19TH ANNUAL GDBBS DSAC STUDENT RESEARCH SYMPOSIUM

Wednesday, April 27th, 2022

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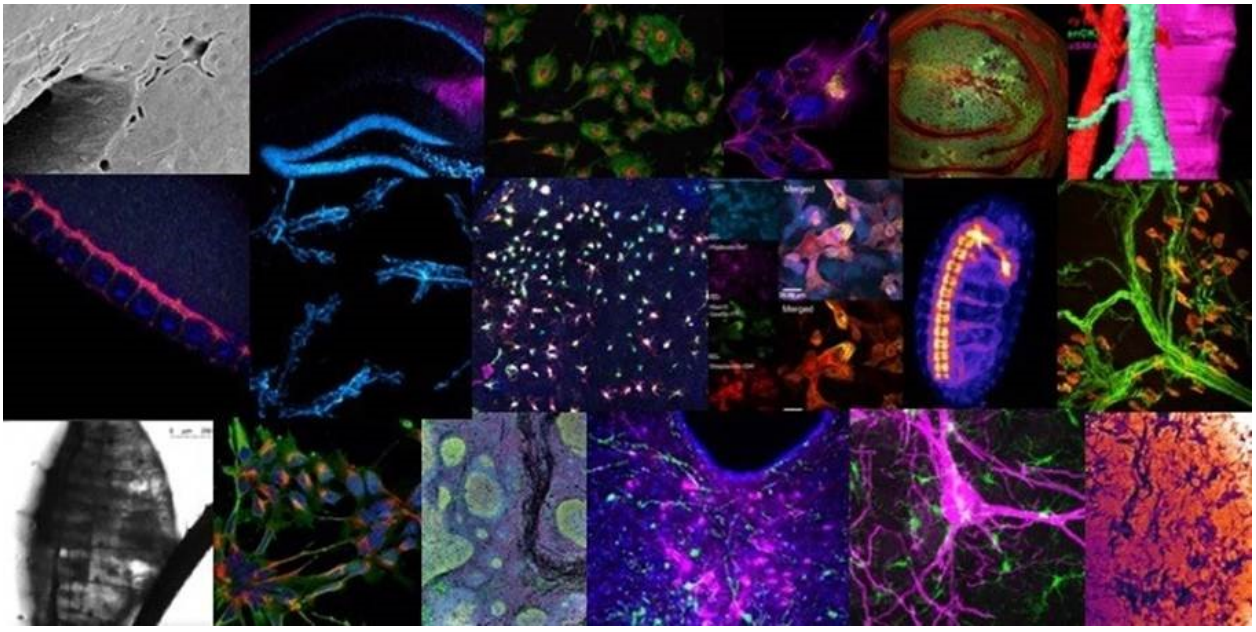
ICI Image Competition



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Oral Presentation Abstracts

Session 1:

Therapeutics

8:30AM

Gianna Branella, CB **8:30AM**

Advancing novel ligand-based CAR T therapy for hematopoietic stem cell transplantation and myeloid-leukemia treatment

Gianna M. Branella[1,2], Jasmine Y. Lee[1,2], Raquel F. Arthuzo[1,2], Andrew Fedanov[1], Christopher B. Doering[1,3], H. Trent Spencer[1,3]

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[2]Cancer Biology Program, Graduate Division of Biological and Biomedical Sciences, School of Medicine, Emory University, Atlanta, GA, USA
[3]Marcus Center for Pediatric Cellular Therapy, Children's Healthcare of Atlanta, Atlanta, GA, USA

There are no known leukemia-specific cell-surface antigens that distinguish healthy from malignant myeloid-lineage cells, making the translation of cell-based immunotherapies challenging for myeloid malignancies. While there has been great success in the implementation of chimeric antigen receptor (CAR) T therapy for the treatment of B-cell malignancies, similar strategies cannot be applied to the treatment of myeloid malignancies like acute myeloid leukemia (AML), as myeloid depletion cannot be managed like B-cell aplasia. Therefore, we have developed a CAR and gene modification platform to target malignant hematopoietic tissue through interaction with c-kit using its cognate ligand, stem cell factor (SCF). Herein, we show i) successful generation of a ligand-based SCF CAR, ii) successful expansion of γδ T cells—a short-lived yet cytotoxic alternative to αβ T cells—the first safety modification, iii) engineering of γδ T cells with robust yet transient CAR expression—the second safety modification, iv) specific *in vitro* SCF CAR-mediated lysis of c-kit+ AML cell lines CMK (79.1 ± 7.2% killing) and Kasumi-1 (78.3 ± 4.4% killing) at low effector-to-target ratios (1:1), and v) non-significant killing of the c-kit- cell line 697 (38.5 ± 7.3% killing), which demonstrates specificity. Additionally, we show treatment of SCF CAR-modified γδ T cells *in vivo* does not induce toxicities in the stem cell compartment in the bone marrow 72 hours after administration. Thus, we aim to further advance the use of CAR T therapy for myeloid malignancies using safety-modified CAR expression to target non-malignant markers as a means to advance curative therapeutic strategies.

Jakob Habib, IMP **8:45AM**

Selective CD28 blockade impacts T cell differentiation during hematopoietic reconstitution following lymphodepletion

Jakob Habib[1], Danya Liu[1], Rebecca L. Crepeau[1], Maylene E. Wagener[1], Mandy L. Ford[1]

[1]Emory Transplant Center, Emory University, Atlanta, GA

Introduction:
Costimulation blockade targeting the CD28 pathway provides improved long-term transplant survival without the nephrotoxicity of calcineurin inhibitors. Directly targeting CD28 while leaving CTLA-4 coinhibition intact may further improve this strategy. CD28 antagonizing domain antibodies (dAb) are currently in clinical trials for renal transplantation and will likely be accompanied by thymoglobulin induction therapy. Therefore, we sought to investigate the impact of T cell depletion (TCD) on T cell phenotype following homeostatic reconstitution in a murine model of skin transplantation treated with anti-CD28dAb.

Methods:
We performed skin grafts from BALB/c donors to C56BL/6 recipients. Recipients were treated with or without 0.2mg anti-CD4 and 10ug anti-CD8 one day prior to transplant and with or without 100ug anti-CD28dAb on days 0, 2, 4, 6, and weekly thereafter. Mice were euthanized six weeks post-transplant and lymphoid cells were analyzed by flow cytometry.

Results:
Mice treated with TCD+anti-CD28dAb exhibited significantly improved skin graft survival compared to TCD alone, anti-CD28dAb alone, and no treatment. In the kidney, the frequencies of tissue-resident $CD69^+CD103^+CD4^+$ T cells were reduced in TCD+CD28dAb compared to TCD alone. We also observed an increased frequency of $CD8^+Foxp3^+$ T cells in the blood and kidney of mice given TCD+anti-CD28dAb compared to TCD alone.

Conclusion:
These data demonstrate that CD28 signaling impacts the differentiation of both of $CD4^+$ and $CD8^+$ T cells during homeostatic reconstitution following lymphodepletion, resulting in a favorable shift towards fewer tissue-resident memory T cells and more $Foxp3^+$ $CD8^+$ T cells, a profile that may underpin the observed prolongation in allograft survival.

Pooja Srinivas, MSP	**9:00AM**

Oxidation alters the architecture of the phenylalanyl-tRNA synthetase editing domain to confer hyperaccuracy

Pooja Srinivas[1,2,3]**,** Mynthia Cabrera Goss[4], Kenneth Keiler[4], and Christine M. Dunham[1,3]

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[3]Antibiotic Resistance Center, Emory University, Atlanta, GA
[4]Biochemistry and Molecular Biology Department, Eberly College of Science, The Pennsylvania State University, University Park, Pennsylvania, USA

Antibiotics have revolutionized modern medicine, but bacterial resistance is growing, with 2.8 million antibiotic-resistant infections per year plaguing humanity. New antibiotics to combat this issue are needed. Targeting novel bacterial pathways is one strategy to develop new antibiotics to combat resistance. One such pathway is *trans*-translation. *trans*-translation is the primary bacterial ribosome rescue pathway that releases ribosomes that have translated to the 3' end of mRNA without terminating at an in-frame stop codon (non-stop ribosomes). *trans*-translation is unique from normal translation as it relies on the protein-nucleic acid complex small protein B (SmpB) and transfer-messenger RNA (tmRNA). A previous high-throughput screen identified acylaminooxadiazole compounds with specific activity against *trans*-translation that displayed potent broad spectrum antibiotic activity. Optimization of this compound was able to successfully treat multi-drug resistant *N. gonorrhoeae* in a mouse infection model. Structure and biochemical studies show that the acylaminooxadiazole binds to the ribosome at the peptidyl transferase center and induces stabilization of ribosomal protein bL27 in a novel conformation ('b' denotes a bacteria specific protein, 'L' denotes a large ribosomal subunit protein), with the bL27 N-terminus playing a role in *trans*-translation inhibition. How these inhibitors are specific to *trans*-translation is not clear, given structural similarities between tmRNA and tRNAs. My work seeks to elucidate the specific mechanism behind MBX-4132 inhibition, including differences in binding properties, peptide bond formation, and translocation, between SmpB-tmRNA and tRNAs. This work will assess how *trans*-translation inhibitors function to better predict resistance mechanisms and provide further insight on how *trans*-translation differs from translation.

Michael Cato, BCDB **9:15AM**

Development and characterization of novel LRH-1 antagonist using structural and computational techniques

Michael L. Cato[1], Racheal M. Spurlin[2], Jeffery L. Cornelison[2], Nathan T. Jui[2], Eric A. Ortlund[1]

[1]Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia
[2]Department of Chemistry, Emory University, Atlanta, Georgia

Nuclear receptors are transcription factors that respond to lipophilic signaling molecules by recruiting coactivators that enhance target gene expression. Liver receptor homolog-1 (LRH-1) is a nuclear receptor that drives steroidogenesis and expression of cell cycle regulatory genes. While this is beneficial for promoting intestinal cell renewal and alleviating gut inflammation, aberrant LRH-1 activity is strongly correlated with breast cancer proliferation. Small molecules that decrease LRH-1 activity are therefore attractive potential therapeutics for the treatment of ERα positive and negative breast cancer. However, mechanistic studies exploring LRH-1 antagonism are incredibly limited due to the lack of structural insight on the few existing compounds that decrease receptor activity. Using structure-guided compound design, we have made modifications to the chemical scaffold of a highly potent LRH-1 agonist to target and disrupt the region of the receptor responsible for coactivator binding. Our strategy effectively decreases LRH-1 thermal stability, coactivator association, and transcriptional activity. Interestingly, molecular dynamics simulations reveal that antagonism is achieved through disruption of allosteric paths of communication unique to LRH-1. Our work therefore demonstrates the utility of using structure-guided modification of small molecule agonists to selectively destabilize receptor activity, characterizes a highly efficacious small molecule LRH-1 antagonist, and provides mechanistic insight into how signaling for this receptor can be effectively disrupted without large structural rearrangements.

Kelsey Bennion, CB **9:30AM**

FcγRIIB expressed on activated CD8+ T cells restrains T cell responsiveness to αPD-1 immune checkpoint blockade in melanoma patients

Kelsey B. Bennion[1], Marvi Tariq[1,2], Megan Wyatt[1,2], Kirsten M. Baecher[1], Chrystal M. Paulos[1,2], Ragini Kudchadkar[2,3], Michael C. Lowe[1,2], Mandy L. Ford[1,2,4]

[1]Department of Surgery, Emory University School of Medicine
[2]Winship Cancer Institute, Emory University School of Medicine
[3]Department of Hematology and Medical Oncology, Emory University School of Medicine
[4]Emory Transplant Center, Department of Surgery, Emory University School of Medicine

Checkpoint inhibition using Fc-containing monoclonal antibodies has emerged as a powerful therapeutic approach to augment anti-tumor immunity. We recently showed that FcγRIIB, the only inhibitory IgG-Fc receptor, is expressed on a subset of differentiated effector CD8+ T cells in mice and humans, raising the possibility that CD8+ T-cell responses may be directly modulated by checkpoint inhibitor binding to T cell-expressed FcγRIIB. Here, we show that FcγRIIB is expressed in human CD8+ T cells in melanoma patient tumors. We show that despite exhibiting strong proliferative and cytokine responses at baseline, human FcγRIIB^{pos} CD8+ T cells exhibited reduced responsiveness to both PD-1 and CTLA-4 checkpoint inhibition as compared to FcγRIIB^{neg} CD8+ T cells. Moreover, frequencies of FcγRIIB^{pos} CD8+ T cells were reduced following treatment of human melanoma patients with nivolumab *in vivo*. This reduced responsiveness was FcγRIIB-dependent, because conditional genetic deletion of FcγRIIB on tumor-specific CD8+ T cells improved response to checkpoint blockade in a B16 mouse melanoma model. The limited responsiveness of FcγRIIB^{pos} CD8+ T cells was dependent on an intact Fc region of the checkpoint inhibitor, in that treatment with Fc-devoid anti-PD-1 F(ab) fragments resulted in a significant increase in proliferation of FcγRIIB^{pos} CD8+ T cells, without altering the response of FcγRIIB^{neg} CD8+ T cells. Finally, blocking FcγRIIB and PD-1 decreased tumor volume by significantly improving anti-tumor CD8+ T cell responses in a B16 mouse model. These results illuminate an FcγRIIB-mediated, cell-autonomous mechanism of CD8+ T-cell suppression which limits the efficacy of checkpoint inhibitors during anti-tumor immune responses *in vivo*.

Session 2:

Genomics and Epigenetics

10:00AM

Yijian Fan, CB **10:00AM**

Developing a sex-biased model for LKB1-mutant lung adenocarcinoma

Yijian Fan[1], Rui Jin[1], Xiuju Liu[1], Liu Yuan[1,2], Chunzi Huang[1], Melissa Gilbert-Ross[1], Adam Marcus[1], Wei Zhou[1]

[1]Department of Hematology and Medical Oncology, School of Medicine; Winship Cancer Institute, Emory University, Atlanta, GA
[2]Department of Biostatistics and Bioinformatics, Emory University Rollins School of Public Health, Atlanta, GA

Liver kinase B1 (LKB1) is the third most frequently mutated gene in lung adenocarcinoma (LUAD). In the TCGA-LUAD dataset, LKB1 was found to be more frequently mutated in males than in females even after correcting for potential confounders ($p < 6.9x10^{-4}$, FDR = 0.033). Similar trends can be found in all other large LUAD datasets, but the mechanism of this bias is unknown, partially due to the lack of a relevant *in vivo* model representing the clinical pattern. Here, we demonstrated that in our Lentivirus-inducing Kras/LKB1-mutant genetically engineered mouse model (GEMM), 97% of male mice (31/32) developed LUAD after lenti-Cre virus infection, but only 58% of female mice developed LUAD (15/26) in a one-year follow-up study (log-rank $p<0.001$). We also developed a syngeneic, tail-vein injection model using tumor cancer cell lines derived from a female host. Sex hormones did not alter the growth of WRJ388 cells *in vitro*, and subcutaneous WRJ388 tumor growth in immunodeficient NSG mice did not show sex-bias. However, tail-vein injection of such cells in syngeneic mice resulted in lung tumor formation in 100% (9/9) of male host GEMM but only 36% (5/14) in the female hosts. Cytokine analysis revealed circulating IL-6 increased 75.6-fold in male vs 16.3-fold in female with lung tumor formation. Together, we have developed two mouse models to evaluate the sex-bias against the formation of LKB1-mutant lung adenocarcinoma.

Lauren Hodkinson, GMB **10:15AM**

Contextual cues drive locus specific function of a context dependent transcription factor

Lauren J. Hodkinson[1,2], Julia Gross[3], and Leila E. Rieder[2]
[1]GMB Graduate Program, Emory University, Atlanta, GA, USA
[2]Department of Biology, Emory University, Atlanta, GA, USA
[3]IMP Graduate Program, Emory University, Atlanta, GA, USA

Despite binding similar *cis* elements, transcription factors can perform context-dependent functions at different loci. We aim to understand how transcription factors integrate *cis* sequence and genomic context to perform their context-dependent functions. One example of a context-dependent transcription factor in *Drosophila* is Chromatin-Linked Adapter for MSL Proteins (CLAMP), which targets similar GA-rich *cis* elements on the X chromosome and at the histone locus but recruits very different, locus-specific transcription factors to each of these contexts. Here we investigate how CLAMP function at the histone locus is impacted by the origin of its *cis* binding elements. CLAMP binds a long GA-repeat element in the bidirectional promoter of histone genes 3 and 4 (H3H4p) and recruits histone locus body (HLB) factors needed for histone gene transcription. We engineered flies to carry a transgenic histone locus in which we replaced the H3H4p GA-repeating element with CLAMP-recruiting GA-rich elements from the X chromosome. We assessed how X-linked *cis* elements impact HLB formation by staining third instar larval polytene chromosomes with antibodies specific to a core HLB protein as well as a X chromosome specific factor. When we replaced the H3H4p with an X-linked CLAMP recruiting region, HLB factors were not recruited but X chromosome factors were recruited to the transgene. However, when we replaced only the GA-repeats with X chromosome GA-rich sequences, the transgene was able to recruit HLB factors or the X-chromosome factors to the transgene. Our observations indicate that both sequence and context dictate CLAMP function at the histone locus.

Emilio Rodriguez, MMG **10:30AM**

Function of gonococcal *ispD* in the meningococcal urethral clade US_NmUC

***Emilio Rodriguez*[1]**, Yih-ling Tzeng[1], David S. Stephens[1]

[1]Department of Medicine, Division of Infectious Diseases, Emory University, Atlanta, GA

Invasive meningococcal disease, caused by the exclusively human pathogen *Neisseria meningitidis* (Nm), results in >100,000 deaths annually. While Nm typically colonizes the nasopharynx, since 2013 increased recognition of meningococcal urethritis cases has occurred worldwide. Many of these cases are caused by an emerging cc11.2 clade of urethritis-causing Nm, US_NmUC. Whole genome sequencing of >200 clade isolates revealed that the Nm ancestor integrated *Neisseria gonorrhoeae* DNA segments into its genome, including a 3.3-kb segment containing 5 genes. One of these gonococcal alleles, *ispD*, encodes a terpenoid precursor synthesis pathway protein. IspD has been shown to be essential in several gram-negative bacteria, including *E. coli*. A viable *ispD* mutation at the native locus was only successfully generated in meningococci when *ispD*, complemented at a distinct genomic location and under the control of an IPTG-inducible promoter, was induced, suggesting that *ispD* is essential in Nm. The transcription of the native *ispD* showed no discernable differences in aerobic cultures of clade (CNM3) and non-clade Nm (MC58) by qRT-PCR. When induced by 0.01 mM IPTG, the CNM3/*ispD* mutant complemented with the non-clade *ispD* (CNM3*ispD*$_{MC58}$) supported robust growth compared to the mutant complemented with the clade *ispD* (CNM3*ispD*$_{CNM3}$). Thus, IspD$_{MC58}$ appeared to have a higher enzymatic activity than IspD$_{CNM3}$ under aerobic conditions. In contrast, in an anaerobic environment supplemented with nitrite, MC58*ispD*$_{CNM3}$ grew significantly better than wild-type MC58 and MC58*ispD*$_{MC58}$. This suggests that the gonococcal *ispD* assists in the clade's growth under anaerobic environments, and thus may contribute to the clade's evolution as a urogenital pathogen.

Benjamin Babcock, CB **10:45AM**

Systemic perturbations to scRNA-seq reveal dropout-susceptible transcripts as the primary driver of batch effects that can be mitigated by normalization strategies.

Benjamin R. Babcock[1], Astrid Kosters[1], Junkai Yang[1], Mackenzie L. White[1], Eliver E. B. Ghosn[1,2]

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[2]Emory Vaccine Center, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, GA 30322, USA

Single-cell RNA sequencing (scRNA-seq) can reveal accurate and sensitive RNA abundance in a single sample, but robust integration of multiple samples remains challenging. Large-scale scRNA-seq data generated by different workflows or laboratories can contain batch-specific systemic variation. Such variation challenges data integration by confounding sample-specific biology with undesirable and batch-specific systemic effects. Therefore, there is a need for guidance in selecting computational and experimental approaches to minimize batch-specific impacts on data interpretation and a need to empirically evaluate the sources of systemic variation in a given dataset. To uncover the contributions of experimental variables to systemic variation, we intentionally perturb potential sources of batch-effect in human peripheral blood samples. Using in-house generated data, we investigate sequencing replicate, sequencing depth, sample donor, sample encapsulation, and the effects of pooling libraries for concurrent sequencing. We confirm our findings in publicly available datasets. To quantify the downstream effects of these variables on data interpretation, we introduced a new scoring metric, the Cell Misclassification Statistic (CMS), which identifies losses to cell-type fidelity that occur when merging datasets of different batches. Using CMS, we reveal undesirable overcorrection by widely adopted methods for batch-effect correction and data integration. We show that a major component of systemic batch effects is donor-specific detection of broadly expressed but low expression-level genes susceptible to dropouts. Optimizing gene expression matrix normalization/scaling and merging can mitigate the influence of these genes, reduce the need for batch-effect correction and minimize the risk of overcorrecting true biological differences between samples.

Ellen Krall, GMB **10:55AM**

CRAWLING ELEPHANT (*CREL*) controls H3K27me3 deposition and gene expression in tomato

Ido Shwartz[1], Chen Yahav[1], Neta Kovetz[1] Matan Levy[1] Alon Israeli[1], Maya Bar[1,7], Katherine L. Duval[2,6], ***Ellen G. Krall***[2,4]*,* Naama Teboul[1], José M. Jiménez-Gómez[3,5] Roger B. Deal[2] and Naomi Ori[1]

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Throughout their lives, plants continuously develop organs such as leaves, roots, and flowers. Both internal and external signals control the morphology and timing of organ formation in a flexible manner. Often, these signals coordinate global gene expression changes through changes to the epigenetic landscape. The Polycomb Repressive Complex 2 (PRC2) is a conserved protein complex that represses gene expression by trimethylating lysine 27 of histone H3 in promoter-associated nucleosomes. Core PRC2 subunits do not contain DNA-binding domains, so other transient subunits must direct PRC2 to appropriate gene promoters in response to specific developmental signals. Here, we investigate the role of CRAWLING ELEPHANT (CREL) in directing PRC2 activity and subsequent gene expression changes in *Solanum lycopersicum* (tomato). The *crel* mutant was previously identified as a suppressor of the simple-leaf phenotype of *entire (e),* a mutant in the ENTIRE/SlIAA9 gene, involved in tomato compound-leaf development. *Crel/(e)* mutants have increased leaf complexity and suppressed ectopic leaf blade growth. *Crel* mutants also have delayed flowering and delayed and aberrant stem, root, and flower development. Utilizing Chromatin ImmunoPrecipitation followed by sequencing (ChIP-Seq) and RNA-sequencing (RNA-Seq), we found that *crel* mutants have drastically reduced H3K27me3 enrichment at approximately half of the 14,789 sites enriched in wild-type plants, along with upregulation of many underlying genes. Interestingly, a subset of sites in *crel* mutants have increased H3K27me3, indicating that PRC2 activity is directed elsewhere in the absence of CREL. These results suggest that CREL is broadly required for PRC2 recruitment and therefore proper organ development in tomato.

Session 3:

Human Disease and Aging

11:30AM

James Rose, GMB **11:30AM**

Distinct transcriptomic and epigenomic modalities underpin human memory T cell subsets and modulate their activation potential

James R. Rose[1], Andrew R. Rahmberg[2], Michael D. Powell[1], Sakeenah L. Hicks[1], Christopher D. Scharer[1], Jeremy M. Boss[1]

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Human memory T cells (MTC), an indispensable part of adaptive immune memory, have historically been categorized by the cell surface proteins CCR7 and CD45RA into specialized subtypes (T_{CM}, T_{EM}, and T_{EMRA}), and are known to be poised to rapidly respond to antigen upon re-exposure. However, the epigenetic characteristics that distinguish the MTC subsets as well as the gene regulatory networks governing each MTC subsets' response to T cell activation remain poorly understood. Here, we derived the transcriptional and epigenetic programs of circulating $CD4^+$ and $CD8^+$ MTC subsets in the blood and following ex vivo activation. A progressive hierarchy of gene expression differences from naïve to T_{CM} to T_{EM} was observed and this was accompanied by corresponding changes in chromatin accessibility. MTC contained unique regulatory modalities comprised of discrete accessible chromatin patterns, transcription factor binding motif enrichment, and evidence of epigenetic priming from prior stimulation. Basic-helix-loop-helix factor motifs for AHR and HIF1A distinguish subsets and predict transcription networks capable of responding to environmental changes. Following simulation, primed accessible chromatin correlated with an augmentation of MTC gene expression as well as effector transcription factor gene expression, including T-BET, EOMES, and MSC. These results identify coordinated epigenetic remodeling and transcriptional changes that enable MTC subsets to ultimately respond to antigen re-encounters more efficiently.

Christine Bowen, BCDB **11:45AM**

Identification of novel Kv1.3 channel interactors in immune cells using proximity labeling

Christine A. Bowen[1], Young Lin[1], Hai Nguyen[3], Tia Dowling[1], Pritha Bagchi[2], Duc D. Duong[2], Prateek Kumar[1], Heike Wulff[3], Nicholas T. Seyfried[2], Srikant Rangaraju[1]

[1]Department of Neurology, Emory University, Atlanta, GA
[2]Department of Biochemistry, Emory University, Atlanta, GA
[3]Department of Pharmacology, University of California, Davis, CA

Kv1.3 is a voltage-activated potassium channel that is highly expressed by pro-inflammatory microglia in Alzheimer's Disease, where it regulates calcium signaling and several immune functions, partly via functional interactions with other proteins on the cell surface. Previous work shows that Kv1.3 blockade dampens proinflammatory responses and reduces neuropathology. The proteins and pathways that functionally interact with Kv1.3 channels in microglia remain unknown. This project aims to identify Kv1.3 interacting proteins in microglia and how microglial activation state impacts the Kv1.3 protein interactome, using proximity labeling (TurboID) coupled with mass spectrometry. We generated N- and C-terminal Kv1.3-TurboID fusion constructs and validated these in HEK293 cells using qPCR, patch-clamp electrophysiology, immunocytochemistry (ICC) and flow cytometry. Biotinylation was observed with both N and C-term Kv1.3-TurboID fusions. Label-free quantitative mass spectrometry (MS) of enriched biotinylated proteins identified distinct protein interactors with the N (n=101) and C terminus (n=146) of Kv1.3. These included antigen processing (PREB1) and N-glycosylation(DAD1) for the N-terminus proteasomal(E2) and actin remodeling(ANK3) proteins for the C-terminus, and receptor tyrosine kinase(EGFR) and signaling proteins(MAPK) for both. Ongoing studies will validate these proteomic findings. We generated BV2 microglia lines stably expressing N- or C-term Kv1.3-TurboID fusions. Kv1.3 expression and biotinylation of proximal proteins was verified by electrophysiology, qPCR, western blot and flow cytometry. Our studies identified novel domain-specific interactors of Kv1.3 channels in mammalian cells and may explain how Kv1.3 channels regulate immune function. Ongoing work within our stably transduced microglia lines will reveal domain-specific interactors, which may vary based on microglial activation state.

Daniel McManus, IMP **12:00PM**

Early generation and anatomical commitment of PD-1+ stem-like CD8 T-cells

Daniel T. McManus[1], Christopher B. Medina[1], Ewelina Sobierajska[1,2,3], Maria A. Cardenas[1,2,3], Nataliya Proknevska[1,2,3], Masao Hashimoto[1], James L. Ross[1], Amanda Gill[1], Haydn T. Kissick[1,2,3], Rafi Ahmed[1,3]

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Stem-like CD8 T cells (PD-1+ TCF-1+ Tim-3- TOX+) sustain the CD8 T cell response during chronic viral infection and cancer, and residency in lymphoid tissues is a key aspect of their biology. When do these cells first emerge after infection and at what point do they commit to positioning in the splenic white pulp? We used the chronic LCMV strain, clone 13, to address these questions. Phenotypical and transcriptional profiling of virus-specific CD8 T cells indicated that stem-like CD8 T cells emerge as early as day 5 after infection. Intravascular labeling and imaging showed that the spatial commitment of these cells to the white pulp had already started on day 5. This observation is particularly striking as it indicates that the sequestration of these cells to the white pulp has been prioritized even before the outcome of the infection has been determined. RNA sequencing revealed considerable transcriptional overlap between PD-1+ stem-like CD8 T cells from the early (day 5) and established phases (>day 45) of clone 13 infection, indicating that the canonical gene signature of these cells manifests early and is maintained throughout infection. These data show that stem-like CD8 T cells emerge early during infection, and that their compartmentalization to lymphoid tissue occurs not only before the infection has gone chronic but before other key features of the stem-like CD8 T cell program have been acquired.

Changtian Ye, NS **12:15PM**

Age and sex dependent long-term brain deficits in a Drosophila head trauma model

[1]***Changtian Ye***, [1]Joseph A. Behnke, [1]Kenneth H. Moberg, and [1,2,3]James Q. Zheng

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Traumatic head injuries, including mild ones such as concussion, represent an environmental risk factor linked to the development of chronic neurodegeneration that can occur years after initial exposure. However, the mechanisms of this long-term process remain to be understood. A better understanding of the brain's response to mild physical insults and the detrimental effects can lend new insight towards the development of neuroprotective strategies. To investigate the life-long effects on brain function and structure after initial exposure to mild head trauma and the underlying mechanisms, we developed the **H**ead **I**mpact **FL**y **I**njury (HIFLI) model, in which mild repetitive headfirst impacts to multiple awake and unrestrained adult *drosophila melanogaster* can be delivered. Flies subjected to our nonlethal injury paradigm, regardless of age and sex, immediately exhibited concussive-like behaviors but recovered within minutes. However, injured flies gradually developed impaired startle-induced climbing and increased brain degeneration throughout their lifetime. We observed substantially worse sensorimotor decline and brain pathology in flies injured at older ages, indicating that aging increases vulnerability to mild head injury. Importantly, these deficits were more profound in females than in males and were most prominent in females in the oldest injury cohort. Lastly, by comparing virgin females with mated flies, we concluded that body size does not contribute to the observed sex differences. Together, our findings validate *Drosophila* as a suitable model system for investigating the long-term effects of head trauma and suggest an increased vulnerability in females and older adults for head trauma-induced brain dysfunction and degeneration.

Katherine Westover, GMB **12:30PM**

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

Katherine Westover[1] and Bing Yao[1]

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

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 underlie AT progression.

Session 4:

Cellular Processing and Pathways

2:15PM

Hannah Smith, MMG **2:15PM**

Teasing apart the evolution of lipoprotein trafficking in Gram-negative bacteria

Hannah Smith[1,2,3,4], Marcin Grabowicz[2,3,4]

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[2]Department of Microbiology & Immunology, Emory University School of Medicine, Atlanta, GA
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The outer membrane (OM) of Gram-negative bacteria like *Escherichia coli* is an essential permeability barrier that is assembled by several conserved biogenesis machines. Each machine requires an essential lipoprotein component to function. The Lol system traffics lipoproteins to the OM in *E. coli*. The LolCDE complex extracts lipoproteins from the inner membrane and delivers them to LolA, a periplasmic chaperone. LolA traffics lipoproteins across the periplasm to LolB, which then inserts lipoproteins into the OM. Curiously, many Gram-negative organisms (e.g., *Caulobacter crescentus*) do not produce LolB. It is therefore unknown how lipoprotein trafficking occurs in such organisms. LolA may act as both a chaperone and OM lipoprotein insertase in these organisms. Consistent with this hypothesis, we show that LolA from *C. crescentus* (*Ccr*LolA) can complement the loss of both LolA and LolB in *E. coli.* A modelled structure of *Ccr*LolA revealed a protruding loop that is absent from *E. coli* LolA. A similar loop is present in *E. coli* LolB. When this loop is disrupted, OM lipoprotein insertion is abolished. We show that when the loop of *Ccr*LolA is disrupted, it can complement loss of LolA but no longer complements loss of LolB in *E. coli*. Our findings suggest that the loop of *Ccr*LolA is necessary for lipoprotein insertion into the OM and is separate from chaperone activity. We show that in bacteria lacking LolB, LolA has two functions in lipoprotein trafficking. Our study suggests that gene duplication and specialization helped to evolve a highly efficient lipoprotein trafficking pathway.

Sarah Strassler, BCDB **2:30PM**

Substrate recognition by the tRNA methyltransferase Trm10

Sarah E. Strassler[1,2] and Graeme L. Conn[1,2]

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Trm10 (TRMT10A in humans) is a tRNA methyltransferase that is ubiquitously expressed but also highly enriched in neuronal cells. Trm10 modifies a subset of tRNAs on the base N1 position of the 9th nucleotide in the core region of the tRNA and is conserved throughout eukarya and archaea. Mutations in the *TRMT10A* gene have been linked to neurological disorders such as microcephaly and intellectual disability, as well as defects in glucose metabolism. Of the 26 tRNAs in yeast with guanosine at position 9, only 14 are substrates for Trm10. However, no common sequence or other posttranscriptional modification similarities have been identified among these substrates, suggesting the presence of some other tRNA feature(s) which allows Trm10 to distinguish substrates from non-substrates to only modify the correct tRNAs. This project tests the hypothesis that tRNA substrate recognition by Trm10 is dependent on specific conformational changes in tRNA. Using the sensitive RNA structure-probing method SHAPE, conformational changes upon binding to Trm10 in tRNA substrates, but not non-substrate, have been identified and mapped onto a model of Trm10-bound tRNA. These changes may play an important role in substrate recognition by allowing the Trm10 to gain access to the tRNA core region. Evidence thus far suggests a novel mechanism of substrate recognition by a highly conserved tRNA modifying enzyme. Further, these studies promise to advance our overall knowledge of discrimination by tRNA-modifying enzymes between structurally similar tRNA species.

Joel Eggert, IMP **2:45PM**

Strong basal TCR signaling mitigates the responsiveness of naive CD8 T cells

Joel Eggert[1], Wendy M. Zinzow-Kramer[1], Byron B. Au-Yeung[1]

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T cells are a powerful component of the immune response that, on the one hand, can protect the host from invading pathogens, but on the other hand, T cells can cause self-damage when regulation fails. There are many autoimmune diseases where T cells are sufficient or necessary for inducing a detrimental immune response toward the host. Hence, the activation of T cells has to be a strictly controlled process where only foreign cognate antigen stimulation of the T cell receptor (TCR) generates a robust response. However, naive T cells constantly interact with self-peptides via their TCR as they scan antigen-presenting cells for their cognate antigen. While persistent antigen stimulation is associated with reduced T cell function and can impair the T cell response against chronic infections or cancer, it remains incompletely understood how chronic basal TCR signaling from self-peptides affects the responsiveness of naive T cells. We investigated the heterogeneity and functional implications of basal TCR signal strength in naive CD8 T cells by utilizing a fluorescent reporter mouse (Nur77-GFP) reflective of TCR signaling. We found that strong basal TCR signaling was associated with diminished cytokine secretion and proliferation during the early phase of an immune response. This result indicates that naive CD8 T cells that experience strong basal TCR signaling likely become de-sensitized to subsequent stimulation. We propose that this de-sensitization of naive T cells may allow the immune system to limit the autoreactive potential of the most self-reactive naive CD8 T cells to prevent autoimmunity.

Nic Janto, GMB	**3:00PM**

Requirement for Tet1 in intestinal stem cell regulation and lineage specification

Nic Janto[1,2], Maria Fonseca[1], Adam Gracz[1,2]

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Intestinal stem cells (ISCs) must balance roles in self-renewal, proliferation, and differentiation in order to maintain the intestinal epithelium and replace differentiated cell lineages that are being constantly turned over. Understanding the regulatory mechanisms governing ISC differentiation has important implications for regenerative medicine, inflammatory disease, infection, tissue metaplasia, and cancer. One of the proposed regulatory mechanisms in ISCs is the DNA hydroxymethylation activity of the chromatin modifying enzyme ten-eleven translocation methylcytosine dioxygenase 1 (Tet1). 5mC is a repressive chromatin modification, but Tet1 converts 5mC to 5hmC as the first step in demethylation, resulting in de-repression of the previously methylated genomic element. Tet1 expression is enriched in ISCs, and global 5hmC levels are generally higher in ISCs than in differentiated intestinal cells. We make use of an inducible intestine-specific Tet1 knockout mouse model to demonstrate Tet1's requirement in ISC differentiation into a variety of intestinal cell types. The Tet1 knockouts produce significantly fewer tuft and enteroendocrine cells (EECs) and significantly more goblet and stem cells than the controls, suggesting Tet1 may be required for proper specification of tuft and EEC lineages. The Tet1 knockouts also show reduced 5hmC levels at cell-type specific genes in progenitor and differentiated cells compared to controls, indicating that the requirement for Tet1 may be dependent on its catalytic role in hydroxymethylation rather than any non-catalytic recruitment functions. Together, our findings indicate that Tet1 is essential for regulating proper differentiation of ISCs, and particularly in the differentiation into tuft and EEC lineages.

Julia L. de Amorim, BCDB **3:15PM**

DDX1 helicase interacts with the nuclear RNA exosome in a DNA damage-dependent manner

***Julia L. de Amorim*[1,2]**, Sara W. Leung[1], Ramona Haji Seyed Javadi[3,4], David S. Yu[3], Anita H. Corbett[1,2,4]

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The RNA exosome is a 10-subunit complex that mediates both RNA processing and degradation. This complex is ubiquitously expressed, essential, and critical for fundamental cellular functions, such as rRNA processing. The RNA exosome subunits have been implicated in neurodegenerative disease that arise from missense mutations, which have been shown to disrupt RNA processing/degradation. Recent studies have illuminated the expansive role of this complex, including degradation of R-loops present at sites of transcription. The mechanism by which the RNA exosome targets RNAs for decay/processing is largely due to interacting protein cofactors. These cofactors have been mostly studied in budding yeast; however, many are conserved in mammalian systems. Thus far, studies in mammalian systems have not been performed in brain or neuronal lines. Due to the neuronal phenotype of RNA exosome-related disease, we employed a mouse neuronal line (N2A) for our studies. We performed immunoprecipitation of the RNA exosome subunit, EXOSC3, followed by mass spectrometry to obtain a snapshot of the RNA exosome interactome. We confirmed enrichment of the RNA exosome complex by identifying all components and several known cofactors in the eluate. We extended the analyses to other proteins and validated an endogenous interaction with EXOSC3 and DDX1, an RNA helicase. DDX1 plays major roles in DNA repair and has also been shown to promote rRNA processing and RNA clearance. We tested whether inducing DNA damage would have an impact on the interaction by treating N2A cells with a topoisomerase inhibitor, confirming that the EXOSC3 and DDX1 interaction is DNA-damage sensitive.

Anders Johnson, MMG **3:30PM**

Pro-IL-18 secreted by keratinocytes detects the group A streptococcal protease SpeB

Anders F. Johnson[1,2]*, Jenna S. Sands[1]*, Keya Trivedi[1], Raedeen Russell[1], Doris L. LaRock[1], Christopher N. LaRock[1,2]

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*Equal contributions

Background: Group A *Streptococcus* (GAS, *Streptococcus pyogenes*) commonly infects the skin and can cause severe infections such as necrotizing fasciitis. Keratinocytes are one of the first cells to contact GAS and are poised to initiate early inflammatory responses. The proinflammatory cytokine IL-18 is typically activated intracellularly by the inflammasome protease caspase-1, but keratinocytes constitutively secrete the inert form (pro-IL-18) which lacks activity unless processed by an extracellular protease.

Methods: Cytokine arrays were used to compare the secretion of IL-18 and other proinflammatory cytokines by common cell lines and primary human keratinocytes. Activation of IL-18 was examined during infection with wild type and knockout strains of GAS and other pathogenic and commensal skin microbiota using reporter cells and western blots. Activation was visualized *in-vitro* with recombinant forms of pro-IL-18 and activating proteases. Whole human blood and keratinocyte/PBMC co-cultures were used to model the ability of IL-18 to promote IFN-γ production by T cells.

Results: Extracellular pro-IL-18 generated by keratinocytes is directly processed into a mature active form by the GAS protease SpeB. This mechanism contributes to the proinflammatory response against GAS, resulting in T cell activation and the secretion of IFN-γ that restricts GAS growth. Most other major bacterial pathogens and microbiota of the skin did not have significant IL-18-maturing ability.

Conclusions: These results suggest keratinocyte-secreted pro-IL-18 is a sentinel that sounds an early alarm to foreign proteases. It is highly sensitive to GAS, yet tolerant to species typically resident on the healthy skin, suggesting a mechanism for pathogen discrimination.

Session 5:

Interactions and Behavior Across Scales

4:00PM

Stephanie Prince, NS	**4:00PM**

Thinking on your feet: rapid updating of spatial trajectories in response to new information

Stephanie Prince[1,2], Navya Katragadda[2], Tyler Roberts[2], Teema Yassine[2], Abigail Paulson[2], Annabelle Singer[2]

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[2]Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

Remembering our past and planning for the future are fundamental cognitive processes that guide our day-to-day lives. The ability to selectively recall relevant past experiences, use these memories to inform our decisions, and update our choices based on new information helps us navigate our world. The hippocampus and prefrontal cortex are two brain regions often studied for their role in memory and planning. In both brain areas, neural representations of past experiences are reactivated on compressed timescales, which is theorized to support planning for upcoming choices. However, the role of prospective codes in decision making is challenging to study since planning can be an internally driven process and it is often unclear when a choice is made. To address this question, we designed a novel decision-making task in virtual reality and performed large-scale neural activity recordings in mice during behavior. We found that mice can learn a complex task which requires them to change their behavioral responses when presented with new information. We then recorded electrophysiological activity from hippocampus and prefrontal cortex during behavior. We are currently testing our hypotheses that prospective codes update when animals are presented with new information and predict correct decisions. The novel task design allows us to precisely control the timing of these behaviors, and this dataset will provide unique insights into how neural codes guide decision making. This novel paradigm can be extended to mouse models of Alzheimer's disease to test how prospective memories are affected in disease.

Gabrielle Delima, MMG **4:15PM**

Beneficial interactions between co-infecting influenza A viruses extend to heterologous strains

Gabrielle K. Delima[1], Ketaki Ganti[1], Anice C. Lowen[1]

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Virus-virus interactions occur when multiple viruses infect the same cell. These interactions can augment or suppress viral progeny production. For influenza A viruses (IAV), delivery of multiple viral genomes per cell substantially increases the frequency of productive infection. We previously found that the magnitude of this benefit is both host and strain dependent. Notably, influenza A/guinea fowl/HK/WF10/99(H9N2) virus (WF10wt) is highly reliant on multiple infection in mammalian cells, needing a coinfecting virus to enable replication from a low multiplicity of infection. Here, we tested the prediction that the outcome of IAV virus-virus interactions differs with strain pairing and to determine what viral traits are associated with a more beneficial interaction. We measured WF10wt replication during co-infection with a panel of IAVs including avian- and mammalian-adapted viruses and strains with high or low reliance on multiple-infection. Co-infection of WF10wt with WF10var, a synonymously mutated variant, was used as a homologous control. We infected mammalian cells with WF10wt and increasing doses of the co-infecting IAV, then quantified WF10wt replication. Our results show WF10wt replication is enhanced by both homologous and phylogenetically distant co-infection partners. However, strains that had the lowest intrinsic reliance on multiple infection were the most effective at augmenting WF10wt replication. Indeed, introduction of a point mutation in the WF10var PA protein that lowers reliance on multiple infection led to significantly greater enhancement of WF10wt replication. Together these data suggest that the extent to which an IAV relies on multiple infections determines its potential to augment the replication of co-infecting strains.

Michelle McCauley, PBEE **4:30PM**

Searching for a signal of antagonistic fluctuating selection in a coevolving host-parasite system

Michelle A. McCauley[1], Amanda K. Gibson[2], McKenna J. Penley[3], Levi T. Morran[1,3]

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The prevalence of sexual reproduction has long puzzled evolutionary biologists because sexual reproduction is costly compared to asexual reproduction, and yet outcrossing (biparental sexual reproduction) is ubiquitous in nature. One of the leading hypotheses for the prevalence of outcrossing is the Red Queen hypothesis, under which parasites select for outcrossing in their coevolving hosts. A key assumption of this hypothesis is that reciprocal selection between host and parasite results in antagonistic fluctuating selection, or changes in the strength or direction of selection which leads to oscillations in host and parasite genotypes, also called Red Queen dynamics. While theoretical work supports these dynamics, empirical testing for antagonistic fluctuating selection remains challenging. Here I use a nematode-bacteria system to show that coevolution between the nematode host and bacterial parasite maintains outcrossing in the host. I then test for antagonistic fluctuating selection using time shift assays, in which hosts are exposed to parasites from their evolutionary past, present, and future. I demonstrate that time shift assays, which are the main tool used to test for antagonistic fluctuating selection, fail to capture evidence for fluctuating selection at the population level, but reveal a signal of fluctuating selection at the genotypic level.

Sena Agezo, NS **4:45PM**

Measuring animal behavior in social contexts using deep learning approaches

Sena Agezo[1,2], Amelie M. Borie[2], Frankie King [2], Larry J. Young[3], Robert C. Liu[2], Gordon J. Berman[2]

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Understanding animal behavior in a social context requires tracking and quantifying their behaviors as they interact with each other. Recently, there has been notable progress in developing deep learning algorithms and software to track multiple animals in social paradigms. Some of these algorithms only track animal identities, which has been shown to be robust. Other algorithms detect key points on the animals' bodies to estimate poses, which can be reasonably successful when the animals are some distance apart or have brief close contact with each other. However, these algorithms exhibit poor tracking accuracy when the animals spend more time with each other, performing key social behaviors such as huddling, mutual-grooming, and mating. To help improve the tracking of animals within social contexts, when the animals are close for long periods, we implemented a pipeline that combines multiple deep-learning-based tracking methods to obtain detailed and high-accuracy postural trajectories of multiple animals. Tested on a data set of prairie voles - a model organism for the study of social interactions - our pipeline robustly maintains animal identities and increases the accuracy of the posture tracking over applying convolutional neural network methods by themselves. With this improved tracking accuracy, we can build a better representation of the behaviors of animals in a social context, isolating behaviors that are key for understanding the dynamics of social interactions.

Poster Presentation Abstracts

Poster Presentations

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

Poster	Name		Program	Poster	Name		Program
1	Alexander	Katie	IMP	**29**	Lanjewar	Samantha	GMB
2	Alexander	Ashley	PBEE	**30**	Lee	Jasmine	CB
3	Antezana	Brenda	MMG	**31**	Lee	Cheyenne	MMG
4	Barbee	Britton	MSP	**32**	Mattingly	Jacob	BCDB
5	Becker	Scott	MSP	**33**	O'Haren	Tommy	GMB
6	Blackmer-Raynolds	Lisa	NS	**34**	Oviedo	Alejandro	BCDB
7	Brockman	Maegan	MSP	**35**	Owyoung	Jordan	GMB
8	Cooper	Garrett	GMB	**36**	Parker	Rebecca	CB
9	Diaz Perez	Kimberly	GMB	**37**	Parwani	Kiran	CB
10	Done	Rachel	MMG	**38**	Phillips	Megan	PBEE
11	Gill	Amanda	IMP	**39**	Pottorf	Tana	NS
12	Goettemoeller	Annie	NS	**40**	Raghuram	Vishnu	MMG
13	Guerra	Stephanie	MMG	**41**	Reece	Monica	MMG
14	Haji-seyed-Javadi	Ramona	GMB	**42**	Robinson	Kelsey	GMB
15	Hamilton	Adam	NS	**43**	Salas	Eliseo	GMB
16	Harbin	Nicholas	MSP	**44**	Santiago	Juliet	NS
17	Hardin	Katherine	BCDB	**45**	Schweibenz	Colby	BCDB
18	Harris	Lynnea	IMP	**46**	Shelton	Debresha	GMB
19	Hartigan	Christina	IMP	**47**	Shiu	Fu Hung	NS
20	Harvey	Brandon	NS	**48**	Shue	Taylor	MMG
21	Heaton	Elizabeth	NS	**49**	Silver	Bri	GMB
22	Hodge	Kenyaita	GMB	**50**	Tanquary	Julia	BCDB
23	Holmes	Kate	MMG	**51**	Tian	Tina	NS
24	Hrncir	Hannah	BCDB	**52**	Tilahun	Kedamawit	GMB
25	Hutchinson	Katherine	BCDB	**53**	Vargas-Cuebas	Germàn	MMG
26	Khatib	Tala	BCDB	**54**	White	Alexandria	NS
27	Lancaster	Carly	BCDB	**55**	Wiggins	Keenan	GMB
28	Lane	Alicia	NS	**56**	Willett	Courtney	GMB

Katie Alexander, IMP Poster #1

Protective immunity against a murine Epstein-Barr virus homolog is preserved during CD11b-CD154 blockade

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

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CD154 pathway antagonism has shown to be a promising target for inducing long-term graft survival, in some cases showing efficacy that is superior to anti-CD40. It has recently been shown that CD11b is an alternate receptor for CD154. In recently published work, we showed that a peptide mimetic of the CD154-binding domain on CD11b (cM7) improved long-term graft survival. However, the impact of CD154:CD11b blockade on protective immunity is not known. The goal of this study was to determine the effects of the CD154:CD11b specific peptide inhibitor on protective immunity to a murine Epstein-Barr virus (EBV) homolog (MHV68). Mice treated with cM7 had significantly higher numbers and frequencies of total CD8+ T cells compared to untreated mice (p=0.0317) at 10 days post-infection in the blood. cM7-treated mice had significantly lower frequencies of short-lived effector cells (p=0.0159) and significantly higher frequencies of memory precursor effector cells (p=0.0159) in the spleen 14 days post-infection. cM7-treated mice were found to have significantly lower frequencies of CD3-CD19+ B cells in the spleen (P=0.0317) but significantly higher levels in the mLNs (p=0.0079). Importantly, when viral burden was analyzed using a YFP-expressing virus, MHV68-YFP, there was no significant difference in the frequency of YFP-expressing infected B cells in the spleens and mLNs of cM7-treated mice as compared to PBS-treated controls. These data suggest despite its ability to impair CD8+ T cell trafficking into allografts, CD154:CD11b blockade does not negatively impact protective immunity to a murine EBV homolog. Targeting this pathway could hold promise for transplant immunosuppression.

Ashley Alexander, PBEE Poster #2

Disruption of aspartate transporter *gltT* enables *Staphylococcus aureus* survival in the presence of *Pseudomonas aeruginosa*

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[3] Division of Infectious Diseases and Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA
[4] Department of Pediatrics, Division of Pulmonology, Asthma, Cystic Fibrosis, and Sleep, Emory University, Atlanta, Georgia, USA

The two most common bacterial pathogens in cystic fibrosis-related lung infections are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. When both opportunistic pathogens persist in a coinfection, patients often experience more severe complications. Analysis of clinical samples has revealed a range of *S. aureus-P. aeruginosa* interaction statuses, suggesting that some strains of *S. aureus* are able to adapt to the selective pressures presented by *P. aeruginosa*. To test this, we designed a serial transfer evolution experiment to observe how *S. aureus* adapts to the presence of *P. aeruginosa* on solid agar. Using *S. aureus* strain, JE2 as our ancestral strain, populations of *S. aureus* were repeatedly cocultured with fresh *P. aeruginosa* at a ratio of 30:1. After 8 coculture periods isolates were taken from evolved populations and whole genome sequencing revealed unique mutations in populations of *S. aureus* whose survival significantly increased. These mutations effectively truncate the sole *S. aureus* aspartate transporter, *gltT*. The evolved phenotype of improved *S. aureus* survivability was replicated in an isogenic *gltT*-transposon mutant. These findings demonstrate that disruption of *gltT* is one adaptive strategy that *S. aureus* can employ to persist in the presence of *P. aeruginosa.* In addition to low survival in the presence of *P. aeruginosa,* wild type *S. aureus* was observed to have a reduced growth rate in chemically defined media when aspartate is present, and glutamate is absent. The results of this study demonstrate the importance of considering the impact of species interactions across multiple scales when studying chronic and recurrent infections.

Brenda Antezana, MMG Poster #3

Extracellular vesicle-mediated transformation of antibiotic resistance among *Streptococcus pneumoniae*

Brenda S. Antezana[1,2], Yih-Ling Tzeng[2], David S. Stephens[2]

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Transformation is an important mechanism for the dissemination of antibiotic resistance among *Streptococcus pneumoniae* (*Spn*). The role of extracellular vesicles (EVs) released from *Spn*, shown to carry nucleic acids and transform corresponding recipients, in the dissemination of antibiotic resistance is not well understood. Quantitative PCR targeting specific antibiotic resistance genes conducted with EVs purified from spent culture media detected 9.84±10.57 ng DNA/µg protein of *tetM* from strain GA16833^{Tn2009} and 5.24±0.91 ng DNA/µg protein of *ermB* from strain D39Δ*ply::ermB*, compatible with reported EVs from other *Spn* strains. *In vitro* transformations of wildtype D39 conducted utilizing 100 ng of D39Δ*ply* EVs resulted in a transformation frequency (tF) of $5.82x10^{-4}$±$6.68x10^{-4}$, confirming that *Spn* EVs could transfer the ~800 bp *ermB* gene. Presence of DNaseI during transformation reduced the tF to <$3.73x10^{-7}$±$5.20x10^{-7}$, suggesting that DNA was not protected by the EVs. EV-mediated transformation utilizing 100 ng of GA16833 EVs did not lead to tetracycline-resistant transformants (tF<$5.71x10^{-6}$), indicating that the large Tn*2009* (23.5 kb) could not be efficiently transformed via EVs. Competence (D39Δ*comE*) or transformation apparatus mutants (D39Δ*comEA/EC*) were unable to be transformed with D39Δ*ply* EVs (tFs <$1.55x10^{-8}$ or <$6.90x10^{-8}$, respectively), supporting the importance of transformation in EV-mediated gene transfer. In conclusion, EVs released from *S. pneumoniae* carry detectable concentrations of genomic DNA and can transform pre-competent *Spn* cells with as large as ~800 bp of DNA.

Britton Barbee, MSP **Poster #4**

Isoform-selective PI3-kinase inhibition confers partial resilience to cocaine cessation-induced anxiety-like behavior

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Phosphoinositide 3-kinase (PI3K) is a multi-subunit signaling complex that phosphorylates phosphoinositides, membrane-embedded second messengers that are critical for synaptic and structural plasticity of neurons. Cocaine potentiates PI3K-Akt-mTOR cascade activity, and this activation persists beyond the period of drug exposure. The PI3K p110β isoform is neuronally enriched and able to control PI3K signal propagation, allowing for manipulation of PI3K activity in a more targeted manner than broad-spectrum PI3K inhibition. Cessation of cocaine use triggers anxiety-like behavior in humans and rodent models, and anxiety can be a causal factor in relapse. Here, we used viral-mediated gene silencing to reduce expression of p110β in the dorsomedial prefrontal cortex (dmPFC). Isoform-selective PI3K inhibition mitigated anxiety-like behavior triggered by acute cocaine. Interestingly, however, a history of repeated cocaine exposure occluded this resilience, presenting an opportunity to compare immediate-early gene expression between cocaine-vulnerable and cocaine-resilient mice. We examined 22 brain regions and found that resilient mice – those displaying less anxiety-like behavior – displayed lower immediate-early gene expression in the claustrum and lateral hypothalamus. We next found that chemogenetic stimulation of the claustrum induced anxiety-like behavior. Future studies will determine whether suppressing p110β in the dmPFC combats anxiety-like behavior via connections with the claustrum. Our findings suggest that isoform-selective PI3K inhibition mitigates cocaine cessation-elicited anxiety-like behavior, likely via coordinated brain regions and circuits.

Scott Becker, MSP **Poster #5**

Development of a therapeutic platform to enhance cytotoxicity of γδ T cells using mRNA electroporation and combination therapy

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Despite the promising success of chimeric antigen receptor (CAR) T cell therapy, there are a number of risks and obstacles that must be addressed to improve patient outcomes and safety. There are limited tumor-specific targets, and the development of memory CAR-T cells and cytokine release syndrome has caused harmful side effects and death. We have developed γδ T cells as a promising option for adoptive cell therapy and can reduce many of these risks because these cells persist for a short duration in vivo, do not produce a memory phenotype, and can be developed as an off the shelf allogenic immunotherapeutic. As a platform technology, we i) developed a robust genetic engineering strategy for transient expression of anti-CD19 and anti-CD22 CARs in γδ T cells, ii) showed robust killing of the CD19+ and CD22+ 697 cell line, iii) showed enhanced killing of cancer cells using unmodified γδ T cells with T cell engagers, such as Blincyto, iv) tested the combined use of Blincyto with CD22 CAR-modified γδ T cells, and v) showed, using an NSG mouse model, that mice treated with CD19 CAR-expressing γδ T cells or unmodified γδ T cells with Blincyto resulted in a reduction in tumor burden. Transient expression of CARs and combining CARs with bispecific T cell engagers offers an attractive platform strategy for treating cancer and can improve upon traditional αβ T cell applications. We are currently using this platform technology to develop treatment options that are not limited to CD19/22 positive cancers.

Lisa Blackmer-Raynolds, NS Poster #6

From Gut to Brain: Evaluating the contribution of select gut bacteria to Alzheimer's disease outcomes

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Alzheimer's disease (AD) patients display alterations in gut microbiome composition that may contribute to disease. Microbiome manipulations are sufficient to modulate AD-like symptoms and pathology in mouse models, suggesting that the microbiome may directly impact disease outcomes. The mechanism(s) by which this occurs is currently unknown, however, one possibility is through the effects of the microbiome on microglia development and activation that, in turn, modulate microglia responses to AD pathology. However, the contribution of individual microbial species to neuroinflammatory tone and AD outcomes is unknown. Therefore, wildtype germ-free mice were mono-colonized with AD-associated (both positively and negatively) bacterial species for two weeks prior to microglia isolation and assessment. RNA-seq analysis of CD11b+ cells displayed robust gene expression changes only in *Escherichia coli* mono-colonized mice, demonstrating a specific *E. coli*-dependent increase in neuroinflammatory processes. Therefore, to explore the consequences of *E. coli* in the context of AD, conventionally raised 5xFAD mice were enriched with *E. coli* for 1 month. Behavioral analysis revealed a trend towards increased cognitive impairment and multi-plexed immunological assessments and western blot analyses revealed significant increases in inflammatory markers and amyloid production in *E. coli* enriched mice. Together, these results identify *E. coli* as an AD-related bacteria with unique consequences on microglia activation in both health and disease. Furthermore, it demonstrates that *E. coli* exposure is sufficient to exacerbate AD-associated markers in the 5xFAD model, highlighting the potential contribution to AD progression.

Maegan Brockman, MSP Poster #7

The role of neutrophils in initiating type I interferon response in myocardial ischemia/reperfusion

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Myocardial ischemia/reperfusion (MI/R) triggers an inflammatory response in which neutrophils are the most rapid and abundant immune cells recruited to the myocardium. While neutrophils play a role in wound healing, they can cause further damage through the generation of reactive oxygen species, proteolytic enzymes, and neutrophil extracellular traps (NETs). Utilizing single-cell RNA sequencing to better characterize the role of neutrophils and NETs in MI/R, we identified a significant population of neutrophils with a strong type I interferon (IFN) signature recruited to the area of infarct 24 hours post MI/R in a murine model. We hypothesize that double stranded DNA from NETs activates cGAS-STING signaling in a murine model of MI/R, leading to the production of type I interferons by neutrophils. Mice underwent MI/R and heart tissues were harvested 6- and 24- hours after reperfusion. Analysis by qPCR shows an increased fold change in gene expression of IFNα (7.226.05, n = 3; 3.832.22, n = 4) and IFNβ (6.745.39, n = 3; 3.642.25, n = 4) at 6- and 24- hours post MI/R respectively, compared to sham mice. Additionally, analysis by ELISA shows a significant increase in interferon β protein levels in the heart after 6- hours (0.41pg/mg0.04, n =8) compared to sham mice (0.16 pg/mg 0.06, n = 7; $p \leq 0.01$, Student's t-test). These data indicate the rapid increase in IFN, which implicates neutrophils in the interferon response. Identifying neutrophils as an early source of IFN can provide a potentially new therapeutic target in treating MI/R.

Garrett Cooper, GMB Poster #8

The role of DPF2 depletion in SMARCB1-deficient cancers

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Rhabdoid tumors are one of the most aggressive and lethal cancers in pediatric oncology with overall 5-year survival rates of ~20-25%. This is despite intensive multi-modal therapies including surgery, radiation and chemotherapy. Loss of *SMARCB1* is the primary recurrent genetic alteration found in over 90% of cases. Recent advances have implicated *SMARCB1* loss in a number of other cancers broadly referred to as SMARCB1-deficient cancers, which similarly have high rates of SMARCB1 loss. SMARCB1 is a critical component of the BAF chromatin remodeling complex, a complex which controls gene transcription by positioning nucleosomes at gene regulatory regions. Deletion of *SMARCB1* has been shown to deplete an adjacent BAF subunit, DPF2. Re-expression of SMARCB1 in a SMARCB1-deficient cell line leads to a robust accumulation of DPF2. The histone reader protein, DPF2, plays a critical role in the recruitment of the BAF complex to regulatory regions and regulates both differentiation and apoptosis, however the role of DPF2 has not yet been studied in the context of SMARCB1-deficient cancers. We hypothesize that loss of *SMARCB1* leads to the depletion of DPF2 through proteasomal degradation and inhibits apoptosis and differentiation by misregulating BAF localization. We have preliminary data suggesting that rescuing DPF2 levels may target the BAF complex to regulatory regions that promote fibrosis and inflammation and prevent cell growth. These data suggest that increasing DPF2 levels through proteasomal inhibition may be a promising intervention and may lead to future therapeutic targets for this devastating set of diseases.

Kimberly Diaz Perez, GMB Poster #9

Estimating the diagnostic yield of 503 genes in orofacial cleft trios using whole-genome sequencing

Kimberly K. Diaz Perez[1], Sarah W. Curtis[1], Alba Sanchis-Juan[2], Taylor Head[1], Samantha Ho[1], Bridget Carter[1,3], Toby McHenry[4], Madison R. Bishop[1], Luz Consuelo Valencia-Ramirez[5], Claudia Restrepo[5], Lina Moreno Uribe[6], George Wehby[7], Terri H. Beaty[8], Jeffrey C. Murray[9], Eleanor Feingold[10], Mary L. Marazita[4,10], David J. Cutler[1], Michael P. Epstein[1], Harrison Brand[2], Elizabeth J. Leslie[1]

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Orofacial clefts (OFCs) are common craniofacial birth defects, including cleft lip (CL), cleft lip and palate (CLP), and cleft palate (CP). The etiological heterogeneity of OFCs complicates clinical diagnostics as it is not always readily apparent if the cause is Mendelian, environmental, or multifactorial. Although sequencing could aid diagnosis, it is not commonly used for 60-70% of OFC cases that are nonsyndromic or lack a strong family history. We aimed to estimate the diagnostic yield of 837 OFC cases and 301 controls in 503 genes associated with primarily Mendelian OFCs using whole-genome sequencing. After filtering and curation, variants were reviewed according to American College of Medical Genetics criteria, blinded to case-control status. We found pathogenic variants in 9.32% of cases and 1.33% of controls ($p<0.0001$), which was almost exclusively driven by variants in autosomal dominant genes. The yield was highest in CP (19.7%) while CL (2.91%) was not significantly different from controls. The pathogenic variants in 41 genes indicate substantial genetic heterogeneity, but interestingly, nine genes alone accounted for 4.66% of OFC cases. Most variants (59%) were classified as "variants of uncertain significance" (VUS) and were more frequent in cases ($p=7.02 \times 10^{-4}$). However, we could not identify individual genes with a significant excess of VUSs. Several genes were nominally significant (e.g., *NSD1*, *PRICKLE1*), and variants in these genes should be subjected to functional validation to determine their true effect. Cumulatively, these results underscore the etiological heterogeneity of OFCs and suggest sequencing could reduce the diagnostic gap in OFCs.

Rachel Done, MMG **Poster #10**

Temperature regulation of a *Pseudomonas aeruginosa* virulence factor

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Pseudomonas aeruginosa is an important opportunistic bacterial pathogen that adapts to living inside a host by producing and regulating virulence factors. One such virulence factor is the secreted serine protease PrpL. Published transcriptomic studies show that *prpL* expression is higher at ambient temperatures (25°-28°C) than human body temperature (37°C). We measured *prpL* expression by RT-qPCR in *P. aeruginosa* PAO1 and found that *prpL* expression is about a hundred times higher at 25°C than 37°C, leading us to hypothesize that *prpL* is thermoregulated at the level of transcriptional regulation. To test this, transcriptional reporters of the *prpL* promoter and green fluorescent protein (GFP) on a multi-copy plasmid were used to measure promoter activity. In PAO1 containing the reporter, GFP production was higher at 25°C than 37°C, indicating that *prpL* thermoregulation occurs at the level of transcription from the promoter. We then tested this reporter in mutants of known transcriptional regulators of *prpL*, MvaT/MvaU and LasR, at 25°C and 37°C. In each mutant strain, GFP levels were equivalent between 25°C and 37°C. However, in the PAO1 *mvaT/U* mutant reporter, GFP production was higher than in PAO1, suggesting that MvaT/U repress *prpL*, while in the PAO1 *lasR* mutant reporter strain, GFP production was lower than in PAO1, suggesting that LasR promotes *prpL* expression. Future studies will focus on the molecular mechanism for how MvaT/U and LasR thermoregulate *prpL*. A better understanding of *prpL* thermoregulation will enhance our knowledge of how *P. aeruginosa* survives inside a human host.

Amanda Gill, IMP **Poster #11**

Does PD-1 blockade during chronic infection deplete the PD-1^{+}TCF-1^{+} stem-like CD8 T cells?

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PD-1^{+}TCF-1^{+} stem-like CD8 T cells provide the proliferative burst following PD-1-directed immunotherapy. PD-1 blockade induces stem-like CD8 T cells to proliferate and differentiate into transitory, effector-like cells, and then into more terminally-differentiated, exhausted cells. We asked the question: does this process result in depletion of the stem-like CD8 T cells? To study this, we treated chronically LCMV-infected mice with αPD-1 for two weeks and analyzed the number and frequency of virus-specific stem-like and exhausted CD8 T cells. We found that stem-like cells decreased in their proportion to exhausted CD8 T cells after two weeks of αPD-1 treatment. Surprisingly, the absolute number of stem-like cells remained constant, and they also maintained Ki67 expression. To extend this, we used a mouse αPD-1 antibody to perform continuous PD-1 blockade in the same setting. Again, we found that treated and untreated mice maintained equal numbers of stem-like cells even after two months of therapy. We next sought to determine whether αPD-1 might transcriptionally or functionally alter stem-like cells, without depleting them. We found that PD-1 blockade did not induce such changes. αPD-1-treated and untreated stem-like cells had nearly identical transcriptional profiles by RNA-seq, and treated stem-like cells also maintained their proliferative potential upon adoptive transfer and viral challenge. PD-1 blockade clearly drives substantial proliferation and differentiation of stem-like cells, but it may also act at another level. These studies suggest that PD-1 may be a regulator of stem-like cells' self-renewal and that PD-1 blockade can influence their homeostatic turnover.

Annie Goettemoeller, NS Poster #12

Mechanisms of altered firing in fast-spiking interneurons during early phase Alzheimer's Disease

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Alzheimer's Disease (AD) is the most common form of dementia, neuronal dysfunction and memory loss, yet there remains no effective preventative treatment. AD research has primarily focused on treating pathological plaques and tangles. However, recent research suggests plaque-and-tangle pathology occurs relatively late in the disease. New evidence in AD patients and mouse models of disease have placed hyperexcitability, increased pyramidal neuron firing, prior to plaque pathology, providing an early point for disease intervention. Pyramidal neuron hyperexcitability is typically prevented by inhibition from GABAergic interneurons. Interestingly, recent literature has shown that distinct interneuron subtypes are disrupted at this early phase in mouse models, specifically fast spiking (FS) parvalbumin interneurons. FS interneurons display altered action potential firing in the prodromal phase in multiple AD mouse models, resulting in pyramidal neuron hyperexcitability. Despite this apparent universal early alteration in FS interneuron firing across models, the mechanistic cause remains unclear. Our preliminary work shows that altered FS interneuron firing in 5xFAD mice (7-8 weeks) occurs through a Kv3-dependent mechanism. To determine which aspect of the 5xFAD model may contribute to the mechanism, we analyzed contributions of wild-type human Amyloid Precursor Protein (WT hAPP) using a viral approach. Using ex-vivo patch-clamp electrophysiology, we once again observed an altered firing phenotype in FS interneurons in 7-8 week old mice (2 wpi). This breakdown of effects of APP on FS interneuron firing will give us potential insight into early mechanisms of the pathogenesis of Alzheimer's Disease, providing a potential point for disease intervention.

Stephanie Guerra, MMG Poster #13

Testing the Goldilocks Affinity Model for Antibiotic Substrate Selection by the *Pseudomonas aeruginosa* MexXY-OprM Efflux System.

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Antimicrobial resistance (AMR) in bacterial pathogens is a growing problem around the world and a burden in clinical settings. An estimated 1.27 million deaths were attributed to bacterial AMR in 2019. *Pseudomonas aeruginosa* is a gram-negative opportunistic bacterial pathogen which uses several efflux pump systems as a mechanism to resist multiple classes of antibiotics. *P. aeruginosa* expresses several Resistance-Nodulation-Division (RND) efflux pumps which possess different but overlapping substrate profiles. For example, the RND efflux pump MexXY-OprM has been found to efflux aminoglycosides, a group of positively charged antibiotics, but not negatively charged ß-lactams, whereas another RND efflux pump, MexAB-OprM, can efflux ß-lactams but not aminoglycosides. The inner membrane bound transporter protein MexY must be able to recognize and bind aminoglycosides in order to efflux these molecules from the cell. We propose that MexXY-OprM maintains 'Goldilocks affinity', where MexY binds with sufficient affinity to support interaction with antibiotic substrates, without impeding movement through the pump. Using a homology model of MexY, we identified areas of interest that may be important for substrate recognition and binding, including the vestibule ceiling. Our model predicts that altering the neutrally charged MexY vestibule ceiling to a negative charge, as found in MexB, will reduce aminoglycoside efflux due to tight binding between the molecule and the vestibule ceiling. In this project, I have successfully created a double mutant MexY with a negatively charged vestibule ceiling that can be used to assess aminoglycoside sensitivity and begin experimentally testing the 'Goldilocks hypothesis'.

Ramona Haji-Seyed-Javadi, GMB Poster #14

A synthetic lethal screen identifies novel DNA damage response proteins that govern etoposide resistance in small cell lung cancer

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Lung cancer is the leading cause of cancer death. Small cell lung cancer (SCLC) is the most aggressive form. Chemotherapeutic agents such as etoposide and ionizing radiation (IR) are the first-line treatments for SCLC. A major cause of unsuccessful cancer therapy is resistance driven DNA repair mechanisms. A rationale-driven therapeutic approach is to exploit specific DNA repair pathways to improve the efficacy of DNA-damaging agents. To identify genes that mediate etoposide resistance, we performed a synthetic lethal screen in an etoposide-resistant SCLC cell line, NCI-H128. An siRNA screen targeting 1,008 nuclear enzymes was carried out. NCI-H128 cells were transfected in 96-wellplates, treated or not with etoposide. After comprehensive statistical analysis 37 drug-resistant knockdown genes were confirmed among which a few DEAD box protein family of RNA helicases proved to sensitize cells to etoposide, IR, CPT, HU and ATB888, suggesting that they respond generally to DNA double-strand breaks (DSBs). We verified their localization to distinct DNA repair foci, impairment of homologous recombination (HR) upon their depletion and interaction with other known DNA repair proteins through mass spectrometry and co-IP experiments. Increased total level of R-loops in the cell implies direct or indirect involvement of RNA helicases with DNA-RNA hybrids. DEAD box family members may function directly in mediating genome integrity by promoting HR at DNA double-strand sites. Uncovering the mechanisms by which they participate in DNA repair pathway will provide new insights into how they may be exploited as novel therapeutic targets for chemotherapy resistant SCLC.

Adam Hamilton, NS **Poster #15**

Spinal cord injury-associated microbiome alterations contribute to impaired immune and neuronal status in the gastrointestinal tract

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Spinal cord injury (SCI) often results in significant alterations to gastrointestinal (GI) physiology, known as neurogenic bowel dysfunction, one of the most cited detriments to quality of life in persons with SCI. The gut microbiome changes significantly after SCI and has been shown to directly impact injury recovery. Interactions between host and microbiome modulate overlapping functions including enteric neurogenesis, endocrine signaling, and inflammation, all of which mediate bowel motility function. We therefore sought to determine if dysbiosis directly contributes to changes in enteric neuromusculature and GI motility, and if targeting the post-injury microbiome could improve SCI outcomes. Adult mice were subjected to a midthoracic (T9) spinal cord injury or a midthoracic laminectomy (sham-surgery controls). Fecal microbiomes derived from mice with SCI or from sham controls were then used to colonize germ-free (GF) mice to determine the impact of the injury-induced microbiome on GI physiology in healthy, injury-naïve, mice. Mice with SCI had significantly impaired intestinal transit and presented with reduced colonic nNOS and increased levels of proinflammatory cytokines. Intriguingly, these findings were recapitulated in ex-GF mice colonized with SCI-derived microbiomes, demonstrating a direct effect of the post-SCI microbiome on physiology. 16S rRNA sequencing was preformed to elucidate distinct bacterial taxa altered by SCI. These taxa were then given as probiotics to mice with SCI. Our data suggests that probiotic enrichment of the post-SCI microbiome ameliorates enteric and central neuroinflammation.

Nicholas Harbin, MSP Poster #16

Regulator of G-protein signaling 14 (RGS14) alters behavioral and pathological responses due to kainic acid-induced status epilepticus

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RGS14 is highly expressed in pyramidal cells of the hippocampus to regulate calcium influx and synaptic plasticity. Recent reports have demonstrated a protective function of RGS14 against injury in the periphery. We hypothesized that RGS14 may be protective within the hippocampus after following status epilepticus (SE). To test this hypothesis, we induced SE in wild-type (WT) and RGS14 knockout (RGS14 KO) mice using kainic acid (KA). We evaluated behavioral responses during SE, probed for RGS14 expression and SE-induced pathology, and used proteomic analysis to determine pathological alterations in the hippocampal proteome after SE. After KA treatment, we found RGS14 KO mice achieve SE faster and die quicker compared to WT mice. Western blotting and immunohistochemistry of hippocampal tissue from WT mice revealed a striking and significant upregulation of RGS14 after SE, notably in pyramidal cells of area CA1. Using MAP2 and PSD95 as proxies for neuronal damage, we observed a significant loss of MAP2 and PSD95 expression in area CA1 of RGS14 KO but not WT mice after SE. Using IBA1 and GFAP expression as markers of neuroinflammation, we found KO mice had a blunted neuroinflammatory response in area CA1 after SE compared to WT mice. Lastly, proteomic analysis on hippocampi from SE WT and RGS14 KO mice revealed altered expression of bioenergetic- and redox-associated processes in RGS14 KO hippocampi. Taken together, these results suggest that RGS14 plays a critical role in hippocampal neuroprotection, likely by modulating oxidative stress and neuroinflammation during and after SE.

Katherine Hardin, BCDB Poster #17

Examining the Role of Fascin1 in Growth Cone Motility and Axon Guidance

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Axon guidance is a critical developmental process in which axonal projections are guided to their specific targets for the precise wiring of the central nervous system. Errors in axon guidance can result in wiring defects that are associated with a wide range of brain disorders including autism and epilepsy. Axon guidance depends on the highly motile tips of elongating axons called growth cones, which are responsible for sensing and responding to extracellular guidance cues to direct axonal growth. Growth cone filopodia are known to function as sensory apparatus as they are equipped with guidance receptors and can sample the surrounding environment. Despite the importance of filopodia in axon guidance, the molecular and cellular mechanism that controls and regulates filopodia formation and dynamics is largely unknown. Fascin1 is a ~55 kDa actin bundling protein that crosslinks actin filaments to form tight F-actin bundles in filopodia and is a known regulator of cell migration. Fascin1 is highly expressed in developing neurons and enriched in growth cone filopodia, but its role in growth cone motility and guidance has not been investigated. e have developed a CRISPR-Cas9-mediated approach to knock out Fascin1 from cultured rat hippocampal neurons, allowing us to assess the effects of Fascin1 on individual growth cone's guidance responses. In addition, we have used a Fascin1 null *Drosophila* fly model to reveal a crucial *in vivo* role for Fascin1 in axon guidance in the *Drosophila* mushroom body, a brain structure that is analogous to the mammalian hippocampus.

Lynnea Harris, MSP **Poster #18**

In vivo characterization of a novel GluN2B-selective negative allosteric modulator of the N-methyl-D-aspartate receptor in the context of analgesic tolerance to morphine

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Drug overdose is the leading cause of accidental death in the US, and over 75% of cases are due to chronic opioid use and subsequent tolerance and addiction. Analgesic tolerance is characterized by a decreased response to the analgesic effects of opioids, requiring increasingly higher doses to maintain the desired level of pain relief. Overactivation of GluN2B-containing NMDA receptors is a key cellular adaptation in the development of analgesic tolerance due to the concentrated expression of the GluN2B subunit in pain processing regions of the brain and spinal cord. This work characterizes a novel GluN2B-selective negative allosteric modulator (NAMs) developed by our lab in the context of analgesic tolerance to morphine. Using the tail-immersion test for rodents, the compound has been assessed for the ability to elicit analgesia, to potentiate the analgesic effects of morphine, to inhibit morphine-induced analgesic tolerance, and to reverse pre-established tolerance in a mouse model. Future structure-activity relationship work around this compound could give rise to compounds that can be co-administered with opioids to inhibit the worsening of and/or delay the onset of tolerance due to chronic opioid use. If successful, future compounds could decrease the likelihood that chronic pain patients will become addicted to their prescribed medication.

Christina Hartigan, IMP Poster #19

TIGIT agonism alleviates costimulation blockade resistant rejection in a Treg-dependent manner

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Belatacept-based immunosuppression in kidney transplantation confers fewer off-target toxicities compared to calcineurin inhibitors but comes at a cost of increased incidence and severity of acute rejection, potentially due to its deleterious effect on both the number and function of Foxp3$^+$ Treg. TIGIT is a coinhibitory receptor expressed on several types of immune cells, including Treg. We evaluated the use of an agonistic αTIGIT antibody to ameliorate costimulation blockade-resistant rejection in a murine model of skin transplantation. We show that TIGIT agonism, when combined with costimulation blockade by CTLA-4Ig, can prolong allograft survival in a minor antigen mismatch model of transplantation compared to CTLA-4Ig treatment alone (MST beyond 80 days compared to MST of 24 days, p value=0.0007). Further, we show that this prolongation of graft survival is accompanied by a reduction in the number of CD8$^+$ T cells in the graft (p=0.03), and an increase in the frequency and number of graft-infiltrating Tregs (1.6 fold increase, p=0.0046). Through the use of Treg-specific TIGIT conditional knockout animals, we show that the TIGIT-mediated reduction in the graft-infiltrating CD8$^+$ T cell response is dependent on signaling of TIGIT on Tregs. Our results highlight the therapeutic potential of TIGIT agonism to function in combination with CTLA-4Ig to optimize costimulation blockade-based immunosuppression for transplant patients.

Brandon Harvey, NS Poster #20

Deep Learning Classifier for the Combined Scoring of Sleep-Wake and Seizures in Mice

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The relationship between sleep and seizure is complex and bidirectional, including seizure-frequency-associated sleep fragmentation as well as sleep-deprivation-induced increases in seizures. In order to provide better throughput for sleep studies in Intra-Amygdalar Kainic Acid (IAKA) Temporal Lobe Epilepsy (TLE) model mice, we have designed a data processing pipeline and Keras-based deep learning classifier for sleep staging and seizure scoring. While sleep-wake classifiers and seizure detection algorithms abound, the combination is difficult given the abnormal EEG background in mice with epilepsy.

We obtained electrocorticogram (ECoG), electromyogram (EMG), video and bilateral hippocampal depth electrode data from mice implanted using a customized 3D-printed headplate designed in our lab. Our dataset included 972 12-hour recordings from a group of 47 mice (including 23 mice that developed spontaneous seizures and 10 control mice), that included 642 seizures. We used 80% of the data for training and 20% for testing. The classifier was trained on manually scored 20-second epochs from existing downsampled recordings (from 2kHz to 200Hz), using Fourier bins of alpha, beta, delta, gamma, and theta frequency bands in recordings from this montage.

With a sequential model, the classifier has achieved >90% scoring accuracy in most categories for our epileptic mice. This reliable classification will allow for rapid combined sleep-wake and seizure scoring in our IAKA sleep-wake paradigm. Using this classifier to improve our throughput, we hope to improve the mechanistic and symptomatic understanding of sleep disruption in epilepsy, leading to improved patient treatment and outcomes.

Elizabeth Heaton, NS Poster #21

Melanocortin-4 receptor control of striatal-dependent action selection

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Goal-directed action refers to behaviors that are dynamic, sensitive to unexpected events, and require the dorsomedial striatum (DMS). Molecular factors underlying an organism's ability to flexibly shift between goal-directed and habitual behavior are incompletely understood. We recently discovered that dorsal striatal melanocortin-4 receptor (*Mc4r)* expression correlates with this behavioral flexibility in adult male and female mice, leading to the hypothesis that the activity of *Mc4r*+ DMS neurons regulates goal-directed action. Stimulation of *Mc4r*+ DMS neurons via Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) facilitated animals' ability to select actions based on reward likelihood. Meanwhile, inhibition via Gi-DREADDs rendered animals insensitive to changes in reward likelihood, promoting habits. MC4R controls GluA2 AMPA receptor subunit availability at the membrane such that *increasing* MC4R activity should *decrease* glutamatergic excitability of MSNs and mimic the behavioral effects of Gi-DREADDs in *Mc4r*+ neurons. Indeed, administration of MC4R agonist setmelanotide facilitated habit formation, while viral-mediated *Mc4r* knockdown in the DMS enhanced the ability of mice to select actions based on reward likelihood. The DMS receives dense glutamatergic projections from the orbitofrontal cortex (OFC), a region necessary for goal-directed action. Initial trans-synaptic retrograde tracing indicates that DMS MC4R+ neurons receive monosynaptic projections from the OFC, and chemogenetic experimentation revealed that the OFC is necessary for MC4R-related changes in behavioral flexibility. These results reveal that striatal MC4R may be a key factor in sustaining *versus* "breaking" habits, and thus could serve as a target for treating maladaptive habits that contribute to neuropsychiatric disease.

Kenyaita Hodge, GMB Poster #22

A Polyepigenetic Glucocorticoid Score is Associated with Bronchopulmonary Disease Severity

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Background: Bronchopulmonary dysplasia (BPD), the most common morbidity among very preterm infants, is a respiratory disorder often necessitating treatment with supplemental oxygen and/or anti-inflammatory corticosteroids [e.g., glucocorticoids (GC)] to improve gas exchange. These interventions can perturb the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response. A polyepigenetic GC score associated with prenatal GC exposure has been developed and is associated with poor mental and behavioral outcomes. This GC score is an innovative epigenetic biomarker for assessing early life stress and health.

Objective: To determine whether the GC score is associated with BPD and if there is a dose response with increasing BPD severity.

Design/Methods: In the Neonatal Neurobehavior and Outcomes in Very Preterm Infants (NOVI) study, buccal cell tissue was collected from 542 very preterm infants (born <30

weeks gestational age) at Neonatal Intensive Care Unit (NICU) discharge. DNA methylation (DNAm) levels were profiled using the Infinium MethylationEPIC BeadChip. We calculated the neonatal GC score by multiplying the DNAm beta values by the 24 CpG weights established by Provençal et al (2020). We used linear regression to test for differences in the GC score with increasing severity of BPD after adjusting for potential confounders.

Results: Increasing BPD severity was associated with the GC score in buccal tissue. Neonatal GC score decreased with increasing BPD severity, with GC scores being significantly lower for severe BPD (β= -0.409; p-value= 0.0438).

Conclusion: Our findings suggest that infants with more severe BPD had lower polyepigenetic GC scores, which is indicative of greater GC exposure.

Kate Holmes, MMG Poster #23

Use of barcoded influenza A virus to evaluate genetic drift within and between hosts

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Stochastic effects can shape the evolution of viruses, both within individual hosts and at transmission bottlenecks between hosts. Here, we use a virus population carrying a fitness-neutral barcode to evaluate patterns of viral diversity within and between experimentally inoculated guinea pigs and their naïve contacts. Our barcoded virus library contains twelve synonymous polymorphisms within the influenza NA segment, resulting in a library with 4096 potential unique barcodes. We inoculated animals with this library and placed direct or aerosol contacts one day later. Nasal washes were collected from all animals daily for seven days and then deep-sequenced. We assessed the diversity of the barcoded virus population in individual animals each day using the Shannon diversity index. In inoculated guinea pigs, a high level of barcode diversity is maintained despite an early decline. Continuity in the barcodes detected across time furthermore indicates that stochastic effects are not pronounced within these hosts. In contrast, a sharp, stochastic decline in viral diversity is observed between inoculated and contact animals, with a stronger effect seen in aerosol contacts. This stringent bottleneck is consistent with prior work; however, our data offer greater temporal resolution than previously reported and show that viral diversity in contact animals is relatively high in the first positive sample. Diversity then decreases substantially on subsequent days as relatively few genotypes go on to establish infection in contact animals. These results suggest that, in addition to loss of virus in the environment, host barriers to initial infection contribute to the viral transmission bottleneck.

Hannah Hrncir, BCDB Poster #24

Intrahepatic bile duct subpopulations exhibit varying levels of *Sox9* expression

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The liver possesses the unique ability to regenerate and withstand substantial damage from drugs and infection. However, often the only treatment for end-stage liver disease is transplantation. In order to combat liver disease and the liver's failed regenerative mechanisms, a deeper understanding of liver homeostasis must be attained. A common phenotype of end stage liver disease is ductular reaction which is characterized by an expansion of biliary epithelial cells (BECs). Regulating this response is the transcription factor sex-determining region Y-box (SRY-box) containing gene 9 (*Sox9*). To explore the role *Sox9* plays in BEC identity, we created a *Sox9*^{EGFP} transgene which reports distinct *Sox9* expression levels. Interestingly, *Sox9*^{EGFP} demonstrated BEC heterogeneity with populations expressing GFP^{sub}, GFP^{low}, and GFP^{high}. To further dissect BEC heterogeneity, we performed single cell RNA-sequencing and spatial transcriptomics which revealed five distinct BEC clusters. From this data, we determined that BECs are composed of subpopulations which exhibit varying levels of *Sox9* expression. To explore the role *Sox9* plays in tissue heterogeneity, we knocked out *Sox9* developmentally which resulted in ductal paucity and impaired organoid formation. These results, further demonstrates *Sox9*'s critical role in maintaining liver homeostasis and proper ductal morphology. These data help in gaining a better understanding of how distinct BEC subpopulations are regulated by *Sox9* and how these mechanisms regulate liver homeostasis and disease.

Katherine Hutchinson, BCDB Poster #25

Mitochondrial deficiencies influence variable penetrance of nuclear Nab3 granule accumulation

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Reorganization of cellular proteins into subcellular compartments, such as the concentration of RNA-binding proteins into cytoplasmic stress granules and P-bodies, is a well-recognized, widely studied physiological process currently under intense investigation. One example of this is the induction of the yeast transcription termination factor, Nab3, to rearrange from its pan-nucleoplasmic distribution to a granule at the nuclear periphery in response to nutrient limitation. Recent work in many cell types has shown that protein condensation in the nucleus is functionally important for transcription initiation, RNA processing, and termination; however, little is known about how subnuclear compartments form. Here we have quantitatively analyzed this dynamic process in living yeast using a high-throughput computational tool and fluorescence microscopy. This analysis revealed that Nab3 granule accumulation varies in penetrance across yeast strains and that levels of granule accumulation were inversely correlated with a defect in growth when oxidative phosphorylation is required. The results indicate that OXPHOS, and presumably the supply of ATP, strongly impacts Nab3 granule accumulation, a precedent for which is seen for cytoplasmic stress granules. In conclusion, we describe an important determinant of granule biogenesis, OXPHOS, and demonstrate its impact on the accumulation of an inducible subnuclear compartment for the RNA-binding transcription factor, Nab3.

Tala Khatib, BCDB **Poster #26**

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

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Metastasis accounts for 90% of cancer-related deaths. One primary mode of metastasis is collective invasion, where packs of cells invade into the stroma while maintaining cell-cell contacts. Cells within the pack are heterogenous, and harbor distinct genetic and phenotypic subpopulations that cooperate to drive invasion. Our lab has established a technique to investigate phenotypic heterogeneity in cells - Spatiotemporal Cellular and Genomic Analysis (**SaGA**). SaGA is an image-guided approach that isolates and purifies live cells based upon *in situ* phenotypic criteria. We have used this technique to isolate NSCLC cells within the collective invasion pack, where cells on the leading edge are 'leaders', and cells within the pack are 'followers'. RNA sequencing identified Interleukin-13 receptor alpha 2, IL13ra2, as a binarily expressed transmembrane protein in followers. IL13ra2 is a decoy receptor to immunoregulatory cytokine, IL13 and 3000-fold higher in followers compared to leaders. Fluorescence active cell sorting of IL13ra2 negative and positive cell populations mimic established leader and follower invasive phenotype across a panel of NSCLC cell lines and patient samples. Likewise, both IL13ra2 positive and negative cells are required for efficacious metastasis *in vivo*. These data suggest that IL13ra2 marks a critical subpopulation, providing a molecular basis to sort and sequence IL13ra2 positive and negative cells in NSCLC. The objective is to present translational evidence of the validity of this biomarker and determine the molecular mechanisms required to maintain each tumor population. Establishing the molecular mechanisms that fuel these subpopulations is critical to understanding and treating tumor metastasis.

Carly Lancaster, BCDB Poster #27

A conserved RNA binding protein regulates RNAs critical for neurodevelopment

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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.

Alicia Lane, NS Poster #28

Metabolic mechanisms of copper-dependent neuronal dysfunction in Menkes disease

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Homeostatic regulation of copper is necessary for a broad range of neuronal functions from neurotransmitter synthesis to cellular respiration. The breakdown of homeostatic mechanisms controlling copper is associated with neurodegenerative conditions including Alzheimer's disease, Parkinson's disease, and Menkes disease, a rare genetic disorder caused by mutations in the copper transporter ATP7A. These conditions are also associated with dysregulation of cell metabolism and bioenergetics. The underlying mechanisms by which copper depletion influences cell metabolism and neuropathology remain unclear. We generated a set of isogenic CRISPR cell models that are depleted of copper via mutations in 2 copper transporters: ATP7A and CTR1. We used ICP mass spectrometry, extracellular flux oximetry, and Nanostring gene expression assays to characterize copper content and metabolism in these cells. These mutants exhibit distinct metabolic responses to genetic copper depletion, with ATP7A mutants exhibiting increased mitochondrial respiration and CTR1 mutants exhibiting decreased mitochondrial respiration. Changes in gene expression were consistent with respiration patterns. Transcript expression in ATP7A mutants revealed downregulation of genes which annotate to the hypoxia inducible factor 1 (HIF-1) pathway, a metal-sensitive pathway which regulates cellular bioenergetics by switching metabolism from mitochondrial oxidative phosphorylation to glycolysis. This was not seen in CTR1 mutants. These results suggest multiple mechanisms by which cells respond to copper depletion. Further investigation will clarify the metabolic pathways responsive to copper and their effects on neuronal function.

Samantha Lanjewar, GMB Poster #29

Identifying drivers of human astrocyte development

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Astrocytes are 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 extrinsic and intrinsic factors that control human 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. We examined whether secreted signals from neurons could drive precocious astrogenesis. We cultured young organoids in conditioned media from mature organoids, engrafted fetal neurons into organoids, and directly cultured organoids with candidate ligands. We found that extrinsic signals on their own appear to be insufficient to drive precocious astrogenesis, indicating the need for intrinsic changes. To determine intracellular factors, we are investigating the possible synergism required between the Notch, BMP, and JAK-STAT pathways, each of which is individually involved in astrocyte production. Using CRISPR-activation human stem cells, we will activate these pathways combinatorially in young organoids and test for precocious astrogenesis. Additionally, we will knockout *NGN2* or *NEUROD1*, master regulators of neurogenesis, to test whether inhibition of neuronal development alters the gliogenic switch. Ultimately, understanding what drives the gliogenic switch will provide insight into how perturbations to the timing and production of astrocytes contribute to the pathogenesis of neurodevelopmental disorders.

Jasmine Lee, CB	**Poster #30**

Identifying PTK7 as a prospective CAR T-cell target for high-risk neuroblastoma

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High-risk Neuroblastoma (NB) is the most common extracranial solid tumor in pediatrics with a 5-year survival of <50%. Half of these patients will relapse after standard treatment, highlighting a need for new and effective therapeutics. We found Protein tyrosine kinase 7 (PTK7) to be consistently and highly expressed both before and after chemotherapy. PTK7 is also highly expressed on the cell surface of multiple NB cell lines and PDXs with low expression levels on normal tissue. Therefore, we developed a lentiviral vector-based, chimeric antigen receptor (CAR) construct specific to PTK7. CAR surface expression and antigen specificity were successfully determined in primary αβ and γδ T cells and the optimized scFv construct for binding PTK7 was determined. Further, anti-PTK7 CAR T cells became activated in the presence of several PTK7-positive NB cell lines. Similar activation was not observed when CAR T cells were co-cultured with a PTK7-negative cell line or NB cell line with CRISPR/Cas9 knockout (KO) of PTK7, showing specific activation of the CAR to antigen-positive cells. Importantly, anti-PTK7 CAR T cells show specific cytotoxicity against PTK7-positive NB cells, IMR5, NLF, and SKNAS, but remain non-cytotoxic against PTK7-negative cells lines, SKNAS PTK7 KO and CMK. In addition, anti-PTK7 CAR T cells showed no adverse effects when administered to mice. The extensive in vitro studies and initial in vivo studies provide a strong rationale to further pursue PTK7 as a target, and our ongoing in vivo studies will assess the therapeutic potential in NB and other PTK7-positive tumors.

Cheyenne Lee, MMG **Poster #31**

Impact of KipI and KipA proteins on *Clostridioides difficile* growth and sporulation

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Clostridioides difficile is a Gram-positive anaerobic pathogen that causes potentially fatal gastrointestinal disease, longer hospital stays, and possible recurrent infections. Efficient transmission of *C. difficile* through an aerobic environment depends on metabolically dormant spores resistant to most common sanitization methods. Sporulation in *C. difficile* and other spore-forming organisms requires phosphorylation of the master transcriptional regulator Spo0A. In *Bacillus* species, Spo0A is phosphorylated via a phosphorelay resulting in sporulation initiation. The initial point of regulation for this relay is KinA where KipI binds and prevents the initial autophosphorylation reaction that is the source of phosphate for this system. KipI negatively regulates sporulation in *Bacillus subtilis* and is antagonized by KipA. *C. difficile* has no phosphorelay as seen in Bacillus species, nor an identified Spo0A activating kinase, but contains orthologs to KipI and KipA. To characterize the effect of KipI and KipA on sporulation in *C. difficile,* we deleted both genes in 630Δ*erm* and found no change of sporulation frequency compared to wild-type. However, previous data in our lab showed that the *kipIA* operon is induced upon exposure to deoxycholate (DCA), a secondary bile acid found in the gut. Exposure of the Δ*kipIA* mutant to 0.02% DCA and chenodeoxycholate (CDCA) results in a 2.5-fold lower sporulation frequency when compared to wild-type treated the same. Exposure of *C. difficile* to DCA and CDCA both induce expression of the *kipIA* operon. These data suggest that KipI and KipA impact sporulation and growth of *C. difficile* through a currently unknown mechanism.

Jacob Mattingly, BCDB Poster #32

Macrolide antibiotics control resistance gene expression by inducing ribosomal frameshifting

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Antibiotic resistance is a 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 antibiotics is critical for the continued usefulness of these drugs in the clinic. Previous studies have demonstrated that bacteria can sense macrolides, an important class of ribosome-binding antibiotics, to induce the translation of macrolide resistance genes. This induction of resistance proceeds through programmed shifts in the messenger RNA (mRNA) reading frame of macrolide-bound bacterial ribosomes translating leader sequences upstream of resistance genes; however, the 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 macrolide resistance and how the bacterial ribosome can sense cellular chemical conditions. Primer extension inhibition (toeprinting) assays demonstrate the ability of macrolide-bound ribosomes to frameshift, 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 uncover the molecular mechanism of macrolide-induced frameshifting. Accompanying ribosomal RNA (rRNA) mutant selection studies will reveal rRNA regions important for macrolide-induced frameshifting, and single-molecule Förster resonance energy transfer (smFRET) experiments will demonstrate the kinetic effects of macrolides on the translation of frameshift-prone mRNAs. The new understandings generated through this study will inform the design of new macrolides and reveal how macrolide antibiotics modulate ribosomal activity to perturb mRNA reading frame maintenance.

Tommy O'Haren, GMB Poster #33

Developmental regulation of histone genes by *Drosophila* pioneer factor Zelda

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Animal embryos are maternally loaded with proteins and RNAs that control early development. However, the zygotic genome must activate and begin transcribing necessary genes to assist in early cellular divisions, a process known as zygotic genome activation (ZGA). The initial organization and transcriptional activation of the zygotic genome is facilitated by pioneering transcription factors that are able to open regions of nucleosome-bound DNA. The *Drosophila* maternally deposited, pioneer factor Zelda is the "master regulator" of ZGA and binds across the genome to "TAGteam" sites, opening chromatin and marking surrounding regions for activation and transcription. The five canonical histone genes are amongst the first zygotic genes activated during development and cluster in animal genomes forming histone loci. A suite of factors known as the Histone Locus Body (HLB) act upon histone loci and regulate histone expression. The genome of *Drosophila melanogaster* carries a single histone locus of 107 tandem copies of the histone gene array. We discovered that Zelda targets the embryonic histone locus around nuclear cycle 8. A histone array transgene in which TAGteam sites are ablated is unable to fully recruit HLB factors suggesting that Zelda is necessary for HLB formation in the early embryo and zygotic histone gene expression. In the future, we will restore Zelda to the transgenic histone array using dCas9 and assay rescue of HLB formation. Overall, our results indicate that one indirect mechanism through which pioneer factors remodel the zygotic genome is through zygotic histone gene expression.

Alejandro Oviedo, BCDB Poster #34

Non-coding RNA 866 (nc886) structure and mechanism of oligoadenylate synthetase 1 (OAS1) activation

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The innate immune system is a critical line of cellular defense against pathogens, comprising a diverse set of pattern recognition receptors (PRRs) that are responsible for sensing specific pathogen-associated molecular patterns (PAMPs). Cytosolic viral double-stranded RNA (dsRNA) is a potent PAMP and its accumulation is an indication of viral infection that leads to downstream antiviral responses once detected by PRRs. Evidence also suggests that cellular RNAs may be involved in regulating cytosolic RNA PRRs, but how they accomplish this remains a major knowledge gap. Human cellular non-coding RNA 886 (nc886) is a 101-nucleotide structured RNA that was first found to inhibit the activity of the dsRNA-activated protein kinase (PKR). Our lab later determined that nc886 not only inhibits PKR activity, but also potently activates the 2',5'-oligoadenylate synthetase 1 (OAS1)/RNase L pathway. nc886 forms two distinct structural conformers that can be natively separated and possess starkly different activities against PKR and OAS1. In particular, only one RNA conformer contains a unique tertiary structure in its apical stem-loop (AS) region that I hypothesize confers these activities against PKR and OAS1. My project will focus on: 1) identifying the RNA sequence(s) necessary for forming the unique RNA tertiary structure, 2) defining the OAS1 binding site(s) on nc886, and 3) determining the first high-resolution structure of the OAS1-nc886 complex. This work will provide new insights into the mechanism of OAS1 regulation by a cellular non-coding RNA via a unique RNA tertiary structure motif.

Jordan Owyoung, GMB Poster #35

The role of the sympathetic nervous system in improving skeletal muscle quality with exercise

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Myopathies are neuromuscular diseases that affect the muscle and result in muscle weakness from dysfunctional muscle fibers. They affect hundreds of thousands of people a year and can cost millions of dollars per patient. Exercise is currently the only therapeutic that partially restores motor function in patients with myopathies; however, how exercise improves locomotive function remains largely unknown. Research shows that exercise influences sympathetic nervous system (SNS) activity. β2-adrenergic receptors (B2ARs) are the main receptors of the SNS and play a role in muscle protein synthesis. Furthermore, aerobic exercise has been shown to increase B2AR (*Adrb2*) expression. I hypothesize that aerobic exercise modulates skeletal muscle metabolic health and function via sympathetic nervous system signaling. I will test my hypothesis in two ways. First, I will evaluate whether exercise can improve skeletal muscle *function* in mice with an ablated sympathetic system and in mice with a tamoxifen-induced homozygous knockout of *Adrb2* in skeletal muscles. For each of the treatments, I will exercise a subset of mice. I hypothesize that a functional SNS is required for exercise to improve skeletal muscle function and therefore expect that exercise will not improve skeletal muscle function in either the sympathectomized or $Adrb2^{-/-}$ mice. Next, I will evaluate whether B2ARs are required for exercise to influence skeletal muscle *health*. I will use my $Adrb2^{-/-}$ mice and exercise a subset. Because B2ARs are essential for SNS signaling in the skeletal muscle, I hypothesize that exercise will not improve skeletal muscle health in $Adrb2^{-/-}$ mice.

Rebecca Parker, CB **Poster #36**

Defining the role of PER1 and circadian rhythm dysregulation in KRAS/LKB1-mutant lung adenocarcinoma

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Lung adenocarcinoma, the most common histological subtype of lung cancer, frequently exhibits mutations in both KRAS and LKB1. To study the unique biology of KL lung adenocarcinoma, we have engineered an isogenic panel of KRAS-mutant (K), LKB1-null (L), and KRAS/LKB1 (KL) human bronchial epithelial cells (HBECs) that recapitulate clinical phenotypes. Cross-referencing gene expression data from these HBECs with patient data from The Cancer Genome Atlas, we found that transcripts of PER1—a core component of the circadian clock that has been reported as a tumor suppressor in lung cancer—are increased in KL HBECs and tumors as compared to K samples. At the protein level, expression of PER1 is lower in KL than in K HBECs. Because PER1 functions in the nucleus to prevent its own transcription, these data are consistent with the hypothesis that LKB1 loss in lung cancer results in a time-shifted circadian period. To understand the functional effects of PER1 on lung cancer, we turned to the H1299 cell line model of collective invasion, where distinct populations of highly invasive leader cells and highly proliferative follower cells cooperate to invade and metastasize. In RNAseq data from these cells, which express LKB1, PER1 expression is higher in leader than in follower cells. In 3D invasion assays, siRNA knockdown of PER1 drastically decreases invasion in leader cells, with much less of an effect on invasion in followers. Future studies will describe nuclear and cytoplasmic localization and temporal expression of PER1 in K versus KL and leader versus follower cells.

Kiran Parwani, CB Poster #37

Histone H3 threonine 45 phosphorylation contributes to the radiation-induced DNA damage response in glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and fatal adult brain tumour. While the standard of care includes surgery and DNA damaging agents such as radiation and/or chemotherapy, radioresistance can be attributed to the DNA damage repair response. This resistance mechanism is governed by chromatin post-translational modifications (PTMs). Some PTMs are regulated by the PI3K/AKT pathway, which is hyperactive in around 90% of GBM tumours. Previous research demonstrated that the PI3K effector AKT phosphorylates histone H3 at threonine 45 (pH3T45) in response to DNA damage. We hypothesize that PI3K/AKT-driven pH3T45 enhances GBM radioresistance by augmenting the DNA damage response. To genetically dissect this hypothesis, we engineered pH3T45 wild-type and phospho-null mutants in a PI3K-activated GBM cell line. Our preliminary data demonstrates that pH3T45 status informs that of H3K36me3, a histone H3 PTM involved in the DNA double strand break repair pathways non-homologous end joining (NHEJ) and homologous recombination (HR). By surveying irradiation-induced 53BP1 foci formation, we demonstrate that loss of pH3T45 compromises irradiation-induced damage repair through NHEJ. We find that the presence of pH3T45 increases damage signalling; loss of H3T45 phosphorylation delays the onset of repair signalling. Supplementing these findings, we show that loss of pH3T45 impairs damage resolution through comet assays, translating to a reduction in GBM cell clonogenicity. Taken together, we demonstrate that pH3T45 functions in damage repair and anticipate that further pH3T45 characterization will inform both our understanding of GBM radioresistance as well as suggest novel therapeutic strategies.

Megan Phillips, PBEE Poster #38

Plasmid-mediated tetracycline resistance in *Staphylococcus aureus* predates clinical tetracycline use

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The pT181 plasmid is responsible for conferring tetracycline resistance in human bacterial pathogen *Staphylococcus aureus*. Recent research on methicillin-resistant *S. aureus* has established that resistance emerged in the pre-antibiotic era and that the prevalence of antibiotic resistances in *S. aureus* has been driven by use of other antibiotics. For example, methicillin resistance increased in prevalence due to widespread use of penicillin, which predates clinical use of methicillin. The availability of bacterial genome analysis tools such as Bactopia and many high-quality *S. aureus* sequences facilitates further study of the pT181 plasmid across decades of samples. Our analysis of 132 publicly available, dated samples of pT181 suggests the existence of a common ancestral plasmid that predates the advent of clinical tetracycline use. The pT181 plasmid underwent an unusual burst of sequence evolution near the root of the phylogenetic tree before diversifying over time as expected. Early diversification of the plasmid occurred in the 1950s and 1960s with significant polytomies emerging in the 1980s. The dated phylogeny recapitulated the early detection of the ancestral type I SCCmec cluster, with later emergence of other types. Further study into the timeline and pattern of sequence evolution in the pT181 plasmid offers a greater understanding of plasmid evolution on a population level, which has important implications for tracking and managing antibiotic resistance. Improved knowledge of how these traits disseminate through a bacterial population may help scientists, health care professionals, and agricultural workers better contain the spread of these infections in humans and livestock.

Tana Pottorf, NS	Poster #39

TREM2 Regulation in Spinal Cord Microglia following Peripheral Nerve Injury

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Following peripheral nerve injury (PNI) spinal cord microglia exhibit a gradient of phenotypic morphologies as they interact with various injured motoneurons (MNs). Within the first week after a PNI, microglia will proliferate, migrate, and extend large processes toward injured MNs. Microglia will then adhere to and scan the MN surface with dynamic filopodia. Many microglia will remain in this “sampling” state as they associate with regenerating neurons, whereas microglia associated with presumed dying MNs transform into an ameboid-like state with minimal processes, and form a unified cluster of microglia, denoted as a “death cluster.” The differentiating signals between regenerating and dying MNs that microglia respond to in order to change morphology and potential functionality are currently unknown. Our evidence suggests that triggering receptor expressed on myeloid cells 2 (TREM2) is differentially regulated in microglia that associate with MNs that have the potential to regenerate compared to microglia involved in death clusters around dying MNs. The upregulation of TREM2 in death clusters is correlated with an increase in phagocytic marker, CD68. Our results provide insights into the signaling cascades involved in different microglia morphology following PNI and suggest altered functionality towards individual injured MNs dependent on MN health.

Vishnu Raghuram, MMG Poster #40

Detecting within-host diversity of Staphylococcus aureus during colonization

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As pathogenic bacteria go through cycles of growth and adaptation within a host, the genetic makeup of the initial population may be altered with time. A straightforward approach to tracking a microbial population within an infected host would be to isolate and characterize a single colony obtained from a clinical sample. However, this method may not capture the complete genetic diversity in the total population. As an alternative, the pool of colonies obtained from a sample can be investigated but this bears the disadvantage of having a non-homogeneous sample, making it difficult to perform specific experiments. To compare the genetic diversity detected between single colonies and pooled colonies, we periodically sampled specific body-sites of patients presenting with *Staphylococcus aureus* skin and soft-tissue infection (SSTI). *S. aureus* is a ubiquitous nosocomial pathogen and one of the leading causes of healthcare associated infections in the US. We found that in most cases, our *S. aureus* skin isolates do not stem from multiple clonal lineages, and the genetic diversity observed within a patient is relatively low, suggesting single strain origins. We also found that the combined diversity of 8 single colonies is usually sufficient to capture the complete diversity in the total population. Further analysis for detecting antibiotic resistance genes, mutation rates, Multi Locus Sequence Types, and quorum sensing groups between single colonies and pooled colonies will be performed. Due to robust sampling of multiple body-sites across different timepoints, this study can provide several insights into tracking a within-host microbial population over time.

Monica Reece, MMG **Poster #41**

Rufinamide-induced autophagy cell death in HIV-1-infected macrophages

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No approved HIV-1 therapeutics prevent or target the viral reservoir, the major barrier to HIV cure. The myeloid reservoir, targeted with poor penetration and efficacy by antiretroviral therapy (ART), is a major source of immune dysregulation in chronic infection contributing to inflammation-induced co-morbidities. The myeloid reservoir is long-lived and actively produces virus while forgoing cell death. Macrophages have innately higher levels of SAMHD1, degrading dNTPs, thus inhibiting reverse transcription and resulting in slower replication kinetics and survival. We have identified an FDA-approved drug, rufinamide, with the ability to increase replication kinetics and induce cell death specifically in HIV-1-infected macrophages.

2700 compounds (FDA approved/phase3) were screened for accelerated viral replication kinetics in infected macrophages using a D3HIV-GFP vector which expresses GFP in late stage replication.

The mechanism of rufinamide was found to be independent of SAMHD1 through immunoblot studies. Rufinamide increases replication kinetics and induces cell death only in infected macrophages. Vpr was examined due to its known role in toxicity and cell death using a Vpr deletant D3HIV-GFP vector. The Vpr deletant replication kinetics were not affected by rufinamide, indicating that Vpr plays a role in the cell death phenotype induced by rufinamide. Autophagosomes were observed via EM in rufinamide-treated D3HIV-GFP transduced primary macrophages but not untreated transduced cells, evidencing autophagy as the cell death mechanism.

Rufinamide induces cell death specific to HIV-1 infected macrophages, independent of SAMHD1. We are investigating the cell death pathway induced by rufinamide with preliminary data indicating autophagy and mechanistic involvement of HIV-1 protein Vpr.

Kelsey Robinson, GMB Poster #42

Family-based GWAS of cleft palate reveals novel associations and subtype-specific effects

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Cleft palate (CP) occurs in approximately 1 in 1700 live births annually and accounts for ~30% of all orofacial clefts (OFCs). As compared to OFCs involving the upper lip, for which there are dozens of known genetic risk loci, only a few risk loci have been identified for CP. Historically, these studies have evaluated CP as a whole, rather than by its heterogenous subtypes that include overt clefts of the hard and/or soft palate, or submucous cleft palate. Here, we performed genome-wide association studies in 429 case-parent trios with various types of CP. No loci met genome-wide significance ($p<5x10^{-8}$), but there were several that reached suggestive significance ($p<1x10^{-5}$). The top locus, near *HOMER1* has no previous association with CP, but *Homer1* is expressed in developing craniofacial tissue, especially within the palatal rugae. When stratified into groups by the affected structure (hard palate or soft palate) additional loci reached suggestive significance thresholds, indicating possible differences in underlying genetic risk factors. A peak on 9q33.3 was the top hit for CP affecting the hard palate; a microdeletion syndrome of these region often includes a CP. A locus near *DACT2* emerged from the GWAS of CP affecting the soft palate; Dact2 interacts with the Wnt signaling pathway, which is essential for normal palatogenesis. This is the first GWAS to evaluate CP in the context of subtype-specific genetic risks, and results support the potential of genetic heterogeneity driving different phenotypes.

Eliseo Salas, GMB **Poster #43**

Elucidating the structural effects of bacterial collided ribosomes on translational fidelity

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Traditionally, protein synthesis or translation has been studied through the lens of a single ribosome translating a single mRNA. However, multiple ribosomes (called polysomes) can translate a single mRNA *in vivo* and the interactions between adjacent ribosomes is now realized to be an important cellular signal. Stalling of ribosomes can occur during translation elongation resulting in collisions between stalled and actively translating ribosomes. The unique interface that forms between two collided ribosomes acts as signal for regulatory pathways. Ribosomal protein bL9 ("b" refers to bacterial) has been implicated as a mediator of accuracy in protein synthesis despite being located distant from where tRNA selection occurs. Recent structures of colliding ribosomes reveal that bL9 can adopt two different positions: either an extended form that directly contacts an upstream adjacent collided ribosome or in a compact form in which bL9 only interacts within its own ribosome. Biochemical and genetic evidence suggests that bL9 may be a sensor for a lack of mRNA reading frame fidelity which may account for the differences in its position on the ribosome. To determine the interactions between bL9 and adjacent collided ribosomes, we generated an *E. coli* colliding ribosome system that undergoes a stalling event. This resulted in four collided ribosomes (tetrasomes) translating on a single mRNA which we visualized using negative stain electron microscopy (EM). Future studies will focus on comparing these tetrasomes to ribosomes lacking bL9 and identifying the connection between colliding ribosomes, processivity errors like mRNA frameshifting, and the influence of antibiotics.

Juliet Santiago, NS **Poster #44**

Identification of state-specific proteomic characteristics of microglia-derived exosomes

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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 exosome release because of their role in the transport of macromolecules between cells to facilitate intercellular communication. Thus, it is possible that microglia-derived exosomes transfer pathogenic cargo which could perpetuate AD pathology. The proteomic profiles and influence of different microglia-derived exosomal populations on AD pathology remain unknown. We hypothesize that different microglia states determine the molecular composition of exosomes. We treated three groups (n=4/group) of a murine microglia cell line, BV2 cells, with either lipopolysaccharide, interleukin 10, or transforming growth factor beta to polarize to a pro-inflammatory, anti-inflammatory, or homeostatic state, respectively. Following 72 hours of treatment, BV2 cells were lysed and cell culture media was collected for exosome isolation and downstream mass spectrometry (MS) analyses. Transmission electron microscopy images and western blotting for exosomal marker, CD9, confirmed exosome purification by our isolation method. In MS studies, we identified 533 proteins in exosome fractions and 1,866 proteins in BV2 cell proteomes. We found that exosome related proteins, Sdcbp and Igsf8, were significantly increased in the exosomal proteome compared to the cell proteome. We identified proteins that are differentially expressed across polarization. Our results indicate that exosomes derived from microglia adopt distinct state-associated protein profiles which may have differential effects on other cell types.

Colby Schweibenz, BCDB Poster #45

Elucidating the mechanism of coactivator Taiman/AIB1-driven cell competition and its relation to the Adenomatous Polyposis Coli (APC) tumor suppressor in *Drosophila*

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The phenomenon of cell competition ensures that the fittest cells populate developing primordia but is also postulated to underlie “field cancerization,” in which cancer cells expressing ‘super-competitor’ genes eliminate slow growing neighbors and take over epithelial tissue. Our prior work demonstrated that cells overexpressing the *Drosophila* protein Taiman (Tai; human NCOA3/AIB1), a transcriptional co-activator of the Ecdysone receptor (EcR), can kill wildtype neighbors within the larval wing epithelium (Byun et al, 2019). Here we use the wing disc to test and confirm the reciprocal hypothesis: cells with reduced Tai expression (Tai^{low}) are competitive ‘losers.’ We used a genetic screen to identify mechanisms required for elimination of these cells by wildtype neighbors. This screen recovered ‘hits’ in the pro-apoptotic genes, *head involution defective*, *reaper,* and *grim*, as dominant suppressors Tai^{low} cell loss, confirming a competitive mechanism that operates through conserved caspase apoptosis. We also recovered alleles of factors involved in cell signaling pathways that could act downstream of Tai’s role in controlling competitive fate. Our current studies focus on the two *Drosophila* Adenomatous polyposis coli (APC) tumor suppressor homologs, Apc1 and Apc2, which are conserved elements of the Wg/Wnt pathway and inhibit field cancerization in the fly midgut (Suijkerbuijk, 2016). We find that Apc1/Apc2 loss rescues elimination of Tai^{low} cells in both eye and wing epithelia, and couple this with evidence that Tai controls Wg-target genes in larval wing cells. Through this research, we aim to define how the Tai/EcR and Wg/Apc pathways intersect to determine winner/loser status in *Drosophila* epithelia.

Debresha Shelton, GMB Poster #46

Subretinal expression of Galectin-3 after light-induced retinal damage

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Purpose: Galectin-3 (Gal-3), is a β-galactoside-binding protein purported to be a prognostic biomarker for severe disease (e.g., heart failure or depression). We hypothesized that manipulation of the Vitamin A cycle during light-induced retinal damage (LIRD) could reveal whether increased Gal-3 expression predicts microglia/macrophage deposition patterns within the RPE.

Methods: High activity Rpe65 (L450 ($^{L/L}$)) and the low activity variant (M450 ($^{M/M}$)) were bred to Cx3CR1-GFP-mice on C57Bl/6J background. Light damage was induced with 50,000 lux for 5 hours. Mice were imaged and sacrificed at days 1, 3, & 7 after damage (age= P60-P365, n=3-4/group). Retinal thickness and morphology were measured. Tissue was collected for RPE flatmounts. GFP+ (microglia/macrophages) and Gal-3+ cells were counted and classified.

Results: There were distinct differences in functional and morphological outcomes after damage between RPE 65 $^{M/M}$ and RPE65$^{L/L}$ mice. At day 1 & 3 post, GFP^{+} cells were not significantly different between genotypes. At day 7, more GFP^{+} cells were observed in the RPE 65 $^{L/L}$ mice compared to RPE65 $^{M/M}$ and no damage controls ($p<0.0001$). Notably, the RPE65$^{L/L}$ mice had significant decreases in ERG amplitudes post LIRD compared to RPE65$^{M/M}$ mice ($p<0.005$). Gal-3 expression in both genotypes was comparable during early stages of damage; however, at day 7, only RPE65$^{L/L}$ animals continued to express Gal-3 heterogeneously in both RPE cells and CX3CR1-GFP^{+} cells. GFP^{+} cell distribution was correlated with Gal-3 expression patterns in RPE cells.
Conclusion: Changes in subretinal expression of Gal-3 correlate to CX3CR1-GFP^{+} cell deposition, corresponding to functional and morphological damage.

Fu Hung Shiu, NS **Poster #47**

Lack of full-length Adgrb1 leads to altered brain development

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The adhesion G protein-coupled receptor BAI1/ADGRB1 interacts with several autism-relevant proteins. Exome sequencing and case report studies have also identified *de novo* and inherent *ADGRB1* variants in patients with autism spectrum disorder (ASD). Previously, we demonstrated that *Adgrb1*$^{-/-}$ mice exhibited impairments in social behavior and increased seizure susceptibility. As Adgrb1 has the highest expression at postnatal 7 (P7) in rodents, we compared hippocampal morphology in P7 and adult *Adgrb1*$^{-/-}$ mice and age-matched WT littermates. We observed increased GFAP immunofluorescence (astrocyte marker) and a higher number of CC3+ cells (apoptosis marker) in P7 *Adgrb1*$^{-/-}$ mice. No differences in GFAP or CC3+ were observed in adult mice. Furthermore, we observed no difference between genotypes in IBA1 immunofluorescence (microglia marker) level at both ages. Since ADGRB1 is an engulfment receptor that is highly expressed in astrocytes but not microglia, we next examined the phagocytosis efficiency of *Adgrb1*$^{-/-}$ astrocytes. We cultured astrocytes from P3-5 *Adgrb1*$^{-/-}$ mice and WT littermates and measured phagocytic capacity. *Adgrb1*$^{-/-}$ astrocytes had less phagocytic capacity compared to WT astrocytes. Reduced phagocytosis can lead to increased uncleared apoptotic cells and could potentially contribute to the observed increase in seizure susceptibility and autistic-like behaviors in *Adgrb1*$^{-/-}$ mutants.

Taylor Shue, MMG **Poster #48**

Inhibition studies of FDA approved COVID-19 drugs against common cold human coronaviruses

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Human coronaviruses are positive-sense, single-stranded RNA viruses that can cause severe disease in humans. Discovery of multiple highly pathogenic coronaviruses within two decades (SARS-CoV-1, MERS-CoV, and SARS-CoV-2) highlights the pandemic potential of this family and need for pan-coronavirus antiviral drugs. Currently all FDA approved antivirals against SARS-CoV-2 (Remdesivir, Paxlovid, and Molnupiravir) act on the highly conserved ORF1ab region of the SARS-CoV-2 genome making them good candidates for inhibition of other human coronaviruses. To study this, we use human coronavirus 229E (HCoV-229E), an endemic coronavirus, which is a good platform for future drug combination and resistance studies. Our goal is to generate inhibitory profiles of these drugs on HCoV-229E to evaluate them as pan-coronavirus antiviral candidates and to have a baseline for further characterization of these drugs. To find the EC_{50} values, HuH7.5 cells were treated with serial dilutions of each antiviral. One hour after treatment, the cells were infected with HCoV-229E. Cells were fixed one day post infection. We used immunofluorescence to stain for cell nuclei and viral dsRNA as a marker of viral infection and determined the EC_{50} values. Remdesivir, Nirmatrelvir (a Paxlovid component), and Molnupiravir (active and pro-drug formula) inhibited HCoV-229E in HuH7.5 cells with EC_{50} values of 8.3 nM, 340.4 nM, 5.4uM, and 8.9 uM respectively. The EC_{50} values from this study will be used as a baseline value for determining the behavior of these drugs in combination and the potential for antiviral resistance via serial passaging followed by introduction of resistance mutations into the SARS-CoV-2 replicons.

Bri Silver, GMB	**Poster #49**

Optimization of a protocol for combining isolation of nuclei tagged in specific cell types (INTACT) with single nucleus RNA-seq analysis

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Arabidopsis thaliana has dozens of unique and specialized cell types, each of which carries out specific functions. These functions are the outcome of each cell type's particular epigenetic programming and the resulting transcriptomes and proteomes. Even within a specific cell type, individual cells may have slightly differing transcriptomes which can be indicative of a yet to be discovered cellular subtype, a particular phase of cellular differentiation, or response to the environment. The system employed here allows for single nucleus level analysis of plant tissues by utilizing a method known as isolation of nuclei tagged in specific cell types (INTACT). This method allows for the selection of nuclei of a specific cell type, which can then be put into a single cell RNA or ATAC-seq platform. Here, INTACT will be paired with single combinatorial indexing-single nucleus-RNA-seq (sci-sn-RNA-seq) from Parse Biosciences to investigate at high resolution of the makeup of the shoot apical meristem. This will allow for identification of cellular subpopulations and insight into differentiation trajectories by way of pseudotime analysis. Ultimately, this will help further resolve spatiotemporal development of the shoot apical meristem and will provide a general method for deep single cell analysis starting with a defined subpopulation of cells.

Julia Tanquary, BCDB Poster #50

Endogenous Activation of bacterial toxins and identification of their mRNA targets

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Toxin-antitoxins are gene pairs located on mobile genetic elements and chromosomes in most bacteria. These gene pairs have diverse functions including involvement in plasmid maintenance, abortive infection, maintenance of CRISPR loci, and antibiotic persistence. However, it is unclear how toxin-antitoxin complexes contribute to these processes as it is difficult to individually activate toxins. Previous studies exogenously overexpress toxins to identify their molecular targets resulting in cell death whereas their endogenous roles are beneficial. Bacterial toxins are activated by cellular stressors that, in turn, activate many other cellular pathways, complicating the identification of the precise effects of toxin activation. In *E. coli,* most identified toxin-antitoxins are type II whereby both the toxin and the antitoxin are proteins and the antitoxin physically binds the toxin to inhibit its toxicity. These toxins are endoribonucleases (RNases) that halt growth by targeting cellular RNAs. A large class specifically targets mRNAs actively being translated by the ribosome. To identify the mRNA targets of chromosomally-encoded type II toxin YafQ, I engineered an *E. coli* strain containing a proteolysis tag (degron) on DinJ, the antitoxin that normally inhibits YafQ. Activation of the degron, causes DinJ proteolysis and the release of YafQ. Preliminary findings reveal that activating YafQ results in bacteriostatic growth, consistent with the proposed beneficial role of toxin activation in bacterial stress responses. Future studies include identifying the protective role of YafQ from diverse stressors and a mechanistic understanding of how the inhibition of growth is achieved via the specific targeting of mRNAs.

Tina Tian, NS	**Poster #51**

Neuronal activity-based therapies fail to enhance sympathetic axon regeneration and functional recovery

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Axonal injuries are common and result in loss of motor, sensory, and autonomic functions that lead to lifelong disabilities. Enhancing axon regeneration is an important goal in the development of novel therapeutics to complement surgical repair of peripheral nerves. However, **the regenerative capacity of post-ganglionic sympathetic axons and their functional recovery has rarely been studied**. Sympathetic innervation plays a major role in muscle strength and thermoregulation. Using a conditioning lesion paradigm, electrical stimulation, and bioluminescent optogenetics approaches, *I studied the regenerative capacity of sympathetic axons in the sciatic nerve after injury.* I additionally investigated sympathetically-mediated functional recovery at the neuromuscular junction and sweat glands by measuring mitochondrial content and sweat production, respectively, after surgical resection of lumbar sympathetic ganglia.

My results suggest that the elongation of sympathetic axons is not enhanced with a conditioning lesion or electrical stimulation. Additionally, selective activation of sympathetic axons with bioluminescence decreases the number of sympathetic axons that have reached 5 mm of growth from the injury site 2 weeks after transection and repair of the sciatic nerve. Thus, activity-based therapies neither enhance sympathetic axonal elongation nor increase the number of sympathetic neurons participating in regeneration.

My preliminary data indicate that neuronal activity-based therapies that have previously been shown to enhance regeneration of motor and sensory axons may be detrimental to sympathetic axon regrowth. This data will have implications for activity-based therapeutic methods, such as electrical stimulation which has reached clinical populations, that can potentially be used to complement nerve repair surgeries.

Kedamawit Tilahun, GMB Poster #52

Antisense repeat RNAs contributes to ALS and FTD caused by C9ORF72 repeat expansion

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are two devastating neurodegenerative disorders arising in a sporadic or familial manner. ALS involves death of motor neurons that lead to muscle twitching, loss, and eventual paralysis whereas FTD is characterized by language dysfunction and memory loss due to neuronal degeneration in the frontotemporal lobe. The most common genetic mutation found in ALS/FTD is a GGGGCC (G4C2) hexanucleotide repeat expansion in the 1st intron of the C9orf72 gene (C9). Healthy individuals carry between 2-30 repeats whereas C9ALS/FTD patients can have 100s-1000s of G4C2 repeats. These expanded repeats are bidirectionally transcribed to produce both sense G4C2 and antisense CCCCGG (C4G2) repeat-containing RNAs. Previous studies demonstrated that intronic repeat-containing RNAs play a central role in disease pathogenesis through RNA-mediated toxicity (by repeat expanded RNA themselves or sequestering RNA binding proteins into RNA foci) or protein-mediated toxicity from dipeptide repeat proteins produced by a non-canonical translation mechanism. The role of antisense C4G2 RNAs in C9ALS/FTD diseases remains to be elucidated. Here we show antisense C4G2 RNA repeats activate stress response pathway via eukaryotic translation initiation factor 2A (eif2α) and induce neural toxicity. Antisense RNA repeats promote (eif2α) phosphorylation via upstream kinase activation, which leads to an inhibition of global protein translation and induction of stress granule formation. Furthermore, the antisense RNA themselves, independent of dipeptide repeat proteins, are sufficient to activate the eif2α pathway. Our results support that antisense C9 C4G2 RNA contribute to ALS/FTD disease pathogenesis and can be targets of therapeutic intervention.

Germàn Vargas-Cuebas, MMG Poster #53

LL-37 activation of glycine catabolism promotes *Clostridioides difficile* pathogenesis

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Clostridioides difficile (*C. difficile)* is the leading cause of antibiotic-associated diarrhea. This human pathogen can ferment amino acids to generate energy through Stickland reactions, in which a donor amino acid is oxidized while an acceptor amino acid is reduced. The glycine Stickland pathway is conserved among *C. difficile* strains, and represents one of the preferred acceptor amino acids in Stickland reactions. We have observed changes in *C. difficile* glycine Stickland transcription in response to the host peptide, LL-37. Even though it is known that Stickland metabolism is important for *C. difficile* growth *in vitro*, how this metabolism impacts pathogenesis and how it is regulated remains largely uncharacterized. We hypothesized that *C. difficile* colonization and pathogenesis is increased by glycine Stickland metabolism, and that this metabolic pathway is modulated in response to LL-37. To test this hypothesis, we generated a Δ*grdAB* mutant and analyzed its growth in minimal media. Our data indicate that the amino acid glycine enhanced *C. difficile* growth through the glycine Stickland pathway. We also found that the ability to catabolize glycine impacts the amount of toxin produced in minimal media. Using transcriptional reporter assays we showed that expression of the *grd* operon is enhanced by LL-37. We assessed transcription of the *grd* operon and investigated direct binding of the LL-37 regulator, ClnR, to the *grdX* promoter. Finally, we observed that the inability to catabolize glycine reduces *C. difficile* virulence in a hamster model of disease.

Alexandria White, NS Poster #54

Oral insecticide exposure induces gastrointestinal dysfunction and alters nigrostriatal dopamine signaling in adult mice

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Pesticide exposure is a risk factor for Parkinson's disease (PD). PD is characterized by the gradual death of the dopamine (DA) neurons in the midbrain, leading to loss of motor function. People with PD experience gastrointestinal (GI) dysfunction, and growing evidence suggests a contributory role for the gut microbiome in PD. Emerging associations between PD, pesticide exposure, and GI abnormalities suggest that some cases of PD might be triggered from within the gut after pesticide exposure. Pyrethroids are commonly used insecticides known to act on the DA system, and ingestion is the most common route of pyrethroid exposure. We therefore predicted that low-dose oral exposure to the pyrethroid deltamethrin induces GI dysfunction and disrupts nigrostriatal DA circuitry in mice. Adult wildtype male and female mice were orally gavaged weekly with deltamethrin or a corn oil vehicle control using subchronic and chronic exposure paradigms. GI function was assessed by measuring fecal output and intestinal transit time, and the composition of the gut microbiome was determined by 16S rRNA sequencing. PD-associated motor behaviors were assessed using a panel of motor function assays. PD-relevant gene and protein expression were quantified in gut and brain tissues via qPCR and western blot. Our data suggest that low-dose oral pyrethroid exposure induces PD-relevant pathology and behaviors in the gut and brain of adult mice. Future studies will identify specific roles for the gut microbiome and possible routes of transmission along the gut-brain axis that are responsible for facilitating pyrethroid-induced neurotoxicity.

Keenan Wiggins, GMB Poster #55

Memory B cells are a heterogenous population regulated by epigenetic programming

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Memory B cells (MBC) are a heterogenous class of immune cells that belong to the adaptive immune response. During secondary infections, these surveilling cells amount a robust secondary response to protect from diseases. It is known that MBC can form throughout an immune response and originate from germinal centers or non-germinal center processes. Understanding how distinct MBC subsets are programmed for development and function is important for better vaccine and MBC therapies. Epigenetic mechanisms play an important role in the development of B cell subsets. EZH2, a histone methyltransferase, deposits methyl groups on histone 3 to repression transcription. EZH2 is a known regulator of B cell development by gene repression, but it is unknown how EZH2 regulates MBC development. To address this, EZH2 was conditionally knocked out of B cells, and the influenza model was used to study MBC development through a live time-course of disease progression. MBC were isolated from the spleen, draining lymph node, and lungs for flow cytometry phenotyping. Tetramers were used to tag antigen specific MBC in the different tissues. Our results showed the MBC response occurred as early as day 7 post infection and the early class switched MBC timepoint appeared by day 14 post infection. In the spleens, there were reductions in germinal centers B cells and class switched MBC from the EZH2 KO mice. Taken together, these results give early insights into MBC development and the role of EZH2 on MBC programming.

Courtney Willett, GMB Poster #56

Differences in transcriptional directionality underlie epigenome differences between plants and animals

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Transcriptional regulation is a universal mechanism to regulate a wide array of biological processes. High-throughput profiling of animal genomes has revealed that RNA Polymerase II appears to initiate transcription in both directions at gene transcription start sites (TSSs), indiscriminate of the position of the gene body flanking the TSS. However, by analyzing histone posttranslational modifications (PTMs) indicative of active transcription, we observe a lack of divergent transcription from TSSs in *Arabidopsis thaliana*, in stark contrast to *Drosophila melanogaster* and *Homo sapiens*. While the four histone PTMs we analyzed are present at TSSs in all three of the species investigated, *A. thaliana* showed a marked depletion of these modifications upstream of the TSS, whereas the animal species exhibit bimodal enrichment. This phenomenon led us to investigate whether these patterns are also observed at cis-regulatory elements, specifically enhancers. In this study, we compare the enrichment of the four histone PTMs associated with enhancers between plant and animal genomes. Regions of intergenic hyperaccessible chromatin were identified through ATAC-seq or DNase-seq and were analyzed as enhancer regions. Through the intersection of these data, it becomes clear that there are distinctions between the epigenetic makeup of plant and animal genomes. While it is known that animal promoters and enhancers have bidirectional transcription, this analysis revealed that plant promoters and enhancers preferentially exhibit histone PTM deposition and transcription only in the sense direction. Overall, the data speak to a fundamental difference in transcriptional initiation in the plant and animal kingdoms that has yet to be elucidated.