined two human H1N1 viruses derived from the 2009 pandemic, and two low-pathogenic avian H1N1 viruses; both human and avian IAV were capable of replicating to high titer in a human bronchial-epithelial cell line (Calu-3). Binding interactions of these IAV to a broad range of bacterial polysaccharides were assessed with microbial glycan microarrays, revealing that seasonal and avian IAV strains exhibit binding diversity to multiple bacterial glycans at both the host and strain level. To examine functional consequences of these binding interactions, IAV were incubated at 33^{o}C and 42^{o}C with LPS derived from *Escherichia coli* or *Pseudomonas aeruginosa*, and assessed for retention of viral infectivity two hours post-incubation. Interestingly, incubation with *P. aeruginosa* resulted in lower IAV infectivity rates compared to incubation with LPS from *E. coli*, in a temperature-dependent manner. Our findings support differential interactions with host bacteria between human and avian H1N1 viruses, suggesting that these interactions may contribute to viral host range restriction.

Julia Bazzano, IMP **Poster #5**

Gm2a as a novel regulator of CD8$^+$ T cell threshold of activation in transplantation

***Julia M. R. Bazzano*[1],** Kirsten M. Baecher[1], Danya Liu[1], Miguel Fribourg[2], Paolo Cravedi[2], Peter Heeger[3], Mandy L. Ford[1]

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Transplant patients must remain on toxic immunosuppression for life, underscoring the need to identify immunotherapeutic targets that may facilitate transplant tolerance. We recently showed that kidney transplant recipients that remained stable following withdrawal of mainstay immunosuppression exhibited increased levels of the glycosphingolipid-catabolizing protein Gm2a, specifically in CD8$^+$ T cells, compared to patients who went on to reject their allografts. While the role of Gm2a in lysosomal glycosphingolipid degradation in neurons is well known, little is known about Gm2a function in the immune system. To investigate this, we performed skin graft surgery in WT vs *Gm2a*$^{-/-}$ mice. Results show that *Gm2a* deficiency significantly increased allograft rejection relative to WT counterparts. Moreover, adoptive transfer of donor-reactive WT vs. *Gm2a*$^{-/-}$ CD8$^+$ T cells into WT hosts resulted in increased accumulation of donor-reactive T cells and accelerated allograft rejection in recipients of *Gm2a*$^{-/-}$ CD8$^+$ T cells as compared to recipients of WT CD8$^+$ T cells. This increased accumulation was likely due to increased proliferation, as in vitro studies revealed enhanced proliferation of CTV-labeled *Gm2a*$^{-/-}$ vs. WT CD8$^+$ T cells. Interestingly, *Gm2a*$^{-/-}$ CD8$^+$ T cells exhibited increased K^b/SIINFEKL tetramer staining and sustained TCR expression following antigen stimulation compared to WT CD8$^+$ T cells. Finally, *Gm2a*$^{-/-}$ CD8$^+$ T cells exhibited increased responsiveness to low dose and low-affinity peptide compared to WT cells. In conclusion, these results identify Gm2a as a novel, CD8$^+$ T cell-intrinsic regulator of the T cell activation threshold that directly impacts CD8$^+$ T cell-mediated allograft rejection.

Kelsey Bennion, CB **Poster #6**

Making their own off switch: CD8$^+$ T cells capable of producing Fgl2 that binds the novel CD8$^+$ T cell inhibitory receptor, FcγRIIB, and induces apoptosis of CD8s

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Cancer immunotherapy has revolutionized patient outcomes in the clinic and effector CD8$^+$ T-cell tumor infiltration is a critical factor to immunotherapeutic success. Our lab discovered that FcγRIIB, the sole inhibitory IgG-Fc receptor, is upregulated on effector-like memory CD8$^+$ T-cells at the tumor in mice and humans. FcγRIIB$^+$ CD8s possess higher proliferative ability and secrete more proinflammatory cytokines than their FcγRIIB$^-$ counterparts in mice and humans, making them imperative to the antitumor response. We have also discovered that fibrinogen-like protein 2, Fgl2, binds FcγRIIB and induces FcγRIIB-mediated cell death of CD8$^+$ T-cells. Fgl2 is known to be produced by several cell types in the tumor microenvironment such as macrophages and regulatory T cells. We have discovered that CD8$^+$ T-cells can express Fgl2 upon activation, raising the possibility that FcγRIIB$^+$ CD8s could regulate themselves through FcγRIIB-Fgl2 binding. Critical clinical data shows that Fgl2 is expressed on patient CD8$^+$ tumor-infiltrating lymphocytes (TIL) and is correlated with decreased immunotherapy response. To that end, we generated a conditional knockout model wherein only the tumor-specific CD8$^+$ T-cells lack Fgl2. When challenged with melanoma, mice given Fgl2-deficient tumor-specific CD8s exhibited enhanced antitumor response, increased cell number of FcγRIIB$^+$ CD8s, and decreased tumor size. These data support the protumor role of Fgl2 is dysregulating the T-cell antitumor response. Furthermore, we have identified a regulatory signaling axis whereby effector-like memory CD8$^+$ T-cells produce their own off-switch. The discovery of this pathway opens several potential avenues to manipulate the expression and signaling of this axis to increase patient treatment options.

Bethany Bogan, MSP **Poster #7**

The role of fatty acid synthase in atherosclerosis

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The contractile phenotype of fully differentiated VSMCs is essential for vascular function. In response to various stimuli, such as cholesterol or lipoproteins, VSMCs dedifferentiate and acquire characteristics of foam cells. These lipid-laden, macrophage-like cells comprise a large portion of the Atherosclerotic plaque. A significant characteristic separating VSMC-derived foam cells from myeloid-derived is their lack of phagocytic capacity, possibly causing lipid uptake to be impaired. We hypothesize that VSMC-derived foam cells acquire characteristics like macrophages but accumulate lipids through intracellular *de novo* Lipogenesis rather than phagocytosis.

Our data shows that when Smooth Muscle Cells are exposed to cholesterol (CHO) *in vitro*, the expression of the critical lipogenic enzymes fatty acid synthase (FASN) (3.6±1 vs. 9.0±0.9 AU $p<0.01$), acetyl-CoA carboxylase (ACC) (2.8±0.5 vs. 10.2±2.3 AU $p=0.02$) is significantly increased. Additionally, cholesterol treatment induced the phosphorylation of ATP Citrate Lyase (ACLY) (1.4±0 vs. 6,2±1 AU). Consistent with the literature, we observed that CHO treatment increases the expression of the macrophage-related marker CD68. Significantly, downregulation of FASN inhibits the CHO-induced CD68 expression upregulation (9.1±0.8 vs. 4.6±1,9 $p=0.002$). Consistent with the reduction in foam cell markers, FASN deficient VSMCs failed to accumulate lipids after CHO treatment measured by Oil Red O staining.

Our study shows lipogenic enzymes are upregulated during the VSMC to foam cell transition triggered by CHO. FASN expression is required for VSMC-derived foam cell formation. Our data suggest that VSMC lipogenesis participates in foam cell formation and may contribute to atherosclerotic plaque accumulation. FASN may represent a novel target for therapeutic intervention.

Maegan Brockman, MSP **Poster #9**

Targeting neutrophil type I interferon response in myocardial ischemia reperfusion injury

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Inflammation following myocardial ischemia/reperfusion (MI/R) plays a significant role in damaging cardiomyocytes and influencing infarct size, with neutrophils being the most rapid and numerous cells in the post-MI/R heart at 24 hours. Preliminary single-cell RNA sequencing data shows type I interferon (IFN) responding neutrophils are present in the hearts of mice 24 hours post-MI/R. We hypothesize that type I IFN responding neutrophils participate in the activation of STING and IFN signaling pathways, promoting inflammation in MI/R. Mice underwent 60 minutes of ischemia followed by reperfusion, and were dosed with either vehicle, a STING inhibitor (H151), or an IFNAR1 antibody at reperfusion and at 24 hours following reperfusion. Cardiac function was assessed two weeks post-reperfusion via echocardiography and tissue samples were collected for histological analysis. Mice treated with H151 or IFNAR1ab showed significant improvement in ejection fraction (43.8% ± 7.1; 44.9% ± 6.8) compared to mice treated with vehicle (31.9% ± 6.2; One-way ANOVA, N=5-6, $p<0.05$). Additionally, mice treated with IFNAR1ab showed significant improvements in LVESD (3.16mm ± 0.52) compared to vehicle treated mice (3.89mm ± 0.40; One-way ANOVA, N=5-6, $p<0.05$), with trending decreases in LVESD for mice treated with H151 (3.23mm ± 0.32, N=5). Treatment with H151 or IFNAR1ab reduced scar size (7.22% ± 3.6; 7.88% ± 3.1%) compared to mice treated with vehicle (9.74% ± 3.7; N=5-6). These data show that targeting the early IFN response in MI/R can improve cardiac function, and provide a basis for studies identifying the effects of the neutrophil-specific IFN response following MI/R.

Joey Buehler, BCDB **Poster #10**

Elucidating the mechanism of Orb2-mediated neural stem cell centrosome asymmetry and division.

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Neural stem cells (NSCs) orchestrate repeated rounds of asymmetric cell division designed to pattern the developing brain with neurons and glia. During each round, the centrosomes facilitate the segregation cell fate determinants that predispose each daughter cell to a different fate. *Drosophila* Orb2 is a cytoplasmic polyadenylation element binding protein (CPEB) ortholog involved in the post-transcriptional regulation of diverse mRNAs across multiple tissues. Our lab recently reported that loss of *orb2* results in NSC centrosome maturation defects and microcephaly, but the mechanisms behind these phenotypes are unknown. To dissect the role of Orb2 in neurodevelopment, we are testing whether Orb2 regulates centrosome activity or brain volume via its role in translational toggling. My preliminary data shows that a functional Orb2 is required for transient suppression of the basal centrosome. However, previous localization experiments revealed that Orb2-GFP does not localize to centrosomes, but rather is diffusely distributed within the NSC cytoplasm. Taken together, these data suggest Orb2 functions indirectly, likely through translational control of one or more mRNA targets, to instruct centrosome activity. Consistent with this idea, an in-silico analysis of crosslinking immunoprecipitation and sequencing data (CLIP-seq) of Orb2 targets within *Drosophila* S2 cells identified an enrichment of RNAs with centrosome ontologies. To identify targets involved in centrosome maturation, we are presently conducting western blots to determine if Orb2 affects steady state protein levels of centrosome activation genes. This work reinforces a model whereby Orb2 contributes to posttranscriptional regulation of centrosome genes critical for NSC asymmetric centrosome maturation, guiding proper neurodevelopment.

Jose Castro, BCDB **Poster #11**

SARS-CoV-2 nsp13 helicase: a novel antiviral drug target

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SARS-CoV-2 is an ongoing global pandemic with an urgent need for novel antivirals. In three years, more than 670 million cases have been reported, of which 6.7 million proved fatal. SARS-CoV-2 has evolved rapidly allowing variants of concern to emerge thus becoming resistance to the available vaccines. Currently in the US only one antiviral has been approved by the FDA: Remdesivir (RDV). RDV is a nucleoside analog that is incorporated into nascent viral mRNA by the viral polymerase (nsp12), inhibiting the synthesis of new viral proteins and thus viral replication. Unfortunately, the high mutation rate and widespread of SARS-CoV-2 require the development of additional antivirals as additional lines of defense. Upon viral replication, nonstructural protein 13 (nsp13) interacts with the replication-transcription complex (RTC, nsp12/nsp7/nsp8) to facilitate unwinding of viral RNA allowing viral replication and translation of additional proteins that ultimately generate more infectious viruses. Therefore, I hypothesize that biochemical and structural studies will elucidate the important atomic details necessary for potent and not toxic antivirals that target nsp13 helicase activity. An initial screening campaign using libraries of FDA approved drugs and bioactive compounds was performed using a FRET-base. For compounds to move downstream in the drug development process it is important to have mechanistic information. Kinetic studies, microscale thermophoresis, HDX-MS and X-ray crystallography are used to further characterize the inhibitors mechanism of action. The long-term goal of my work is to identify and characterize nsp13 inhibitors that can be further optimized and developed into antiviral candidates for treating coronavirus infection.

Diane Choi, MSP **Poster #12**

Anatomical characterization of glutamate delta receptor 1 in the lateral habenula

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Glutamate delta receptors (GluD) are synaptogenic molecules that form trans-synaptic complexes with proteins neurexin and cerebellin to regulate the formation and maintenance of synapses. Recent studies have suggested synaptogenic functions of GluD1 in the striatum, hippocampus, and amygdala. Despite its strong expression, nothing is known about the function of GluD1 in the lateral habenula (LHb), a subcortical structure that processes reward-related behaviors and regulates monoaminergic systems. Mutations of *GRID1*, the gene that encodes for GluD1, and LHb dysregulation are associated with psychiatric disorders such as depression and schizophrenia suggesting that disruption of GluD1 signaling in the LHb may contribute to the pathobiology of these disorders. A deeper understanding of GluD1 function in LHb requires a detailed map of its subcellular localization at the ultrastructural level. To address this issue, we used electron microscopy (EM) immunogold and immunoperoxidase methods in monkey and mice tissue. Preliminary EM data from immunoperoxidase-stained LHb tissue demonstrated that GluD1 is mainly expressed in dendrites, with lower expression in neuronal somata, spines, and glial processes in monkeys and mice. Immunogold data showed a strong expression of GluD1 at a subset of axo-dendritic and axo-somatic symmetric and asymmetric synapses. Extra-synaptic and intracellular dendritic labeling was also found. Studies are in progress to determine the source(s) of LHb afferents that express GluD1. Our results lay the foundation to determine how GluD1 regulates synaptic transmission in the LHb and how GluD1 disruption may contribute to psychiatric disorders associated with LHb dysfunction.

Amber Coats, MMG **Poster #13**

Identifying Potential Compensatory Interactions in Seasonal Human Coronaviruses

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Despite the worldwide implementation of non-pharmaceutical interventions, and, more recently, vaccination, the SARS-CoV-2 pandemic has not been contained, and this virus will continue circulating within the human population for years to come. Phylogenetic and experimental analyses from the last two years have indicated that this virus has been adapting to humans, and more recently, evolving to escape immunity. A subset of these analyses has shown that insertions and deletions (indels) may play a role in immune escape either directly (with additional compensatory substitutions elsewhere in the genome) or by themselves compensating for fitness losses arising from substitutions that enable immune escape. Because SARS-CoV-2's ability to sustain continuous human-to-human transmission mirrors that of the four endemic seasonal coronaviruses (HCoV-229e, HCoV-HKU1, HCoV-NL63, HCoV-OC43), we aimed to assess whether indels may play a similar role in these seasonal coronaviruses that have been circulating in humans for hundreds of years. We hypothesized that seasonal coronaviruses display evolutionary patterns consistent with compensatory interactions between indels and amino acid substitutions, allowing the virus to repeatedly escape population level immunity. Our preliminary results show, in all four seasonal coronaviruses, a consistent pattern of indels that originate within 5 years of point mutations near epitope sites. These results indicate that there may be a fitness advantage to these two mutations co-occurring. Overall, this consistency in patterns indicates that viruses in *Coronaviridae* are likely subject to strong evolutionary constraints and that compensatory mutations are likely needed to escape population level immunity over longer time scales.

Ananya Dash, IMP **Poster #14**

SpeB-matured M protein in Group A *Streptococcus* impairs neutrophil killing

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M protein is one of the most abundant surface virulence factors of *group A Streptococcus* (GAS). Encoded by *emm* gene, over 200 distinct genotypes of GAS have been classified based on N-terminal hypervariability. The M1 antigenic strain is a widely circulating variant of GAS and is a leading cause of streptococcal toxic shock syndrome (STSS). Previous studies have explored the interactions of the membrane-bound form of M1 protein with multiple host proteins. However, it is unclear whether similar mechanisms govern the released soluble M protein during GAS infections. In our studies, we identify a unique cleavage product mediated by SpeB to address downstream immune dysregulations. Preliminary studies point towards immune dysregulation wherein GAS evades phagocytosis by neutrophils. Future investigations aim at revealing the molecular mechanisms of GAS resistance to neutrophils via soluble M protein. This, in turn, can provide insights on immune evasion strategies by GAS and enable us to prevent severe disease outcomes.

Elizabeth Feldman, GMB **Poster #15**

Genome-wide association studies of Down-Syndrome associated AVSD, ASD, and VSD

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Down Syndrome (DS) is a leading cause of structural birth defects and 40-50% of affected children have a congenital heart defect (CHD). Atrioventricular septal defect (AVSD) is a form of CHD occurring in 1/5 infants with DS compared to 1/10,000 euploid infants. While increased dosage of chromosome 21 genes clearly contributes to this risk, trisomy 21 is not sufficient to cause CHD as about 50% of infants with DS have structurally normal hearts (NH). Thus, additional modifying factors may exist. In order to characterize the genetic architecture of CHD in DS, we sequenced genomes of children with DS and a structurally NH (NH+DS, n=572) or DS and a CHD (AVSD+DS, n=438; ASD+DS, n=122; VSD+DS, n=170). We performed subtype specific genome-wide association studies (GWASs) for common variants (MAF>0.05) using logistic regression and adjusting for principal components of ancestry. Although no SNP achieved genome-wide significance ($p<5x10^{-8}$), multiple loci achieved suggestive significance ($p<2x10^{-6}$) in each analysis. Some of these regions include: 5q35.2 ($p=7.67x10^{-8}$, OR=1.97) in the combined CHD GWAS, near *MSX2*, which regulates proliferation of cardiac neural crest cells; 12q21.2 ($p=9.76x10^{-7}$, OR=0.57) in the AVSD+DS GWAS, near *NAV3*, which is required for zebrafish heart development; 1p35.1 ($p=3.32x10^{-7}$, OR=3.15) in the ASD+DS GWAS, near *RBBP4,* a complex of the NuRD complex which interacts with known heart development gene; and 5q33.1($p=8.74x10^{-7}$, OR=1.98) in the VSD+DS GWAS, near known heart development genes *HAND1* and *SAP30L*. The results from this study will generate new hypotheses about biological pathways associated with abnormal heart development due to trisomy 21.

Characterizing the landscape of DENV antigenic evolution

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Dengue virus (DENV), a positive-sense RNA flavivirus, infects more than 390 million people annually. DENV consists primarily of four serotypes, which are genetically related but differ antigenically. Quadrivalent vaccine development has been based on the assumption that including a representative virus from each serotype can generate neutralizing immunity to all variants within all serotypes. However, recent studies indicate variable neutralization of variants within a serotype after vaccination. Therefore, this study aims to determine how genetic variation within a serotype leads to distinct antigenicity. To test this, we have obtained paired patient serum and virus samples from two Colombian cities (Cali and La Virginia) with different disease burdens (6658 and 18 cases, respectively) during the 2021 outbreak. DENV positive samples underwent full genome sequencing using metagenomic library preparation and reference-based assembly. From these samples, we have identified: serotype 1, genotype V (n=17); serotype 2, genotype III (n=3); and serotype 3, genotype III (n=6). While most sequences are serotype 1, genotype V, sequences from Cali and La Virginia within serotype 1 form different clades on a phylogenetic tree, indicating different variants. To test the capacity of our variant mutants to evade existing immunity we have established a pliable, single-cycle, GFP reporter virus particle (RVP) system in which to express variant E protein sequences. In further studies, neutralizing antibody titers in serum from Cali or La Virginia cases will be measured against the RVP panel to assess antigenic variability between these two populations. Our findings will improve surveillance of emergent variants of concerns.

Saahj Gosrani, NS **Poster #17**

Investigating how inhibition of the histone demethylase LSD1 leads to neurodegeneration in Alzheimer's disease

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H3K4me1/2 is associated with active transcription. The histone demethylase LSD1/KDM1A removes this modification to repress transcription. Data from our lab suggest that pathological neurofibrillary tau tangles (NFTs) may drive neurodegeneration in Alzheimer's disease (AD) by sequestering LSD1 in the neuronal cytoplasm, interfering with continuous LSD1-dependent repression of neurodegenerative transcriptional pathways. To investigate this further, we are taking a two-pronged approach. First, we performed H3K4me2 chromatin immunoprecipitation (ChIP) in the hippocampus of our inducible LSD1 knockout mice to determine how LSD1 loss affects transcription. Surprisingly, H3K4me2 increases not only at genes that are activated following LSD1 deletion, but at genes that are unchanged and repressed. This suggests that LSD1 maintains the terminally differentiated state of neurons by demethylating H3K4me2 and providing a thermodynamic barrier to transcription genome-wide. Required neuronal genes easily overcome this barrier. However, in AD when LSD1 is inhibited, neurodegenerative pathways vulnerable to activation are inappropriately expressed. We will perform the assay for transposase-accessible chromatin (ATAC-Seq) to further determine transcription factor binding and chromatin accessibility changes following the loss of LSD1. In addition, to examine how neuronal cell death occurs when LSD1 is inhibited, we incorporated a Thy1-YFP neuronal reporter into our LSD1 inducible mice. We have shown that axons and dendrites are completely lost from hippocampal neurons upon loss of LSD1 in our inducible knockout mice. Using two-photon live imaging in awake animals, we will determine whether this is an active part of neuronal cell death and interrogate the role of the microglia activation in this process.

Kyndal Goss, IMP **Poster #18**

Multiomic analysis of γMSC-suppressed alloreactive T cells

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A major complication of hematopoietic cell transplantation is the development of graft-versus-host disease (GVHD), an immune-mediated disorder which arises when adoptively transferred donor naïve T cells become activated by host antigen presenting cells and damage host tissues. It is crucial to find an alternative GVHD prophylaxis that is more effective and less toxic than what is currently available. Mesenchymal stromal cells (MSCs) have powerful immunomodulatory and tissue regenerative capabilities and suppress alloreactive T cells when primed with IFNγ (γMSCs); however, an FDA-approved cellular therapy for GVHD has not been developed, in part, because a full understanding of the mechanism of action hasn't been elucidated. Preliminary experiments with γMSC-suppressed alloreactive T cells revealed excess reactive oxygen species (ROS) within the first 24 hours of suppression which seemingly induce prompt cell cycle exit without simultaneously suppressing differentiation. Thus, we hypothesize that γMSCs induce a complex systems effect driven by ROS-induced changes in chromatin architecture, which governs gene expression. We utilized 10X Genomics Multiome technology (scRNA-seq and snATAC-seq) to analyze γMSC suppressed T cells to identify the T cell genetic and epigenic changes which underlies the suppressive effect. Our data indicate that γMSCs induce cell cycle exit and transiently repress some activation/differentiation pathways. All repressed pathways recover or even surpass expression levels in controls once γMSCs have waned. These data will continue to be analyzed using the SCENIC+ pipeline to build enhancer-driven gene regulatory networks (eGRNs). Comparing eGRNs between γMSC-suppressed and control T cells will reveal the networks that are disrupted by γMSCs.

Alexander Gulka, GMB **Poster #19**

Defining the molecular features of BAF-regulated chromatin loci

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Chromatin remodeling is essential for eukaryotic transcriptional regulation, and mutations in genes encoding components of the chromatin remodeling machinery are prevalent causes of cancer and neurodevelopmental disorders. Changes to a cell's transcriptional program rely on activation of appropriate sets of *cis*-regulatory sequence elements (cREs). An early step in cRE activation is local remodeling of chromatin resulting in increased accessibility. Chromatin is remodeled at cREs by the BRG-1/BRM-Associated Factors (BAF) complex. The mechanisms by which BAF complexes are localized to chromatin remain unclear, as this complex has no inherent ability to recognize specific DNA sequences. To investigate factors that may play a role in recruiting BAF complexes to cREs, I identified genomic sites that depend on BAF activity for their chromatin accessibility and characterized patterns of transcription factor (TF) binding and histone modifications at these loci. Specifically, I measured genome-wide changes in chromatin accessibility following inhibition of BAF activity in GM12878 cells, a cell line in which the genomic distributions of over 200 TFs and histone modifications have been profiled. BAF inhibition resulted in altered accessibility at tens of thousands of loci, with the most pronounced trend being loss of accessibility at transcription start site-distal elements. These sites of lost accessibility were specifically enriched for binding of members of the Activator Protein 1 (AP-1) TF family, while unaffected sites were enriched for CCCTC-binding factor (CTCF) and cohesin complex binding. These results highlight a potential role for AP-1 TFs in BAF localization, inviting further mechanistic study.

Lydia Gutema, BCDB **Poster #20**

Investigating DNA features involved in centromere specification

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Centromeres are chromosomal loci that bind to spindles to facilitate chromosome segregation and ensure the faithful inheritance of genetic information in daughter cells. DNA sequences at the endogenous centromeric locus are highly repetitive, and while they have some sequence similarity, centromeric DNA sequences at different chromosomes are substantially different. Despite this, centromeric proteins are recruited to correct sites during each round of cell division. How centromeric DNA encodes the centromere in the absence of conserved sequences is not well understood. Defects in centromere localization cause ectopic centromeres, which are often associated with chromosomal breakages. This genomic instability is a hallmark of cancer and ectopic centromeres are found in many developmental disorders. Ectopic centromeres have a non-random distribution across the genome, which raises interesting questions about how the location of the centromere is specified. However, conserved features of the DNA or chromatin at these sites and at the endogenous centromeric DNA have not been discovered yet. We hypothesize that the centromeric nucleosomes that define the location of the centromere are targeted to unique DNA structures. Our early data indicate that DNA structures may play an underlying role in centromere specification at endogenous centromeres. Future work will assess the relationship between centromeric proteins and DNA structure within known centromeric sites. This work will allow us to address longstanding questions in the field about how the localization of centromeres is determined in humans, and better understand how unique DNA structures play a role in cellular function.

Melissa Gutierrez, IMP **Poster #21**

Chronic alcohol exposure alters the immune landscape of mice

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Chronic alcohol consumption has been shown to significantly increase sepsis mortality. However, mechanisms by which alcohol consumption influences immune dysregulation worsening septic outcomes are poorly understood. Since alcohol and sepsis may exert independent effects on the immune system, it is important to understand how alcohol alters immune cell composition and function in isolation. C57BL/6 mice were given alcohol in their drinking water starting with 5% ethanol and increasing by 5% every five days to reach 20%. Control animals drank water. Mice were bled every fifth day and samples stained for flow cytometry. Bulk $CD4^+$ T cell frequencies increased from 3.2% in mice drinking 5% ethanol to 7.3% in mice drinking 20% ethanol (p=0.01). Additionally, naïve $CD44^{lo}CD4^+$ T cell frequencies were consistently increased (p=0.006) while effector/memory $CD44^{hi}CD4^+$ T cells were decreased following alcohol (=0.006). $FoxP3^+CD25^+CD4^+$ regulatory T cells were increased in alcohol-drinking mice with a mean of 245.4 cells/uL compared to 158.6 cells/uL at 20% ethanol (p=0.01). While frequencies of bulk $CD8^+$ T cells were not altered, naive $CD44^{lo}CD8^+$ T cells were increased with alcohol (p=0.04), while effector/memory $CD44^{hi}CD8^+$ T cells were decreased (p=0.04). Moreover, the number of $FoxP3^+CD8^+$ T cells in alcohol-drinking mice was increased at with an average of 44.3 cells/uL opposed to 26.8 cells/uL in water-drinking mice at 20% ethanol (p=0.01). Given the decrease in effector/memory $CD4^+$ and $CD8^+$ T cells and increase in regulatory T cells, these results demonstrate that chronic alcohol skews the immune landscape towards an immunosuppressive state and could influence immune dysregulation during sepsis.

Dominick Hellen, GMB **Poster #22**

LiverQuant: Automated quantitative whole slide histologic analysis of liver digital pathology

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Current means to quantify cells, gene expression, and fibrosis of liver histological slides are not standardized in the research community and typically rely upon data acquired from a selection of random regions identified in each slide. As such, the data are subject to unconscious bias of selection as well as limited subsets of available data elements throughout the slide. An ability to provide a whole slide analysis of cells and fibrosis would provide for a more accurate and complete quantitative analysis, along with minimization of intra-and inter-experimental variables. Herein, we present LiverQuant, a method that utilizes whole-slide scanning of digitized histologic images to render a more comprehensive analysis of presented data elements. This process is available and readily adaptable by most laboratories, requires minimal optimization, and makes use of customizable GROOVY-based scripts within Qupath. After loading images and preparing the project in the Qupath program, researchers are provided with 1-2 scripts per analysis that generate an average intensity threshold for their staining, automated tissue annotation, and downstream detection of their anticipated cellular matrices—here we highlight its utility to quantify liver cell counts of macrophages and resident cholangiocytes, along with quantitation of inter-cellular fibrosis. The workflow presented within this method will improve the reliability and reproducibility of experimental results while ultimately reducing the amount of time required by scientists to perform bulk analysis of liver histology.

Megan Hinrichsen, BCDB **Poster #23**

Unraveling the aminoglycoside-dependent regulation of the MexXY-OprM efflux pump in *Pseudomonas aeruginosa*

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The emergence of multidrug-resistant bacterial pathogens–predominantly through the expression of efflux pumps that expel multiple classes of antibiotics out of the cell–is a major public health concern due to increased antibiotic treatment failure. The serious threat pathogen, *P. aeruginosa,* encodes the resistance-nodulation-division efflux pump MexXY-OprM,which is uniquely upregulated by one of its efflux substrates: aminoglycoside antibiotics. Currently, the molecular mechanism behind this regulation is undefined. When aminoglycosides bind their ribosomal target site, the ribosome stalls at an attenuator sequence of PA5471.1, recruiting ribosome-associated protein SuhB. However, the role of the putative release factor, PrfH, is poorly understood and it remains to be determined if specific classes of aminoglycosides differentially induce *mexXY* expression. First, we performed *in vitro* translation of a template protein in the presence and absence of SuhB and aminoglycosides to understand if PrfH works as a release factor in their presence. Second, we performed *in-vivo* antimicrobial susceptibility assays and RT-qPCR in *P. aeruginosa* PAO1 and an isogenic Δ*mexXY* strain in the presence of two structurally distinct aminoglycoside classes and the m^7G1405 16S rRNA methyltransferase, RmtA, to block drug-ribosome interaction and thus tease apart aminoglycoside-dependent regulation. Our data suggests that PrfH putatively hydrolyzes the peptidyl-tRNA from the ribosome when aminoglycosides or SuhB are present. Additionally, we observed aminoglycoside-dependent upregulation in the presence of RmtA in wild-type *P. aeruginosa* PAO1 but not in the isogenic Δ*mexX*Y. This study promises to provide insights on aminoglycoside-induced resistance in *P. aeruginosa* and to ultimately elucidate potential targets for novel therapeutics.

Twinfilin, formin and capping protein form a multicomponent *Ménage à Trois* at the actin barbed end

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The actin cytoskeleton and its associated regulatory proteins are crucial for neuronal development. While it is well-accepted that actin dynamics are essential in growth cones, dendritic spines, and axon terminals, the underlying molecular mechanisms regulating actin dynamics within these structures have yet to be uncovered. Actin elongators (formin), blockers (capping protein), and depolymerases (twinfilin) all exist within a neuronal cell, in a shared cytoplasm. On their own these proteins have distinct activities and have long been thought to bind barbed ends in a mutually exclusive manner i.e. one at a time. However, using microfluidic assisted - total internal reflection fluorescence microscopy and multispectral single molecule imaging, we have discovered that polymerases, depolymerases and blockers can simultaneously bind the same filament barbed end. To our knowledge this is the first report of a three-protein multicomponent complex at the actin filament barbed end. We find that simultaneous presence of these proteins at the barbed end leads to much faster protein transitions at the barbed end and allows for fine control of elongation rate as well as filament lengths and might help explain how the wide diversity in size and dynamics of intracellular actin structures is achieved *in vivo*. Using separation of function mutants, we further show that twinfilin destabilizes the capping protein and stabilizes formin at the barbed end, and as a result acts as a pro-elongation factor in spite of being a barbed end depolymerase on its own.

Lauren Hodkinson, GMB **Poster #25**

An undergraduate driven bioinformatics screen reveals Hox and chromatin remodeling candidates at the *Drosophila* histone locus

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Cells orchestrate histone biogenesis with strict temporal and quantitative control. To efficiently regulate coordinated gene expression, the repetitive *Drosophila melanogaster* replication-dependent histone genes are arrayed and clustered at a single locus. Transcription and regulatory factors concentrate around the locus forming a nuclear body known as the histone locus body (HLB). Historically, HLB factors are largely discovered by chance, but few are known to interact directly with DNA. It is therefore unclear how the histone genes are specifically targeted for their unique and coordinated regulation. To expand the list of known HLB factors, we initiated a bioinformatics candidate-based screen as part of a course-based undergraduate research experience (CURE), where students mapped 30 publicly available ChIP datasets of 27 DNA-binding factors to the *Drosophila* histone gene array. We identified several novel transcription factor candidates, including the *Drosophila* Hox proteins Ultrabithorax (Ubx), Abdominal-A (Abd-A) and Abdominal-B (Abd-B), suggesting a new pathway for these factors in influencing body plan morphogenesis. Additionally, we identified six other transcription factors that target the histone gene array: the JIL-1 kinase, the hormone-like receptor Hr78, the long isoform of a BET family protein fs(1)h as well as the generalized transcription factors TAF-1, TFIIB, and TFIIF. Our current work involves following up on our candidates by performing immunofluorescence staining experiments on *Drosophila* larvae and embryos. This screen provides several candidates as well as a pipeline for future studies into factors that influence histone biogenesis.

Claire Holden, MSP **Poster #26**

The role of peroxisomes and bile acid-initiated signaling in vascular smooth muscle cell phenotype

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Background: Atherosclerosis is a chronic inflammatory process characterized by the accumulation of foam cells. Vascular smooth muscle cells (VSMCs) are not terminally differentiated; their phenotype is modulated in response to stimuli, such as cholesterol (CHO), increasing susceptibility to vascular diseases. Modulated SMCs contribute to foam cell formation within atherosclerotic plaques. Intracellular metabolism of CHO in hepatocytes produces bile acids which agonize FXR, regulating bile acid, glucose, and lipid homeostasis, and LXR, facilitating the first step of reverse CHO transport. It is unknown if VSMCs synthesize bile acids and whether peroxisomes and the bile acids they produce play a role in VSMC-derived foam cell formation. **Results:** CHO treatment of human aortic VSMC (HASMCs) decreased expression of several peroxisomal proteins. Peroxisomal loss-of-function experiments demonstrated increased intracellular lipid accumulation, as visualized with Oil Red O (ORO) staining. Peroxisomal biogenesis associated with fenofibrate treatment eradicated intracellular ORO. We have also shown that HASMCs synthesize bile acids after CHO exposure. HASMCs treated with LXR agonists increased expression of CHO efflux transporter ABCA1, increased differentiation marker expression, and decreased expression of macrophage markers. **Conclusions:** VSMCs have the metabolic capacity to synthesize bile acids, which is impaired if peroxisomes are non-functional or absent. Our data indicates that activation of bile acid receptors upregulates ABCA1, favoring a differentiated VSMC phenotype. Together, these data suggest that bile acid-mediated activation of FXR and LXR have differential roles in CHO-induced VSMC to foam cell transition and may represent a novel atheroprotective target which increases cellular efflux of CHO, promoting VSMC differentiation.

Apical integrins as a target for restoring barrier function in lung epithelium

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Acute respiratory distress syndrome (ARDS) is a life-threatening inflammatory lung disease that has high mortality rates. Chronic alcohol abuse exacerbates the severity and likelihood of ARDS. A major regulator of lung function are tight junctions, and their morphology and function are disrupted in response to chronic alcohol abuse. Changes in tight junction morphology from the established smooth cobblestone patterning to a ruffled junction are associated with an increase in barrier permeability. The tight junction ruffle formation is correlated specifically with a change in claudin-18 interacting partners. We utilized BioID to investigate the proximal claudin-18 proteome. Proteins of interest include those in the talin and filamin family due to their function in integrin signaling. Evidence suggests that integrins have the capability to regulate epithelial barrier function and can function as a possible target for correcting barrier function. We seeded alcohol-treated and control human bronchial epithelial cells (hBECs) on Transwells and measured barrier function via transepithelial electrical resistance (TER). The experimental and control cells were treated with either the 9EG7 β1-integrin activating antibody or a non-specific IgG and the effect on TER was measured. The TER data showed an increase in barrier function after treatment with 9EG7 β1-integrin antibody. After treatment, we fixed cells and stained them to determine if 9EG7 β1-integrin restored the smooth cobblestone patterning of epithelial cells. We saw a straightening of ruffles caused by chronic alcohol treatment. These findings suggest a role for β1-integrin in barrier function and may help reveal links between integrins and tight junctions.

Kyeong Ran Jang, MSP **Poster #28**

Decreased TrkB-mediated responses in primary sensory neurons following spinal cord injury

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BDNF and its receptor TrkB have been shown to promote pronociceptive effects, although their contribution to neuropathic pain after spinal cord injury (SCI) remains unknown. We previously reported that inhibiting TrkB immediately after SCI delayed mechanical hypersensitivity development and improved locomotor recovery, suggesting that not spinal but peripheral TrkB signaling is a potential contributor to pain after SCI. In this study we evaluated TrkB signaling in small diameter dorsal root ganglion (DRG) neurons, using TrkBF616A mice for reversible inhibition of TrkB signaling with a kinase inhibitor, 1NM-PP1. After contusion SCI at the thoracic (T) 10 level, mice were immediately treated with vehicle or 1NM-PP1 in drinking water. T4-T12 DRGs were collected for dissociation from 1NM-PP1 or vehicle-treated uninjured mice and after SCI. Whole-cell patch clamp recordings were made from the cultured neurons, and responses to the TrkB agonist 7, 8-dihydroxyflavone (DHF; 50-500 μM) were assessed. In uninjured mice, DHF induced an inward current that was significantly reduced in 1NM-PP1-treated mice compared to vehicle treatment ($p<.001$; ANOVA). Interestingly, after SCI, DHF-induced current was reduced compared to uninjured mice, and the current was further decreased by 1NM-PP1 treatment. These findings suggest that nociceptors are less sensitive to TrkB manipulations after SCI, similarly to previous results observed in the spinal cord. Although these results fail to identify primary nociceptors as critical to TrkB signaling in pain after SCI, future studies are needed to provide additional insight into peripheral TrkB signaling and the mechanisms by which it promotes maladaptive plasticity and pain after SCI.

Nicolas Janto, GMB **Poster #29**

Tet1 safeguards lineage allocation in intestinal stem cells

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The intestinal epithelium is a highly proliferative tissue possessing critical absorptive, secretory, and barrier functions maintained in homeostasis by intestinal stem cells (ISCs). Understanding the regulatory mechanisms governing ISC differentiation has important implications for regenerative medicine, inflammatory disease, and cancer. Given that ISCs must undergo drastic changes in gene expression to differentiate into mature cell types, we looked to the chromatin-modifying enzyme ten-eleven translocation methylcytosine dioxygenase 1 (*Tet1*) as a potential genome-wide regulator of this process. TET1 converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) during DNA demethylation and interacts with other chromatin-modifying enzymes to activate or repress transcription. *Tet1* is enriched in ISCs and global 5hmC levels are higher in intestinal epithelial cells (IECs) following differentiation. Here, we show that *Tet1* is necessary for proper ISC differentiation in the adult intestine. We generated an inducible IEC-specific *Tet1* knockout mouse model (Tet1iKO) and observed altered proportions of IECs by immunofluorescence and scRNA-seq. Tet1iKO mice exhibited elevated numbers of enterocytes accompanied by early bias towards absorptive lineage transcriptional signatures. We also observed a reduction in ISCs and progenitors in Tet1iKOs, corroborated by premature loss of stem cell and progenitor transcriptional signatures. Assaying genome-wide 5hmC distribution by hmC-seal revealed an unexpected reduction of 5hmC over absorptive genes in Tet1iKO intestines. Because intragenic 5hmC is positively correlated with gene expression, our data suggest a non-catalytic role for *Tet1* in ISC differentiation, potentially through co-recruitment of other chromatin-modifying enzymes. Together, our results support a broad role for *Tet1* in regulating adult ISC differentiation through non-catalytic mechanisms.

Xiyu Li, MSP	**Poster #30**

Enhancement of Trans-Tympanic Drug Delivery by Pharmacological Induction of Inflammation

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Drug delivery directly across the tympanic membrane (TM) could eliminate systemic exposure to antibiotics prescribed for otitis media, the most common reason for pediatricians to prescribe antibiotics. Here, we hypothesized that inducing inflammation of the TM could enhance drug flux across the TM. We demonstrated that the flux of ciprofloxacin across the TM was greatly increased by treatment with the proinflammatory agent histamine. That enhancement was blocked by concurrent treatment with blockers of histamine receptor 1. Treatment of the TM with histamine was able to enhance drug flux sufficiently to eradicate otitis media in vivo in chinchillas, but only if the histamine was applied prior to treatment with antibiotics.
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Yonina Loskove, GMB **Poster #31**

Investigating the role of *ARID1B* in gene regulation and Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is highly prevalent neurological disorder that affects ~1 in 100 children worldwide. This disorder is highly heterogenous and presents with a wide range of cognitive, social, and behavioral deficits**.** Many classes of genes are mutated in ASD patients, especially those coding for chromatin remodelers. However, it is not well understood why this class of genes are so often mutated in ASDs, nor which gene regulatory networks are disrupted when chromatin remodeling is impaired during neuronal development. To bridge these gaps in knowledge, I will investigate *ARID1B,* which encodes a subunit of the chromatin remodeling complex BRG1/BRM-Associated Factor (BAF) and is one of the most enriched genes for *de novo* mutations in ASD. I will characterize E9.5-E15.5 *Arid1b* halpoinsufficient (*Arid1b*$^{+/-}$) mice at the single-cell level that are known to exhibit cognitive and behavioral deficits reminiscent of those seen in ASD patients. This will allow me to identify patterns of dysregulation to chromatin accessibility and gene expression at multiple timepoints of development. Currently we have assayed six wildtype whole mouse embryos at E9.5 and leveraged snMultiome data (snRNA + snATAC-seq) to profile their transcriptomes and regions of accessible chromatin. Using previous literature and mouse scRNA datasets, we have annotated specific cell types and gene expression profiles observed at E9.5. These data provide a rich landscape to explore cell type composition and gene regulatory programs that are necessary for proper neuronal development to identify cell-type specific defects in *Arid1b*$^{+/-}$ mice.

Lester Manly, MSP **Poster #32**

Utilization of proteomics to investigate the pharmacodynamics of Cu(ATSM) in treating an ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is characterized by the loss of muscle control as caused by motor neuron degeneration, especially within the spinal cord. An estimated 25,000 to 32,000 Americans are suffering with ALS and most individuals pass within two to five years after diagnosis. Current therapeutic options only provide modest benefits, extending the lifespan of patients by several months. A lead ALS therapeutic candidate is copper(II)bis(thiosemicarbazone) (Cu(ATSM)), which is currently in clinical trial (NCT04082832). Cu(ATSM) has demonstrated efficacy in preserving motor function and extending the lifespan of preclinical mouse models of ALS. However, the pharmacodynamic mechanism(s) of Cu(ATSM) to elicit these efficacious outcomes have yet to be elucidated. To investigate the pharmacodynamics of Cu(ATSM), we performed a pilot exploratory proteomics study utilizing high-resolution mass spectrometry on spinal cord collected from ALS transgenic mice treated, or not, with Cu(ATSM). Tandem Mass Tag isobaric labeling and offline high pH fractionation was utilized to increase throughput and improve protein quantitation & identification of the mouse spinal cord proteome. The bioinformatics employed included: differential protein abundance, gene ontology, weighted correlation network, and active sub-network analyses. Briefly, we have identified 9874 high-confidence proteins across our samples, of which 400 proteins had differential abundance. The differential protein abundances were used in gene ontology and network analyses, which predicted that Cu(ATSM) treatment of our transgenic mice affected processes such as: protein phosphorylation, lipid metabolism, and mitochondrial bioenergetics. These proteomic insights will provide hypotheses for investigating the ALS pathogenesis and provide mechanistic evidence for next generation ALS therapeutics.

Veronika Matsuk, CB **Poster #33**

Tumor initiation and lineage plasticity in NSCLC subpopulations

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Metastasis accounts for 90% of cancer-related deaths, however, the molecular mechanisms by which cancer cells invade into the adjacent stroma remain poorly understood. Metastases are often seeded by heterogeneous cells that invade collectively in cellular packs. However, most studies and treatments focus on the bulk cellular populations and so smaller more aggressive subpopulations are masked.

Our lab has established a technique to investigate phenotypic heterogeneity in cells, termed Spatiotemporal Cellular and Genomic Analysis (**SaGA**). We have used this technique to isolate subpopulations of collectively invading lung cancer cells, comprised of invasive 'leader cells' and proliferative 'follower cells' trailing behind.

To identify novel anti-metastatic treatment options, we conducted a chemical biology screen where we found leaders are chemo-resistant to top 5 lung cancer chemotherapeutic agents. In addition to chemoresistance properties, leader cells have stemness signature and phenotype in vitro as they express high levels of stem cell-like gene expression profiles including CD70, JAG1, and NOTCH1/2-all traits that suggest a cancer stem cell phenotype. Based upon these data, we tested the hypothesis that subpopulations of leader-like cells are tumor initiating cells. In in vivo xenografting studies, we identified that mice implanted with a heterogenous parental population developed tumors with higher incidence rate compared with mice implanted with leader-like and follower-like subpopulations, consistent with the possibility that heterogeneity is critical even in tumor initiation. Taken together, our data suggests a model where subpopulations within the "bulk" with unique but complementary phenotypes cooperate to provide resilience during tumor progression and subsequent metastatic disease.

Macrolide antibiotics induce ribosomal frameshifting on slippery messenger RNA sequences

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Antibiotic resistance is a significant and growing threat to public health, with deaths from drug-resistant infections projected to increase from 700,000 per year currently to over 10 million annually by 2050 without preventive action. Therefore, understanding how bacteria gain resistance to antibiotic treatment is critical for ensuring the continued usefulness of these drugs in the clinic. Previous studies have demonstrated that bacteria can sense macrolides, an important class of antibiotics which bind the large ribosomal subunit and disrupt peptide bond formation, and induce the translation of macrolide resistance genes in response to them. This induction of resistance proceeds through programmed shifts in the messenger RNA (mRNA) reading frame of macrolide-bound bacterial ribosomes at specific frameshift-prone mRNA sequences, but the underlying mechanism of this frameshifting phenomenon is unknown. Structural and biochemical studies to uncover the mechanism of macrolide-induced frameshifting are therefore important for understanding how bacteria regulate resistance to macrolides and how the bacterial ribosome can sense and respond to cellular chemical conditions. Primer extension inhibition (toeprinting) assays demonstrate the ability of macrolide-bound ribosomes to frameshift *in vitro*, and high-resolution single-particle cryogenic electron microscopy (cryo-EM) studies of actively translating ribosome complexes containing frameshift-prone mRNA and a macrolide antibiotic will demonstrate the mechanism by which macrolide-induced frameshifting proceeds during the elongation cycle of translation. The new understandings generated through this study will inform the design of new macrolide compounds and reveal how macrolide antibiotics modulate ribosomal activity to perturb mRNA reading frame maintenance.

William McFadden, BCDB **Poster #35**

Characterizing the attachment of a fluorosulfate probe to a critical tyrosine residue in the hinge region of the HIV-1 capsid protein.

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The HIV-1 capsid is a major component of the mature virion and has numerous essential roles in both the early and late stages of HIV-1 infection. Over 1500 monomeric capsid proteins (CA) assemble into the mature capsid core, a fullerene-shaped conical lattice encasing the HIV-1 genomes and replicative proteins. Capsid-targeting antiretrovirals have recently gained significant interest with the 2023 approval of Sunlenca, a long-acting antiretroviral that perturbs capsid assembly. In addition to clinical applications, many questions remain about the specific functions of CA in HIV-1 biology. Thus, we aimed to identify "clickable" covalent modifiers of CA for future functionalization. We screened a library of 472 compounds that can undergo Sulfur(VI) Fluoride Exchange (SuFEx) reactions, and of them, five were identified as initial hits. These hits were further characterized for biophysical and antiviral effects. One compound, BBS-103, is shown to covalently bind CA *via* a SuFEx reaction with residue Tyr145 and has antiviral activity in cell-based assays. The compound perturbed the *in vitro* assembly of CA in a dose-dependent manner as determined by an A_{350} plate-reader assay. We determined the structure of BBS-103 using small molecule crystallography and used this to perform covalent docking simulations between the compound and CA. Overall, the covalent binding of compounds that target the HIV-1 capsid could aid in the future design of antiretroviral drugs or chemical probes that will help study fundamental aspects of HIV-1 replication.

Olivia Morrison, GMB **Poster #37**

Adaptive evolution of centromeric proteins and their functional consequences in mammals

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Faithful chromosome segregation is necessary to pass along genetic information during mitosis and meiosis. Centromeres, the site of chromosome segregation, have rapidly evolving DNA despite their important function. In humans, centromere defects lead to aneuploidies, commonly a cause of miscarriages and a hallmark in many cancers. To understand the disease pathogenesis associated with centromere defects, it is necessary to have a better understanding of how rapid evolution affects centromere function.
Chromosome segregation machinery is highly conserved across eukaryotes; however, centromeric DNA is evolving rapidly and lacks conserved motifs. Centromeric DNA binds to centromeric proteins to form a complex called the kinetochore that binds to spindles to facilitate chromosome segregation. The centromere drive model predicts that centromeric proteins are evolving to balance the rapid evolution of centromeric DNA. We have identified centromeric proteins and codon sites undergoing adaptive evolution in the Mus genus. We hypothesize that these changes along with changes in centromeric DNA contribute to optimize centromere function in diverging species.
Further experimentation will study the functional consequences of changes observed in centromeric proteins on centromere function. This fundamental knowledge can then be applied to the study of human centromeres and may allow new insights into how defective centromeres lead to aneuploidies.

Roy Mulpur, GMB **Poster #38**

CRISPR/Cas9 screen of B cell differentiation pathways

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To provide immunity to pathogens, naïve B cells undergo differentiation to several states such as plasma cells and memory B cells. During differentiation, B cells undergo extensive changes in transcription factor networks and gene expression that are underpinned by changes in the epigenome. One such example is a transition from expression of glycolysis related genes to increased expression of genes related to oxidative phosphorylation metabolism pathways. We previously demonstrated that in vivo, naïve B cells take 8 cell divisions to reach the plasma cell terminal differentiation and that at each division, the B cells also go through a hierarchy of reprogramming before becoming plasma cells after exposure to T independent antigens. Our data also showed that at division 3-4 a second branch emerged that expressed genes associated with the memory B cell fate. In order to find genes that play a key role in committing a naïve B cell to the plasma cell or memory B cell fate, cells committed to the two branches after T independent antigen exposure were sorted and analyzed with RNA sequencing. Analysis of this data revealed 67 genes involved in transcriptional and epigenetic regulation that were significantly differentially expressed between the two branches. In order to determine the role these genes play in B cell lineage path commitment, retrovirus libraries containing gRNAs for each of the 67 genes were constructed and tested in a targeted CRISPR/Cas9 knock out screen. These data identify mechanisms of early memory B cell fate commitment.

Grace Neilsen, BCDB **Poster #39**

Nirmatrelvir resistance in SARS-CoV-2 Omicron_BA.1 and WA1 replicons and escape strategies

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a worldwide pandemic and continues to affect millions. To treat this virus, Pfizer, Inc. has developed Paxlovid which the FDA has authorized for emergency use. The antiviral component of Paxlovid, nirmatrelvir (NIR), forms a covalent bond with Cys145 of SARS-CoV-2 non-structural protein 5 (nsp5), the main viral protease. To explore resistance to NIR we designed mutations to impair binding of NIR over substrate. Using Omicron (BA.1) and WA1 SARS-CoV-2 replicons, cell-based complementation, and enzymatic assays, we showed that in both strains, E166V imparted high NIR resistance (~55-fold). E166V lead to a major decrease in WA1 replicon fitness (~20-fold), but not BA.1 replicon fitness (~2-fold). WA1 replicon fitness was restored by L50F. These differences may contribute to a lower barrier to resistance in Omicron than WA1 strains. E166V is rare in untreated patients, albeit more prevalent in Paxlovid-treated EPIC-HR clinical trial patients. Importantly, NIR-resistant replicons with E166V or E166V/L50F remained susceptible to a) the flexible GC376, and b) PF-00835231 which forms additional interactions. Molecular dynamics simulations show steric clashes between the rigid and bulky NIR t-butyl and β-branched V166, distancing the NIR warhead from its Cys145 target. In contrast, GC376, through "wiggling and jiggling," accommodates V166 and can still covalently binds Cys145. PF-00835231 uses its strategically positioned methoxy-indole to form a β-sheet and overcome E166V. Drug design based on strategic flexibility and main chain-targeting may help develop second-generation nsp5-targeting antivirals efficient against NIR-resistant viruses.

Probing the chromatin landscape of a repetitive locus using DiMeLo-seq

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Histone proteins are critical for organizing the genome, and precise regulation of their expression is essential during development. The canonical histone genes cluster in the genome as tandem, repetitive units (collectively called the histone locus) to allow for quick and precise regulation. Epigenetic signatures of the genome, such as histone modifications, emerge during early embryogenesis and contribute to the control of gene expression in these earliest rounds of cell division. However, the epigenetic landscape of the histone locus is unknown as the repetitive nature of the histone genes confounds traditional short-read techniques, such as ChIP-seq. The newly developed DiMeLo-seq (Directed Methylation with Long-read sequencing) circumvents this issue by utilizing antibody-directed DNA methylation as opposed to immunoprecipitation in order to probe regions of protein-DNA interactions. This allows for the capture and sequencing of long-reads (~10-20kb) containing sufficient, unique sequence to map to even highly repetitive regions of the genome like the histone locus. Using antibodies targeting transcriptional machinery and well-studied histone modifications involved in epigenetic control of gene expression, the chromatin landscape of the histone locus can be revealed. Mapping H3K9me3, a marker of heterochromatin typically found at centromeric regions, in *Drosophila* reveals how spreading of the mark may regulate histone gene expression and may explain the presence of over 100 copies of each replication dependent histone gene in the genome. Overall, DiMeLo-seq reveals the epigenetic landscape of previously unmappable genomic regions to better understand how these modifications control gene expression of repetitive loci in the early embryo.

Chiemela Ohanele, BCDB **Poster #41**

Establishing the mitochondrial citrate transporter as a regulator of cardiac morphogenesis

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Congenital heart disease (CHD) results from structural and functional defects of the heart that arise during embryonic development. CHD represents the most common type of birth defect and in the United States alone, affects ~1% of births per year. One common genetic cause of CHD is 22q11.2 deletion syndrome (22q11.2DS), where ~75% of 22q11.2DS patients present with CHD. SLC25A1, which is found in this deletion region, codes for the mitochondrial citrate carrier that is found on the inner mitochondrial membrane. In developing mouse models to study the in vivo functions of SLC25A1, we uncovered an unexpected perinatal-lethal knockout phenotype that suggested roles for mitochondrial function in heart development. Hearts from E18.5 *Slc25a1* knockout embryos display a striking array of cardiac malformations including ventricular noncompaction, and ventricular septal defects. Additionally, cardiomyocytes from *Slc25a1* knockout embryos display higher rates of cell death. Analysis of mitochondrial structure and function reveal that loss of *Slc25a1* causes mitochondrial ultrastructural defects and depresses mitochondrial respiration. Lastly, transcriptomics analyses of metabolism-related genes revealed that *Slc25a1* deletion altered expression of oxidative phosphorylation, glycolysis, lipid metabolism, and hypoxia-related genes in a dosage-dependent manner. Our results highlight a novel role for SLC25A1 in the transcriptional control of metabolic networks in the developing heart and point to a new role for SLC25A1 as a mitochondrial regulator of cardiac morphogenesis.

Jonathan Owen, MMG **Poster #42**

How a virus builds a house: identifying host factors which influence flavivirus replication organelle biogenesis and maintenance

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Flaviviruses, a viral family which includes dengue virus (DENV) and Zika virus (ZIKV), are human pathogens which cause diseases of global importance, affecting upwards of 100 million people per year. All flaviviruses replicate in association with the endoplasmic reticulum (ER) of host cells, forming single-membrane invaginations in which genome replication is thought to occur. The mechanistic interactions underlying the formation of these viral replication organelles (vROs) are unclear; furthermore, although viral proteins are known to play a role, the contribution of host proteins to vRO formation is unknown. The goal of our study is to identify and characterize host proteins which influence flavivirus vRO formation. We have previously found that atlastin-2 (ATL2), a host GTPase typically responsible for mediating three-way junctions in the ER periphery, has a critical role in vRO biogenesis. Knockout of ATL2 reduces the titers of DENV and ZIKV, and dysregulates vRO spatial organization. Building on this initial work, we have probed the ATL2 interactome, identifying 30 host proteins that show changes in ATL2 association during DENV infection. Using a high-throughput microscopy-based siRNA screen, we show that knockdown of several of these proteins has even greater impacts upon viral replication than knockdown of ATL2. These results have identified candidate host proteins which may contribute to vRO formation.

Jordan Owyoung, GMB **Poster #43**

The sympathetic nervous system modulates skeletal muscle mitochondrial health

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Dual innervation of the sympathetic nervous system with the motor neuron at the neuromuscular junction in skeletal muscle was first discovered in the late 1800s. However, the dual innervation hypothesis was later rejected in the 1930s and it was not until *nearly 90 years later* in 2016 that it was reestablished and supported with both functional and anatomical data. Because of the novelty of this field, almost nothing is known about the functional interactions between the sympathetic nervous system and skeletal muscle. Given that exercise influences both skeletal muscle hypertrophy and sympathetic activity, I wanted to mechanistically probe this interaction. To investigate this, I performed surgical sympathectomies (sympx) to ablate the sympathetic nervous system in mice. I then exposed a subset of these mice and naïve mice to an aerobic treadmill running protocol (ex) resulting in 4 experimental groups: sympx + sed, sympx + ex, sed, ex. Sympathectomized groups showed mitochondrial enrichment in differentially expressed genes suggesting mitochondrial changes in the absence of sympathetic activity. Sympathectomies also resulted in a decrease of mitochondria at the neuromuscular junction. Mitochondria are also important markers of skeletal muscle health. In sympx mice, there is a decrease in evoked muscle force and an increase in muscle fatigue, indicating decreased muscle health. My results point to a novel role of the sympathetic nervous system in mitochondrial health, which could be an extremely important finding for neurodegenerative diseases and aging.

Cynthia Perez, GMB **Poster #44**

Weighted gene co-expression analysis of placental transcriptomics and associations with birthweight and PFAS

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Per- and polyfluoroalkyl substances (PFAS) are pervasive chemicals associated with several health impairments including low birthweight. The exact mechanisms by which PFAS impairs fetal growth are undefined, but the placenta is a likely target. In this study, we identified placental mRNA expression modules (gene sets) associated with PFAS and birthweight using a systems biology approach. Study participants were enrolled in the Glowing study, and 141 placental samples were processed for mRNA-sequencing. As a pilot study, level of seven PFAS were measured in 29 placental samples using liquid chromatography-tandem mass spectrometry. We constructed a weighted gene correlation network based on Spearman correlation tests between 13,831 genes. Network analysis revealed 24 highly correlated gene sets, or modules. We then assessed the relationship between the first principal components of each module with birthweight and PFAS using linear regression. We identified 12 modules that were associated with an individual PFAS chemical and/or their sum. Two modules, module1 (p-value = 0.04, n = 77 genes) and module2 (p-value = 0.001, n = 56 genes) were found to be associated with birthweight. Interestingly, with our pilot data, both were also associated with PFNA (module1 p-value = 0.037, module2 p-value=0.007) and the sum of PFAS (module1 p-value=0.031, module2 p-value = 0.044). GO term analysis showed module1 to be representative of metabolic signaling while, module2 represents signal transduction. These associations could explain some of the relationship between birthweight and PFAS. We are building upon these findings by assessing additional PFAS in all 141 placental samples.

Tori Placentra, GMB **Poster #45**

Homeostatic control of intestinal stem cell renewal by two transcriptional regulators

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In mammals, the intestinal epithelium completely turns over every 4-8 days, making it one of the most rapidly cycling tissues. This tissue turnover relies on intestinal stem cells and tight regulation by complex cell signaling cascades. However, the mechanism by which homeostatic and developmental growth pathways cooperate to precisely calibrate tissue renewal remains unclear. In the common fruit fly, *D. melanogaster,* we investigate how the highly conserved Hippo and Ecdysone (steroid hormone) pathways interact to regulate tissue turnover in intestines. The downstream effector of the Hippo pathway in flies is the protein Yorkie (Yki), which enters the nucleus and binds the transcription factor scalloped (Sd) to activate transcription of Hippo pathway targets. The primary steroid hormone in *D. melanogaster* is called ecdysone and timed pulses of its active form temporally coordinate organism-wide development by triggering major developmental transitions. We have previously determined that a physical interaction between Yki and the Taiman protein, which acts as the protein coactivator of the ecdysone receptor (EcR), facilitates coregulation of shared Hippo-Ecdysone transcription targets. Intriguingly, a number of these shared targets have homeostatic roles in wound repair and tissue renewal. To test significance of the Yki-Tai interaction in tissue renewal, we have used Crispr to create a non-Yki binding version of Tai (two amino acid edits) and found evidence of defective tissue turnover in the adult midgut. Based on this data, we hypothesize the Hippo and Ecdysone pathways cooperate to activate transcription of genes required to guide stem cell-based gut renewal.

Sayalee Potdar, CB **Poster #46**

High-dimensional analyses identify distinct subsets of T cells enriched in the bone marrow of patients with plasma cell disorders

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Multiple myeloma (MM) is a hematologic malignancy characterized by growth of malignant plasma cells in the bone marrow. All cases of MM are preceded by a precursor state termed monoclonal gammopathy of undetermined significance (MGUS). Outcomes for MM have improved in recent years, but properties of immune cells in survivors remain poorly characterized. We developed and utilized a 35-marker panel to characterize immune cells in the blood and bone marrow of newly diagnosed MM (n=15), or MGUS (n=13) patients, as well as long-term survivors (LTS; n=26) by mass cytometry. Immunophenotypes of circulating T cells resembled those in the marrow, except for a distinct subset that was selectively enriched in the bone marrow. This subset consisted of predominantly CD8+ T cells, and had a distinct phenotype characterized by the co-expression of Eomes, CD69, and NKG2D. Further analysis of these cells identified heterogeneity within this compartment, with subsets of TCF1+ stem like T cells, as well as more differentiated effector T cells. Preliminary data suggest that phenotypes of marrow-enriched compartments differ considerably between MGUS, MM and LTS, with enrichment of more differentiated T cells in MM relative to other cohorts. Ongoing studies are utilizing complementary tools (such as single cell transcriptomics) and functional studies to further characterize these cells. In summary, our data identify distinct subsets of human T cells specifically enriched in the bone marrow in plasma cell disorders that correlate with malignant transformation and outcome and may serve as targets for improving immune therapy or prevention of myeloma.

Tana Pottorf, NS **Poster #47**

TREM2 regulation of spinal cord microglia morphology and motoneuron fate following Peripheral Nerve Injury

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Motoneuron (MN) degeneration is a common hallmark of many neuropathologies including but not limited to Amyotrophic Lateral Sclerosis, Spinal Muscle Atrophy, and Peripheral Nerve Injuries (PNI). PNI can induce MN death with varying severity. Permanent loss of MNs limits functional recovery. Therefore, identification of mechanisms that govern selective MN loss following PNI is crucial for therapeutic advancement and may have implications for other MN pathologies. We utilize a PNI mouse model to investigate diverse microglia interactions with MNs of varying health states. Within the first few days after PNI, injured MNs release colony stimulating factor 1 (CSF1) activating local microglia. Microglia proliferate, migrate, and extend processes towards MNs, and thereafter, adhere to and scan the MN surface with dynamic filopodia. Microglia that associate with regenerating MNs remain in this “sampling” state, whereas microglia associated with degenerating MNs transform into ameboid-like cells with minimal processes. These microglia tightly associate in groups we denoted as “death clusters.” The differentiating signals between regenerating and dying MNs that microglia respond to while changing morphology and potential functionality are currently unknown. Our evidence suggests that triggering receptor expressed on myeloid cells 2 (TREM2) is differentially upregulated in microglia that associate with regenerating MNs compared to microglia in death clusters around dying MNs. TREM2 levels correlate with increases in the phagocytic marker, CD68. Our results provide insights into signaling cascades that regulate microglia activation and function around injured MNs that survive or die and suggest possible therapeutics to increase MN survival in pathological conditions.

Vedhika Raghunathan, MMG **Poster #48**

HA - NA epistasis shapes the evolution of influenza A virus antigenic variant

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Rapid evolution of influenza A virus (IAV) hemagglutinin (HA) yields antigenically distinct viruses each year. Epistatic interactions between the two major surface glycoproteins, HA and neuraminidase (NA), are important for viral fitness of antigenic variants. The 2014-2015 influenza season was marked by substantial antigenic change in the H3N2 lineage due to the mutation HA F159S. Population data shows polymorphism at residue 93 of NA during this same period. We hypothesize that epistasis between residues HA 159 and NA 93 enabled positive selection of the antigenic variant. To test this hypothesis, we examined the within-host evolution of influenza A/Texas/50/2012 (H3N2; Tx/12) viruses carrying either HA F159 or S159. We inoculated immunologically naïve guinea pigs and collected nasal washes. Plaque assay and whole genome sequencing were used to determine viral titer and identify mutations. Both Tx/12 HA F159 and S159 replicated robustly in guinea pigs with comparable peak viral loads. In all Tx/12 HA F159 infected animals, minor variants were detected throughout infection, and none were shared across animals. By contrast, in all Tx/12 HA S159 infected animals, NA G93S was observed early and increased in frequency over time, reaching >75% by the resolution of infection. These dynamics indicate strong positive selection acting on NA only in the HA S159 background. Prior studies have shown that HA F159S decreases the avidity of HA for sialic acid receptors; NA G93S may therefore act to rebalance HA and NA functions. This study highlights the importance of considering HA and NA epistasis in antigenic evolution.

Monica Reeves, GMB **Poster #49**

Understanding how the maternal epigenetic reprogramming function of LSD1 contributes to inherited developmental disease

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At fertilization, histone modifying enzymes drive massive epigenetic reprogramming that is vital for appropriate embryonic and postnatal development. In *C. elegans* and mouse, the lysine specific demethylase 1 (LSD1/KDM1A) acts as a repressor during this reprogramming by removing H3K4me1/2 and preventing the inappropriate inheritance of transcriptional patterns. In mouse, we showed that the maternal loss of LSD1 results in embryonic arrest at the 2-cell stage, stemming from a failure to repress maternal genes. However, it is unclear how histone modifying enzymes are regulated during maternal reprogramming and whether defects in this reprogramming can lead to inherited disease. To address these questions, we developed a hypomorphic allele of *Lsd1*, that predominantly affects the binding of LSD1 to the CoREST repressor complex. Preliminary data from this new model show that progeny from mothers with maternally hypomorphic LSD1 exhibit increased perinatal lethality, as well as developmental delay and craniofacial abnormalities. This suggests that the maternal function of LSD1 is CoREST dependent and that partial loss of LSD1 can lead to inherited phenotypes. Intriguingly, developmental delay and craniofacial abnormalities are phenotypes observed in in neurodevelopmental disorders, such as Kabuki Syndrome and mutated LSD1 patients, but developmental delay has not been observed in previous Kabuki Syndrome mouse models. This raises the possibility that defective maternal epigenetic reprogramming contributes to neurodevelopmental phenotypes. We are currently using our new mouse model to understand mechanistically how defects in the maternal reprogramming of histone methylation at fertilization gives rise to inherited disease.

Juliet Santiago, NS **Poster #50**

Identification of state-specific proteomic and transcriptomic characteristics of microglia-derived extracellular vesicles

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Alzheimer's disease (AD) is the most common neurodegenerative disorder defined by progressive pathological protein aggregation and deterioration of cognitive function. Microglia-mediated neuroinflammation is a key pathological component of AD; however, there are critical gaps in our understanding of how microglia perpetuate AD pathology. One proposed mechanism is extracellular vesicle (EV) release because of their role in the transport of macromolecules between cells. The molecular profiles and influence of different microglia-derived EV populations on AD pathology remain unknown. We hypothesize that different microglia states determine the molecular composition of EVs. We treated three groups of a murine microglia cell line, BV2 cells, with either lipopolysaccharide, interleukin 10, or transforming growth factor beta, to polarize microglia to a pro-inflammatory, anti-inflammatory, or homeostatic state, respectively. Following treatment, BV2 cells were lysed and cell culture media was collected for EV isolation and downstream mass spectrometry (MS). We validated our enrichment of EVs, by using western blot, nano tracking analysis, transmission electron microscopy, and immunogold labeling. In MS studies, we identified 533 proteins in the EV proteome and 1,866 proteins in the cell proteome. We found that EV related proteins were significantly increased in the EV proteome compared to the cell proteome. We identified proteins that are differentially expressed across polarization. Next, we sought to replicate this experiment for RNA sequencing of polarized BV2 cells and their EVs. These results are currently underway. Overall, our results indicate that EVs derived from microglia adopt distinct state-associated profiles which may have differential effects on other cell types.

Taylor Shue, MMG **Poster #51**

Identification of a novel host-dependency factor for human coronaviruses

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Human coronaviruses are positive-sense, single-stranded RNA viruses that are a major global public health threat. Over the last twenty years, three highly pathogenic coronaviruses have spilled over into humans causing severe human health and economic impacts. SARS-CoV-2 alone has resulted in over six million deaths worldwide. This impact indicates a need for a better understanding of how coronaviruses interact with the host innate immune system which may lead to novel therapeutics. Coronavirus infections trigger the expression of type I interferons (IFNs) and downstream effectors, interferon-stimulated genes (ISGs). Our goal was to identify and validate ISGs that function as host-restriction or dependency factors during coronavirus infections. To identify potential genes of interest, we performed a transient genome-wide CRISPR knockout (KO) screen in Huh7.5 cells with SARS-CoV-2 infection. This screen showed that a KO of the ISG c19orf66, of Shiftless (SHFL), reduces viral infection. This indicates that it functions as a host-dependency factor for SARS-CoV-2. To validate this finding, we used recombinant Cas9 protein to make stable single-cell KO clones of SHFL in Huh7.5 cells (Huh7.5-SHFL KO). Experiments using wild-type Huh7.5 cells and Huh7.5-SHFL KO cells validated the screening phenotype that SHFL acts as a host-dependency factor across multiple coronaviruses. Ongoing experiments will focus on identifying the mechanism of action for SHFL in the enhancement of coronavirus infections. This knowledge will expand our understanding of virus-host interactions, coronavirus biology, and the innate immune system.

Elucidating the Epigenetic Mechanisms of Pluripotency in the Plant Shoot Apical Meristem

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Pluripotency, or the ability of a cell to further differentiate into one of several specific cell types, is imparted by a cell's chromatin landscape. Generally, it is thought that stem cells contain highly accessible and dynamic chromatin, thereby allowing for quick activation of a particular subset of genes to begin the differentiation process. However, the role of various chromatin remodeling complexes, the specific patterning of histone variants, and presence of post-translational modifications – and how they function to both create and maintain the stem cell chromatin landscape - is yet to be elucidated. Plants are an ideal model organism for this research, as they have accessible stem cell pools known as meristems, and continue to make organs post-embryonically. In my research, I plan to explore the role of both the histone variant H2A.Z, as well as several chromatin remodeler complex components in the maintenance and establishment of the meristem in *Arabidopsis thaliana*. So far, I have been developing tools for answering these questions in real time. Rather than using complete knockout lines from which it is difficult to parse out primary and secondary effects, I am leveraging inducible knockout systems to study the direct consequences of losing key chromatin regulators. Here, I present the progress I have made on developing a GFP nanobody degron to inducibly delete GFP-tagged H2A.Z to ask how the meristem is affected in its absence, as well as the troubleshooting of assays I will use to assess these effects, such as single-nucleus RNA-sequencing.

Tina Tian, NS **Poster #53**

Sparse labeling of the sympathetic nervous system is exhibited in *ThCre:mTmG* mice

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The global double-fluorescent cre-dependent reporter mouse *mT/mG* has been a valuable tool for differential identification of tissues and cells. This line was previously shown to have EGFP expression in a cre-dependent fashion in single neurons. Here, we demonstrate very sparse labeling in a *ThCre:mT/mG* cross. This is evident in sweat glands, thoracic spinal cord, sciatic nerve, and lumbar sympathetic ganglia. Therefore, this model may be suitable for imaging or electrophysiology studies of postganglionic sympathetic neuron cell bodies but ineffective for visualizing peripheral axons and tissue targets of the sympathetic nervous system.

Elijah Ullman, MSP **Poster #54**

Single channel characteristics of conductance and non-conductance modifying allosteric modulators of the NMDA receptor at the channel gate

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The N-methyl-D-aspartate receptor (NMDAR) is a ligand gated ion channel that is permeable to Na^+ and $Ca2^+$ and mediates a slow component of excitatory neurotransmission. NMDARs are implicated in synaptic plasticity and disease states such as in Parkinson's, Alzheimer's, Schizophrenia, and stroke. Here we have evaluated the characteristics at the single channel level of three series of allosteric modulators with structural determinants of bindings in regions that participate in NMDAR channel gating. We have generated two series of positive allosteric modulators (PAMs), EU1622 and EU1794 that generate two and three subconductance states respectively, and reduce the Ca^{2+}:Na^+ permeability ratio (Perszyk et al., 2018, 2020) – a first in class features of NMDAR modulators. We are exploring the mechanisms underlying these two classes of unique conductance-modifying allosteric modulators. Specifically, we are measuring effects on open probability, conductance, mean open time, deactivation time course, and agonist potency for conductance modifying modulators for comparison to modulators (EU1180 series) that do not alter conductance. In addition, the reduction of calcium permeability holds interesting therapeutic potential due to reduced neurotoxic risk of NMDA receptor potentiation compared to non-conductance modifying modulators. Ongoing efforts by our group are working to identify the mechanisms by which these modulators mediate changes in calcium permeability, which may be related to changes in calcium binding affinity to residues within or near the pore, or changes in pore diameter.

Sarah Webster, BCDB **Poster #55**

The influence of snoRNA and snoRNA-mediated modifications on NSCLC Invasion

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Recent studies have revealed a novel layer of translational control in cancer at the level of ribosomes. Changes in ribosome quality or quantity can impact the aggressiveness of tumor cells. A fundamental aspect of translational control occurs at the level of ribosomal RNA (rRNA), which comprises the bulk of the ribosomal mass and is chemically modified at functionally important sites. Emerging data indicate that changes in the modification pattern of the rRNAs can tune the mRNA preference of ribosomes. How changes in rRNA modification pattern and ribosomes contribute to the invasiveness of cancer cells is currently unknown. In eukaryotes, most rRNA modifications are guided by evolutionarily conserved small nucleolar RNAs (snoRNAs) that are abundantly present in the nucleus. My project exploits a cell culture-based model of NSCLC invasion to characterize the contributions of snoRNAs and their guided modifications to rRNA heterogeneity and translation. Tumor subpopulations in NSCLC cooperate to drive tumor progression through a process known as collective invasion. We have established a technique to isolate and characterize cells based on live phenotypic criteria called Spatiotemporal Genomic and Cellular Analysis (SaGA). This image-guided technique identified leader and follower cells within the collective invasion pack. Exciting preliminary data from our lab shows that subpopulations within NSCLC have distinct snoRNA expression patterns and exhibit variability in the 2'-O-methylation status of their ribosomes. The results from this work will reveal whether changes in snoRNA expressions and rRNA modifications in lung tumor subpopulations can be exploited to selectively block tumor cell growth and invasion.

Katherine Westover, GMB **Poster #56**

Genome-wide dysregulation of R-loops in Ataxia Telangiectasia neurological pathogenesis

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Ataxia Telangiectasia (AT), a neurodegenerative disease characterized by cerebellar degeneration of Purkinje cells that control balance and movement, affects up to 1 in 40,000 to 100,000 people worldwide. A recessive early childhood onset disorder, AT is caused by mutations within the ataxia telangiectasia mutated (ATM) threonine/serine kinase which plays crucial roles within the DNA damage response (DDR). However, the precise molecular mechanisms underlying AT pathogenesis and how ATM loss-of-function leads to deficient DDR remain elusive. R-loops, three stranded RNA-DNA structures composed of an DNA-RNA hybrid and a non-template DNA strand, have emerged as key components of double strand break (DSB)-induced DDR. Mounting evidence has documented critical roles of R-loops in both causing and responding to DSBs. As DSBs and the failure of their repair play major roles in the pathology of AT, R-loop dysregulation is likely to contribute to AT pathogenesis. One recently identified kinase substrate of ATM is methyltransferase like 3 (METTL3) protein, a N^6-methyladenosine (m6A) methyltransferase. m6A on the RNA strand of R-loops is present inside nuclei and affects R-loop formation during DSB repair. The relationship between ATM-METTL3 phosphorylation in response to DNA damage and regulation of R-loop formation through m6A deposition, which could play crucial roles in AT pathogenesis, has yet to be defined. Our preliminary data has demonstrated a global trend of R-loops decreasing in AT patient-derived neurons compared to healthy controls. We hypothesize that in AT, the lack of METTL3 phosphorylation by ATM could globally dysregulate R-loop formation and underly AT progression.

Megen Wittling, CB **Poster #57**

The type of lymphodepletion prior to adoptive immunotherapy differentially impacts antitumor efficacy and protective immunity

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We sought to determine if two types of lymphodepletion commonly used in the clinic differentially impact the efficacy of our novel CD4+ Th17 cellular therapy. Our team has previously found that the preconditioning method prior to CD8 T cell therapy does not appear to have a major impact on therapy response, however, our new work shows preconditioning may differentially regulate CD4 ACT Therapy. Using total body irradiation (TBI)(5 Gy), cyclophosphamide (CTX)(200 mg/kg), or no preconditioning prior to ACT with antigen-specific Th17 cells, we found that preconditioning with TBI led to better long-term engraftment in the mice. This engraftment increase was importantly linked with enhanced antitumor responses in TBI treated mice (*with 15/16 TBI mice living >30 days, 6/16 CTX mice living >30 days, and only 1/6 mice with no preconditioning living >30 days*). Also of note, the cytokine profile at day 10 post-Th17 injection varied between the preconditioning methods, notably with increased G-CSF, IL-6, MCP-1, MCP-5, IL-5, and KC in the TBI treated mice. Also, IL-17 and IFN-γ were greatly increased in animals given either TBI and CTX but were nearly absent in those mice not preconditioned prior to ACT. Our results indicate the antitumor response, engraftment in multiple organs, and cytokine profile differ between these different preconditioning regimens and that further investigation into the optimal preconditioning regimen may be needed prior to ACT involving Th17 cells. This discovery could inform next generation clinical trials with CD4 T cell therapy.