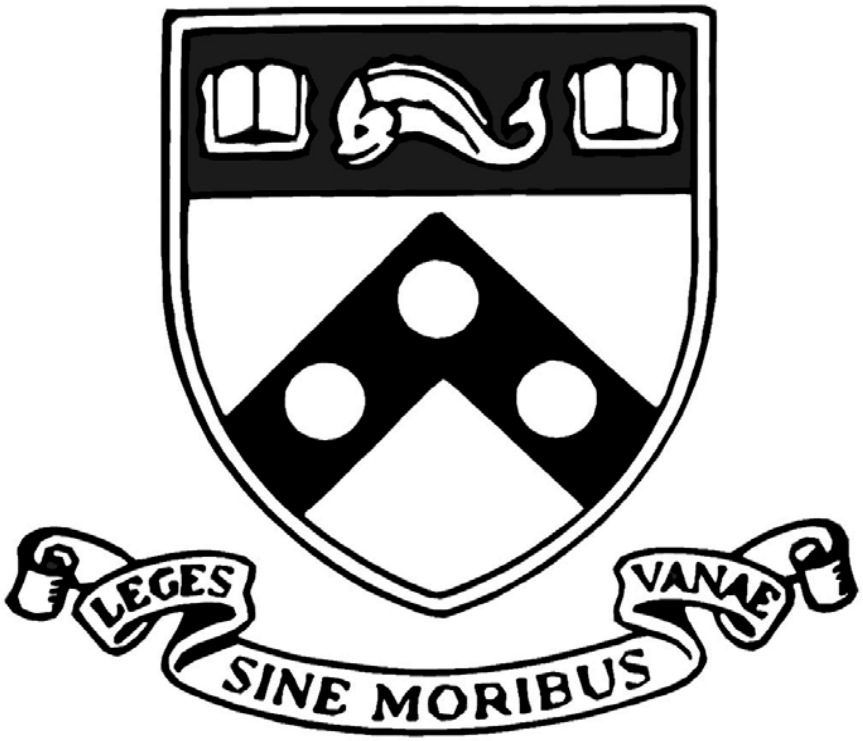
Perelman School of Medicine
at the University of Pennsylvania

Combined Degree Program Annual Retreat



August 4, 2023
Villanova University
Villanova, Pennsylvania

The Combined Degree and Physician Scholar Programs Administration

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Hope Charney, MA	Administrative Coordinator
David Bittner, MA	Coordinator, MD-PhD Program
Anastasia Brown	Coordinator, VMD-PhD Program

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Welcome to the Retreat – from the MD-PhD Program Director

Welcome to the annual Penn MSTP retreat for 2023. Whether you are a current student, a recently arrived first year student, a future student, a long-time-affiliated Penn faculty member or a faculty member joining us for the first time, we are delighted to have you here. This coming year there will be 226 MD/PhD students on campus but if you are one of the 30 new MD/PhD students and 3 VMD/PhD students starting today, know that you are especially welcome. Take the time to start to know as many people as possible. Listen to the talks. Look at the posters. Get advice. Start building your personal networks. Good times are ahead.

Lots has happened since our last retreat, including a decision by the U.S. Supreme Court in July that has us thinking hard about ways to ensure diversity in our community of scholars and future physician-scientists. We've come a long way since where we were a decade ago but our commitment to diversity remains as strong as ever.

Other events of note include another greatly appreciated 5 years of NIH funding for the MD/PhD and VMD/PhD programs. The MSTP grant we had held for nearly 50 years has been retired, replaced by a new grant of equal size. The proposal that we submitted for the next 5 years includes a strengthened commitment to mentorship training and to the use of program evaluation metrics to improve the program. The study section members who reviewed our proposal were enthusiastic about the success of our MD/PhD and VMD/PhD alumni and the talents that all of you bring to the program. Rahul, Aimee, Maggie, David, Francia, Carina and I look forward to working with you. So does Cosmo the Chimera.

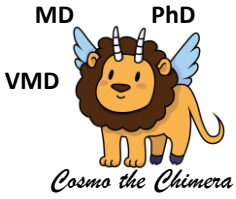
I will conclude by noting 3 anniversaries that have affected all of us. This year and next is the 10th anniversary since the writing and release of the NIH Physician-Scientist Workforce Working Group report. That working group was especially laudatory about the success of MD/PhD programs in training physician-scientists. Their recommendations included expanding NIH support for MD/PhD programs and other programs that provide graduate school training for clinicians, including veterinarians. That has happened. This coming year is also the 10th anniversary of data collection for the National MD/PhD Outcomes Study. The data that were collected include career path information for thousands of MD/PhD program alumni and provide the basis for a lot of the career advice that we send your way. Think p charts. Finally, I'll note in passing that this year marks the 25th anniversary of my term as MSTP director. It has been (and continues to be) a blast. Thanks for letting me hang out with you.

All the best,

Skip Brass, MD PhD

MSTP Director

7/20/23



P.S. Special thanks to Jasmine Larrick for Cosmo's facelift.

Welcome to the Retreat – from the VMD-PhD Program Director

Welcome to the Penn Combined Degree Retreat 2023!

Hi folks! This has been an exceptionally busy and productive year. Perhaps due to pandemic phasing coupled with vet school curriculum changes, we had a bumper crop of PhD defenses the past year. Those who defended their PhD in the past year include Monica Jimenez, Philip Hicks, Jaclyn Carlson, Brinkley Raynor, Ariel Shepley-McTaggart, and Ashley Vanderbeck (6 total). Plus, there are three defending in the next several months: Suna Li, Nate Sotuyo, and Megan Clark). Nine defenses in 15 months is a record for the VMD-PhD program. Two students graduated from the program in May 2023 (Elise Peauroi and Gregory Sousa), and three students are entering the Program this Fall (Audrey Griffith, Erin DeNardo, and Tiffany Wu).

For me, this has been a year for NIH training grant competitive renewals. Three T-grant competitive renewals over 2022 and 2023 was a bit overwhelming, but all three were renewed, and that is very satisfying. Penn Vet rolled out a new curriculum since we last met, and that has kept us all busy as well. But all things considered, this has been an excellent year!

I hope you enjoy this MSTP retreat day. This is the largest MSTP program in the nation, and the only one that includes veterinary combined degree students. I hope you have a great day and enjoy the advantages of the large critical mass and diversities that our program enjoys.

Welcome again to all current and incoming students.

Sincerely,

Michael Atchison, PhD

Many, Many Thanks To the Retreat Planning Committee

We asked the third and fourth year MD-PhD and equivalent VMD-PhD students to take responsibility for planning this event. They did a fabulous job, and we'd especially like to thank the students who were most active in attending the meetings and organizing.

Noa Erlitzki
Claudia Heymach
Emerson Hunter
Rachit Kumar
Maria Merolle
Han-Seul Ryu
Jonathan Sussman
Caroline Wechsler
Daniel Yen
David Zhang

2023 Incoming Class

MD/PhD

Diana Abraham	Cell and Molecular Biology	NYU / NIH
Jessica Anderson	Cell and Molecular Biology	Penn / Cornell
Jamie Benson	Epidemiology and Biostatistics	U Vermont
Chase Chen	Cell and Molecular Biology	Brandeis / NIH
Chelsey Chen	Cell and Molecular Biology	Hopkins
Noah Chen	Immunology	Swarthmore / Penn
Yuen Ting Chow	Chemistry	Harvard
Vasiliki Courelli	Bioengineering	UCSD / Penn
Harry Dang	Bioengineering	U Richmond
Julia Gardner	Pharmacology	Duke
Min Jae Kim	Neuroscience	Hopkins / BWH
Leonie Kurzlechner	Genomics & Computational Biology	Duke
Octavio Lopez	Bioengineering	Harvard
Quinlen Marshall	Cell and Molecular Biology	Saint Johns / CHOP
Vainavi Mukkamala	Bioengineering	MIT
Tai Nguyen	Cell and Molecular Biology	U Washington / NIH
Michelle Onyekaba	Immunology	Harvard / Broad
Maria Isabella Panse	Cell and Molecular Biology	U of Michigan
Jailene Paredes Casado	Cell and Molecular Biology	CUNY - Brooklyn / Mt. Sinai

2023 Incoming Class

Shridhar Parthasarathy	Genomics & Computational Biology	College of NJ / CHOP
Sriya Potluri	Genomics & Computational Biology	U Maryland
Carlos Rodriguez	Neuroscience	UVA / Penn
Martin Rosenfeld	Neuroscience	Cornell College / Yale
Tavian Sanchez	Neuroscience	Creighton
Jay Sastry	Health Care Management	Harvard / U Cambridge
Michelle Schroeder	Immunology	Georgia Tech / NIH
Alexandra Silverman	Bioengineering	Northeastern
Madhav Subramanian	Immunology	Wash U
Josephine Thrasher	Cell and Molecular Biology	Swarthmore / CHOP
Seth Walensky	Biochemistry & Molecular Biophysics	Princeton / Weizmann Inst

VMD/PhD

Erin DeNardo	Cell and Molecular Biology	Wash U
Audrey Griffith	Cell and Molecular Biology	Tulane U
Tiffany Wu	Bioengineering	UC Berkeley

Graduating Students and Thesis Information

MD/PhDs

Joseph Aicher
Improving molecular diagnosis of suspected Mendelian disorders with RNA splicing analysis
Thesis Advisors: Drs. Yoseph Barash and Elizabeth Bhoj

Daniel Akuma
Structural and functional determinants of caspase-11 inflammasome assembly during innate immune defense
Thesis Advisor: Dr. Igor E. Brodsky

Saisai Chen
The Role of PAQR8 in Breast Cancer Recurrence and Therapy Resistance
Thesis Advisor: Lewis Chodosh

Michael Duong
Brain metabolic responses to Alzheimer pathologies with molecular imaging and machine learning
Thesis Advisors: Drs. Ilya Nasrallah and David Wolk

Nicolette Johnson
Understanding Cellular Plasticity within the Intestinal Epithelium
Thesis Advisor: Chris Lengner

Karun Kiani
A Systematic Analysis of the Concordance Between Chromatin Accessibility and Gene Expression Changes
Thesis Advisor: Dr. Arjun Raj

Joe Martinez
Organizational Change, Payment Reform, and the Value of Hospital Care
Thesis Advisors: Atul Gupta and Amol Navathe

Daniel Park
Identification and Characterization of Novel Regulators of Genome Folding
Thesis Advisor: Dr. Eric F. Joyce

Sai Phyo
Regulation of the Tubulin Code in Heart Disease
Thesis Advisor: Dr. Benjamin Prosser

Andy Revell
Localizing Seizure Onset With Diffusion Models
Thesis Advisors: Kate Davis and Brian Litt

Juan Serrano
Rational Design of Dumbbell Protacs for Targeted Degradation of the Genomic Mutator Apobec3a
Thesis Advisor: Dr. Rahul M. Kohli

Derek Sung
Molecular Interplay of VEGF-C and VE-cadherin Controls Sinusoidal, Lymphatic, and Placental Vascular Growth
Thesis Advisor: Dr. Mark Kahn

Stacy Thomas
Liver Macrophages in Pancreatic Cancer Metastasis
Thesis Advisor: Dr. Gregory Beatty

Selen Uman
Injectable Hydrogels to Deliver Extracellular Vesicles After Myocardial Infarction
Thesis Advisor: Jason Burdick

Kevin Zhang
The Impact of Iron on Lysosomal Function in the Retinal Pigment Epithelium
Thesis Advisor: Dr. Joshua Dunaief

VMD/PhDs

Jaclyn Carlson
Fibrillar Collagen Modulation of Extracellular Matrix Structure and Organization Following Tendon Injury
Thesis Advisor: Dr. Louis Soslowsky

Phillip Hicks
A genetic strategy to improve VSV vectored vaccines for emerging bandaviruses
Thesis Advisor: Dr. Paul Bates

Monica Jimenez
microRNA-mediated control of inflammation and energy homeostasis by the gut microbiota
Thesis Advisor: Dr. Jorge Henao-Mejia

Brinkley Raynor
Epidemiology and control of canine rabies in heterogeneous populations
Thesis Advisor: Dr. Ricardo Castillo

Agenda

Please note – all the rooms listed below are located in Villanova's Connelly Center

Student Arrival and Poster Set Up
Villanova Room
8:00 – 9:00am

Opening Remarks
Drs. Skip Brass, MD-PhD
Dr. Laurel Redding, VMD, PhD
Cinema Room
9:00 – 9:15am

Faculty Talks
Cinema Room
9:15 – 10:15am

Robert A. Aronowitz, MD
Walter H. and Leonore C. Annenberg Professor in the Social Sciences
University of Pennsylvania

Not getting "lost in translation": the view from a career spanning clinical medicine and the social sciences

Laurel E. Redding, VMD, PhD
Assistant Professor of Epidemiology,
University of Pennsylvania, School of Veterinary Medicine
Diplomate, American College of Veterinary Preventive Medicine

The (straight) road not taken

Group & Incoming Class Photos
10:20 – 10:55am

Student Group Activity*
Villanova Room
11:00 – 11:45am

Lunch
Villanova Room
11:50 – 12:50pm

**group # is on your nametag*

Student Talks
Cinema Room
12:55 – 1:40pm

Alex Chen	*Generous or Wasted Space? Expertise, Emotion, and Equity in the Design of a COVID-19 Testing Laboratory*
Daniel Connolly	*Progressive disruption of gene expression underlies Rett syndrome pathophysiology*
Jenna Zhang	*A TNF-IL-1 circuit controls Yersinia within intestinal pyogranulomas*

Lightning Talks/Poster Pitches
Cinema Room
1:40 – 1:55pm

Margaret Tamburro	*Structural and Functional Impacts of Early Type III Collagen Reduction on Tendon Healing*
Lev Litichevskiy	*Interactions between the gut microbiome, dietary restriction, and aging in more than 900 genetically diverse mice over the lifespan*
Danielle Kellier	*Predicting migraine in the pediatric emergency room*

Student Poster Sessions (with snacks & adult beverages!)
Villanova Room

Presenters will stand by posters during their session

Poster Session A	**Poster Session B**
2:00 – 2:45pm	2:45 – 3:30pm

Hobby Talks
Villanova Room
3:30 – 4:15pm

David Hill, MD, PhD, FAAP, FACAAI, FAAAAI	*Urban Gardening*
Chloe Winston	*Music - Playing in an ensemble*
Shreya Parchure	*Drawing and Painting*

Departure from Villanova

Post Retreat Happy Hour

Morgan's Pier
221 N. Christopher Columbus Blvd.
5:30 – 7:30pm

Student Talks

Alex Chen

Generous or Wasted Space? Expertise, Emotion, and Equity in the Design of a COVID-19 Testing Laboratory
Advisor: Dr. Adriana Petryna

Daniel Connolly

Progressive disruption of gene expression underlies Rett syndrome pathophysiology
Advisor: Dr. Joe (Zhaolan) Zhou

Jenna Zhang

A TNF-IL-1 circuit controls Yersinia within intestinal pyogranulomas
Advisors: Drs. Sunny Shin and Igor Brosdky

Generous or Wasted Space? Expertise, Emotion, and Equity in the Design of a COVID-19 Testing Laboratory

Chuan Hao Chen

Submitted by: Chuan Hao (Alex) Chen, Anthropology
Email: chuanhao.chen@pennmedicine.upenn.edu; achenchc@sas.upenn.edu
Advisor: Dr. Adriana Petryna

The dearth of testing, essential supplies, and even building materials during the COVID-19 pandemic revealed not only the fragility of global supply chains, but also the complete dependence of medical care on contingencies outside of medicine's control. While public health experts have long debated pandemic "preparedness" (Lakoff 2008), the Coronavirus overwhelmed the U.S. healthcare system, pushing "staff, stuff, space, and systems" (Farmer 2014) of healthcare infrastructures to their limit. Under pressure, how have people responded to the increased demand on healthcare capacity, such as for diagnostic testing? This project is a case study in how an urban American hospital approached the design of a clinical COVID testing lab during the pandemic. Focusing on the design process – how a group of hospital leaders, laboratory technicians, and designers discussed and debated issues of safety and capacity – and specifically on the issue of how to "fit" several new COVID testing machines into limited existing space, this project explores the trade-off between the "flow" of testing and the "flow" of laboratorians who make testing possible. While showing how the physical laboratory environment is critical to pandemic response, this project also unpacks issues of expertise, emotions, and equity. Experts in the disciplinary silos of "medicine" and "architecture" confront gaps in their understanding of each other's field, and (the feeling of) urgency prompt the prioritization of design options that limit impact on the building itself. Finally, where the design process was motivated by the desire to support school reopening, this project provides further evidence of how equitable healthcare is perilously perched on market logics and faith in individual "innovation."

Progressive disruption of gene expression underlies Rett syndrome pathophysiology

Daniel R Connolly*, Zijie Xia*, Remy Stuckey, Maria Fasolino, Emily Fabyanic, Hao Wu, Wanding Zhou, and Zhaolan Zhou

Submitted by: Daniel Connolly, Neuroscience
Email: Daniel.Connolly@pennmedicine.upenn.edu
Advisor: Dr. Zhaolan (Joe) Zhou

Rett syndrome (RTT) is a progressive childhood neurological disorder caused by loss-of-function mutations in the X-linked *MECP2* gene, encoding the predominant DNA methylation reader protein in the brain. Loss of MeCP2 leads to widespread, subtle changes in gene expression, but how these changes occur across disease progression and how they lead to RTT remains unclear. Here, using cell type-specific approaches in a female mouse model of RTT, we comprehensively characterized changes in gene expression in cortical excitatory neurons across disease progression. We identified many subtle changes that accumulated gradually, beginning during the developmental increase of non-CG DNA methylation in neurons, and continuing to accumulate across postnatal life, even after the establishment of DNA methylation patterns in the adult brain. To better understand how specific genes were affected, we simultaneously profiled MeCP2 occupancy and performed multi-omic epigenomic profiling in cortical excitatory neurons. We found that, although MeCP2 bound broadly across the genome, MeCP2-sensitive genes were enriched for non-CG DNA methylation and chromatin signatures of Polycomb repression. Despite MeCP2's broad occupancy pattern, however, binding was depleted across H3K9me3-marked regions of heterochromatin, suggesting that MeCP2 functions outside of these regions to maintain proper gene expression. Taken together, our findings support a "snowball" model for the molecular pathogenesis of RTT, where MeCP2 is unable to maintain precise gene expression as neurons mature, leading to progressive accumulation of subtle transcriptional changes that eventually exceed a threshold and lead to impaired neuronal function.

A TNF-IL-1 circuit controls *Yersinia* within intestinal pyogranulomas

Jenna Zhang[1], Rina Matsuda[1], Daniel Sorobetea[1], Stefan T. Peterson, James P. Grayczyk, Beatrice Herrmann, Winslow Yost, Rosemary O'Neill, Andrea C. Bohrer, Matthew Lanza, Charles-Antoine Assenmacher, Katrin D. Meyer-Barber, Sunny Shin, Igor E. Brodsky
[1]These authors contributed equally

Submitted by: Jenna Zhang, CAMB – Microbiology, Virology, Parasitology
Email: jenna.zhang@pennmedicine.upenn.edu
Advisors: Drs. Sunny Shin and Igor Brodsky

Tumor necrosis factor (TNF) is a pleiotropic inflammatory cytokine that mediates antimicrobial defense and granuloma formation in response to infection by numerous pathogens. *Yersinia pseudotuberculosis* colonizes the intestinal mucosa and induces recruitment of neutrophils and inflammatory monocytes into organized immune structures termed pyogranulomas that control the bacterial infection. Inflammatory monocytes are essential for control and clearance of *Yersinia* within intestinal pyogranulomas, but how monocytes mediate *Yersinia* restriction is poorly understood. We demonstrate that TNF signaling in monocytes is required for bacterial containment following enteric *Yersinia* infection. We further show that monocyte-intrinsic TNFR1 signaling drives production of monocyte-derived interleukin-1 (IL-1), which signals through IL-1 receptor on non-hematopoietic cells to enable pyogranuloma-mediated control of *Yersinia* infection. Altogether, our work reveals a monocyte-intrinsic TNF-IL-1 collaborative circuit as a crucial driver of intestinal granuloma function, and defines the cellular target of TNF signaling that restricts intestinal *Yersinia* infection.

Poster Pitches/Short Talks

Margaret Tamburro

Structural and Functional Impacts of Early Type III Collagen Reduction on Tendon Healing
Advisor: Dr. Louis Soslowsky

Lev Litichevskiy

Interactions between the gut microbiome, dietary restriction, and aging in more than 900 genetically diverse mice over the lifespan
Advisors: Drs. Mingyao Li and Christoph Thaiss

Danielle Kellier

Predicting migraine in the pediatric emergency room
Advisors: Drs. John T. Farrar and Christina L. Szperka

Poster Session A

Biochemistry & Molecular Biophysics

Poster 1A
Small Molecule Activation of Valosin-containing protein (VCP)
Presenter: Benjamin (Ben) Creekmore | Advisors: Drs. Edward Lee and Yi-Wei Chang

Poster 2A
Signatures of 5-methylcytosine modification in DNA by the tumor suppressor TET2
Presenter: Noa Erlitzki | Advisor: Dr. Rahul Kohli

Poster 3A
Towards the Targeted Degradation of a-Synuclein Fibrils in Models of Oxidative Neurodegeneration
Presenter: Andres Fernandez del Castillo | Advisor: Dr. Mark Sellmyer

Bioengineering

Poster 4A
Subject-level weights for detecting brain volume differences
Presenter: Christina Chen | Advisor: Dr. Taki Shinohara

Poster 5A
MRI-based quantification of renal oxygen consumption
Presenter: Rajiv Deshpande | Advisor: Dr. Felix Wehrli

Poster 6A
Microfabrication of a microLED array based on SU-8 for implantable optogenetics
Presenter: Royce Dong | Advisor: Drs. Brian Litt and Flavia Vitale

Poster 7A
Engineering Two-Dimensional and Three-Dimensional Nanopore Devices for Immunomagnetic Extracellular Vesicle Sorting in Multiple Disease Contexts
Presenter: Andrew Lin | Advisor: Dr. Dave Issadore

Poster 8A
Improved Seizure Onset-Zone Lateralization in Temporal Lobe Epilepsy using 7T Resting-State fMRI: A Direct Comparison with 3T
Presenter: Alfredo Lucas | Advisor: Dr. Kate Davis

Cell and Molecular Biology

Cancer Biology

Poster 9A
Optimization of an in vivo CRISPR screen in CAR T
Presenter: Robert (Rob) Hapke | Advisors: Drs. Andy Minn and Evan Weber

Poster 10A
N-glycosylation imposes a targetable constraint on T cell killing of cancer cells
Presenter: Erin Hollander | Advisor: Dr. Ben Stanger

Poster 11A
Investigating the role of tumor transcriptional variability in immune evasion in melanoma
Presenter: Raymond Ng | Advisor: Dr. Sydney Shaffer

Poster 35A
Route-specific immune surveillance during metastasis
Presenter: Benjamin (Ben) Kahn | Advisor: Dr. Ben Stanger

Genetics & Epigenetics

Poster 12A
Mapping the Cellular Biogeography of Human Bone Marrow Niches Using Single-Cell Transcriptomics and Proteomic Imaging
Presenter: Shovik Bandyopadhyay | Advisor: Dr. Kai Tan

Poster 13A
Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma
Presenter: Nathan (Nate) Coffey | Advisors: Drs. Zolt Arany and Celeste Simon

Poster 14A
Intestinal transit amplifying cells require METTL3 for growth factor signaling, KRAS expression, and cell survival
Presenter: Charles Danan | Advisor: Dr. Kate Hamilton

Poster 15A
Delineating the Developmental Trajectory of Brown Fat in the Mouse Embryo
Presenter: Ethan Fein | Advisor: Dr. Patrick Seale

Poster 16A
Understanding the role of STRN3 in lymphatic development
Presenter: Maxwell (Max) Frankfurter | Advisor: Dr. Mark Kahn

Poster 17A
Mapping of neural connectome in health and disease
Presenter: Brian Franklin | Advisor: Dr. Jennifer Phillips-Cremins

Poster 18A
A novel mendelian neurodevelopmental disorder caused by germline variants in MAP2K4
Presenter: Elizabeth (Eli) Gonzalez | Advisor: Dr. Elizabeth Bhoj

Gene Therapy & Vaccines

Poster 19A
Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease
Presenter: Blake Jardin | Advisor: Dr. Jonathan Epstein

Microbiology, Virology, and Parasitology

Poster 20A
The Role of TRIM72 In Regulating Neuronal Antiviral Responses
Presenter: Carl Bannerman | Advisor: Dr. Kellie Jurado

Poster 21A
Understanding intestinal CD4+ T cell immunity using Cryptosporidium infection
Presenter: Ian Cohn | Advisors: Drs. Chris Hunter and Boris Striepen

Poster 22A
The Branched Chain Amino Acids Control Macrophage Inflammation Through Translational Regulation of Cytokine Production
Presenter: Brian Goldspiel | Advisor: Dr. Will Bailis

Chemistry

Poster 23A
Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase
Presenter: Diane Rafizadeh | Advisor: Dr. Dave Chenoweth

Epidemiology & Biostatistics

Poster 24A
DeepCombat: A Statistically-Motivated, Hyperparameter-Robust, Deep Learning Approach to Harmonization of Neuroimaging Data
Presenter: Fengling (Feng) Hu | Advisor: Dr. Taki Shinohara

Poster 25A
Predicting migraine in the pediatric emergency room
Presenter: Danielle Kellier | Advisors: Drs. Christina Szperka and John Farrar

Genomics & Computational Biology

Poster 26A
Characterizing ERV transcripts across healthy human tissues
Presenter: Emerson Hunter | Advisor: Dr. Yi Xing

Poster 27A
Exploiting cell cycle dynamics to interrogate YY1's role in spatiotemporal chromatin organization
Presenter: Jessica Lam | Advisor: Dr. Gerd Blobel

History and Sociology of Science

Poster 28A
"No one had ever told us of the human problems we should be called upon to face": The Role of Medical Students in the 1918 Influenza Pandemic in Philadelphia
Presenter: Caroline Wechsler | Advisor: Dr. Robert Aronowitz

Immunology

Poster 37A
Dysregulation of the hepatic CD4 T cell compartment during childhood obesity
Presenter: Thomas (Sam) Barnett Dubensky | Advisor: Dr. Jorge Henao-Mejia

Poster 29A
Autosomal dominant C-terminal TREX1 frameshift mutants inhibit homology-directed repair and increase risk of breast cancer
Presenter: Samuel (Sam) Chauvin | Advisor: Dr. Jonathan Miner

Poster 30A
Hepatic CD9 regulates adipose tissue function and inflammation during obesity
Presenter: Julia Chini | Advisor: Dr. David Hill

Poster 31A
Understanding the Role of Germinal Centers in the Generation and Durability of nAbs to mRNA-LNP vaccination
Presenter: Joy Chiu | Advisor: Dr. Michaela Locci

Poster 32A
Can microbial molecular mimics protect from type 1 diabetes?
Presenter: B. (John) Deschaine | Advisor: Dr. Michael Silverman

Neuroscience

Poster 33A
Lysosomal lipid accumulation leads to macrophage dysfunction in Krabbe disease
Presenter: Venkata (Sai) Chaluvadi | Advisor: Dr. F. Chris Bennett

Poster 34A
Cell type-specific vulnerabilities in behavioral-variant Frontotemporal Dementia caused by C9orf72 expansion mutations
Presenter: David Dai | Advisor: Dr. Edward Lee

Poster 36A
The Aged Microbiome Drives Cognitive Decline via Intestinal Inflammation and Vagal Inhibition
Presenter: Timothy (Tim) Cox | Advisors: Drs. Christoph Thaiss and Virginia Lee

Poster 1A | Biochemistry & Molecular Biophysics

Small Molecule Activation of Valosin-containing protein (VCP)

Benjamin C. Creekmore, Jessica M. Phan, Nabil F. Darwich, and Edward B. Lee

Submitted by: Benjamin Creekmore, Biochemistry and Molecular Biophysics
Email: Benjamin.Creekmore@pennmedicine.upenn.edu
Advisors: Drs. Edward B. Lee and Yi-Wei Chang

Valosin-containing protein (VCP) is a AAA+ ATPase that plays a crucial role in protein quality control and membrane trafficking/fusion. Mutations in *VCP* have been associated with frontotemporal dementia clinically. Multisystem Proteinopathy (MSP) mutations in *VCP* lead to TDP-43 aggregates and increased ATPase activity, while a Vacuolar Tauopathy (VT) leads to tau aggregates and decreased ATPase activity. Despite MSP mutations increasing ATPase activity, the unifying hypothesis is that VCP loses function in crucial proteostasis pathways, leading to neurodegenerative disease. As such, there is potential therapeutic value to compounds that increase VCP activity.

We identified compounds that increase VCP activity, validated with two orthogonal *in vitro* ATPase assays. We determined dose-dependence of all active compounds against WT VCP with and without *in vivo* relevant cofactors Ufd1 and Nploc4. We also determined effect of ATP concentration on activation and specificity to VCP by using AAA+ ATPase NSF. Walker B mutations of the D1 and D2 ATPase domains and truncations isolating the D1 and D2 ATPase domains of VCP were used to determine which ATPase domain is most effected.

We identified novel activators of VCP that have variable potency and maximum activation. Addition of Ufd1 and Nploc4 has variable effect on the potency of compounds. All compounds exhibit specificity for VCP over NSF. All compounds increase D2 ATPase activity. Interestingly, some compounds significantly decrease D1 ATPase activity with a D2 Walker B mutation and one compound, UP12, increases D1 ATPase activity in a truncated VCP.

Our data characterizes novel activators of VCP that have variable potency, maximum activity, and effect on ATPase domains.

Poster 2A | Biochemistry & Molecular Biophysics

Signatures of 5-methylcytosine modification in DNA by the tumor suppressor TET2

Noa Erlitzki and Rahul Kohli

Submitted by: Noa Erlitzki, Biochemistry and Molecular Biophysics
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The importance of epigenetic DNA modifications in normal physiology and disease has prompted interest in understanding the mechanisms underlying their generation and maintenance. 5-methylcytosine (5mC) is a critical epigenetic DNA modification associated with gene silencing when present in certain regulatory elements of genes, such as CpG islands (CGIs) within promoter regions. Reversal of this gene silencing program allows for dynamic gene regulation and is facilitated by Ten-Eleven Translocase (TET) enzymes, which oxidize 5mC in a step-wise fashion to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC). Both 5fC and 5caC are substrates for base excision repair and replacement with unmodified cytosine, offering a pathway for active DNA demethylation. 5hmC, however, does not share this redundancy and instead feeds into a DNA replication-dependent passive pathway for DNA demethylation. 5hmC is also posited to have additional independent epigenetic functions beyond passive demethylation, in light of evidence that it is a stable and heritable mark as well as the observation that it is the most abundant of the oxidized methylcytosines. 5hmC is thought to play an important role in various developmental pathways including neuronal development, and its dysregulation is prominently associated with oncogenesis, with loss of 5hmC being a hallmark of AML and other cancers. Recognition of 5hmC as an important yet understudied epigenetic node has led to great interest in deciphering its functions and biological footprint. Despite the importance of distinguishing 5hmC from 5fC/5caC, the influence of local sequence context on the generation of these oxidized 5mC bases remains poorly understood. Comprehensive characterization of TET processivity has been limited due to a lack of methods available to discriminate between 5hmC and other cytosine modifications on single DNA molecules and at base resolution. Here, we employed a novel epigenetic sequencing technology to evaluate the *in vitro* dynamics of TET oxidation and observed, for the first time, the generation and depletion of 5hmC at base resolution. An analysis of iterative TET activity at single CpGs and across clusters of CpGs reveals distinct sequence context preferences and provides rationale for exploring the role of local sequence context in the genome in governing the process of gene reactivation in the setting of normal development or in disease.

Poster 3A | Biochemistry & Molecular Biophysics

Towards the Targeted Degradation of a-Synuclein Fibrils in Models of Oxidative Neurodegeneration

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Alpha synuclein (a-syn) is a small (15kDa) intrinsically disordered protein believed to play roles in vesicular trafficking and mediating mitochondrial oxidative stress, and its dysregulation or overexpression leads to proteotoxic stress and cell death. a-syn has the propensity to aggregate into oligomers or fibrils. a-syn has histological and genetic associations with neurodegenerative disorders, including Parkinson's disease. There are no currently approved medications to slow or treat the underlying pathologies of a-syn-associated neurodegenerative diseases. For this reason, a-syn has been the focus of intense study to elucidate its contributions to the progression of neurodegeneration. Oxidative stress and mitochondrial dysfunction have been shown to increase a-syn aggregation in-vitro and in-vivo; Thus, there is evidence that oxidative stress is a driver of a-syn pathology and plays a role in some neurodegenerative disorders such as Parkinson's. Using a a-syn fibril binder developed and synthesized by the Petersson lab, I will design and synthesize Proteolysis Targeting Chimeras (PROTACs) to hijack the cellular Ubiquitin/Proteasome degradation pathway for the destruction of a-syn fibrils. I will additionally design and synthesize Autophagy Targeting Chimeras (AUTACs) to degrade a-syn fibrils via autophagic and lysosomal pathways. I will validate the biological mechanism behind both targeted degradation modalities. I will then apply the above described PROTACs and AUTACs to selectively degrade a-syn fibrils in cellular models of oxidative stress in Parkinson's Disease. Using chronic low-dose rotenone, I will subject neuronal cell models to oxidative stress. I will then use anti-a-syn fibril PROTACs and AUTACs to selectively degrade a-syn fibrils. These studies will explore the interplay of a-syn aggregation, cellular dysfunction, and cytotoxicity.

Poster 4A | Bioengineering - Imaging

Subject-level weights for detecting brain volume differences

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Multi-atlas image segmentation is a widely used approach in imaging analyses that involve estimating the volume of a region of interest (ROI). However, current practices typically treat these images equally without incorporating any information about variations in segmentation precision among the study images. We propose a novel method that estimates the variance of the ROI volume estimate for each subject due to the multi-atlas segmentation procedure and thus provides a way of reweighting these estimates to increase efficiency in downstream inference.

Poster 5A | Bioengineering - Imaging

MRI-based quantification of renal oxygen consumption

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During the early stages of diabetes, kidney oxygen utilization increases. The mismatch between oxygen demand and supply contributes to tissue hypoxia, a key driver of chronic kidney disease. Thus, whole-organ renal metabolic rate of oxygen ($rMRO_2$) is a potentially valuable parameter of kidney function. The key parameters required to determine $rMRO_2$ include renal blood flow rate (RBF) in the feeding artery and oxygen saturation in the draining renal vein (SvO_2). However, there is currently no noninvasive method to quantify $rMRO_2$ in absolute physiologic units. Here, a new MRI pulse sequence, termed K-MOTIVE (Kidney Metabolism of Oxygen via T_2 and Interleaved Velocity Encoding), is described, along with evaluation of its performance in the human kidney in vivo. K-MOTIVE interleaves a phase-contrast module before a background-suppressed T_2-prepared balanced-steady-state-free-precession (bSSFP) readout to measure RBF and SvO_2 in a single breath-hold period of 22 seconds, yielding $rMRO_2$ via Fick's Principle. Variants of K-MOTIVE to evaluate alternative bSSFP readout strategies were studied. Healthy subjects were recruited to quantify $rMRO_2$ of the left kidney at 3T field strength (N = 15). Assessments of repeat reproducibility and comparisons with individual measurements of RBF and SvO_2 were performed, and the method's sensitivity was evaluated with a high-protein meal challenge and serum biomarker analyses (N = 8). K-MOTIVE yielded the following metabolic parameters: T_2 = 157 ± 19ms; SvO_2 = 92 ± 6%; RBF = 400 ± 110mL/min; and $rMRO_2$ = 114±117(μmol O_2/min)/100g tissue. Reproducibility studies of T_2 and RBF (parameters directly measured by K-MOTIVE) resulted in coefficients of variation < 10% and intraclass correlation coefficients > 0.75. The high-protein meal elicited an increase in $rMRO_2$, which was corroborated by an increase in cystatin-C-derived eGFR. The K-MOTIVE sequence measures SvO_2 and RBF, the parameters necessary to quantify whole-organ $rMRO_2$, in a single breath-hold. The present work demonstrates that $rMRO_2$ quantification is feasible with good reproducibility. $rMRO_2$ is a potentially valuable physiological biomarker of kidney function.

Poster 6A | Bioengineering

Microfabrication of a microLED array based on SU-8 for implantable optogenetics

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Optogenetics is a technique in neuroscience that enables *in vivo* neuromodulation with cell type-specificity and high temporal resolution. It is an established method in mice, but scaling up to nonhuman primates and humans has presented a technical challenge given the larger volume, greater mobility, and more complex immune system of the animal models. Optogenetics in nonhuman primates typically require subjects to remain fixed in primate chairs to protect the cables affixed to their heads. Compared with fiber optic light delivery, implanted biocompatible microLED arrays allow for optogenetic experiments in freely moving primates that study naturalistic behavior. This poster details the microfabrication of a microLED array based on SU-8, as well as its electrical, optical, and thermal characterization.

Poster 7A | Bioengineering

Engineering Two-Dimensional and Three-Dimensional Nanopore Devices for Immunomagnetic Extracellular Vesicle Sorting in Multiple Disease Contexts

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Extracellular vesicles (EVs) hold multiple protein and nucleic acid biomarker cargoes which reflect their cells of origin while circulating in peripheral bodily fluids such as blood, urine, and saliva. Although EVs and EV-derived biomarkers have shown potential for diagnosing multiple cancers and other diseases, their high number of circulating background EVs relative to target (~10^10 - 10^12 EVs/mL in human plasma) and nanoscale size (30 - 200 nm) has restricted their clinical utility. To address this issue, we present EV enrichment using nanopore isolation, in which millions of small (600 nm - 3 µm) pores operate in parallel to sort and isolate immunomagnetically tagged EVs bearing specific surface proteins while maintaining high and clinically-relevant sample throughput (>10 mL/hr). This abstract describes the physical scaling laws underlying immunomagnetic nanopore EV isolation and their application in multiple clinical scenarios. Finite-element simulations were first performed to model physical scaling laws for two-dimensional and three-dimensional nanopore EV isolation devices, scanning across conditions such as flow rate, number of pores in series, pore size, and immunomagnetic labeling strength. These EV isolation scaling laws were then experimentally-validated in a model system of pancreatic cancer, and the results closely matched the trends predicted by finite-element simulation. This platform was in turn validated versus both device and versus sample controls in three model systems of cancer (pancreatic, liver, and lung) via cell culture media spiked into complex background (FBS, human plasma). Through this work, we have developed a robust, high-throughput, and tunable EV subpopulation isolation platform for which we are now performing pilot clinical studies in lung cancer (n = 80 patients) and Alzheimer's disease (n = 157 patients).

Poster 8A | Bioengineering

Improved Seizure Onset-Zone Lateralization in Temporal Lobe Epilepsy using 7T Resting-State fMRI: A Direct Comparison with 3T

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Background and Objectives: Seizure-onset zone (SOZ) localization is crucial for targeting therapy in drug-resistant epilepsy (DRE), yet current functional neuroimaging techniques remain limited in their utility for localizing the SOZ. Resting-state functional magnetic resonance imaging (rs-fMRI) at ultra-high-field strengths (≥7T) is known to provide superior signal-to-noise and statistical power than comparable acquisitions at lower field strengths. In this study, we provide a direct comparison of the SOZ lateralizing ability of 7T rs-fMRI compared to that of 3T rs-fMRI.

Methods: Our cohort consisted of drug-resistant temporal lobe epilepsy (TLE) patients who received both 3T and 7T structural and functional neuroimaging during presurgical evaluation. For each patient, we quantified the functional connectivity between the hippocampus and other nodes within the default mode network (DMN) using seed-to-voxel connectivity. We used hippocampo-DMN connectivity to inform SOZ lateralization at 7T and 3T field strengths. We also compared lateralizing functional connectivity at 3T and 7T with standard-of-care FDG-PET lateralizing hypometabolism.

Results: We included a cohort of 70 TLE patients. A paired sub-group of 19 patients had 3T and 7T rs-fMRI acquisitions for direct comparison between the two field strengths. Forty-three patients had only 3T, and 8 patients had only 7T rs-fMRI acquisitions. Differences in hippocampo-DMN connectivity ipsilateral and contralateral to the SOZ were significantly higher at 7T (Cohen's d=0.51, pFWER=0.008) than at 3T (Cohen's d=0.26, pFWER=0.68) when measured in the same subjects. We found that SOZ lateralization was superior at 7T (ROC AUC=0.97, 95% CI: 0.92-1.00) than 3T (ROC AUC=0.67, 95% CI: 0.36-0.98), for the same subjects scanned at both field strengths. These findings replicated in extended cohorts of subjects scanned at either 3T or 7T. Lateralizing findings at 7T were consistent and correlated with clinical FDG-PET lateralizing hypometabolism (Spearman Rho=0.65, p=0.01), but the findings at 3T were not (Spearman Rho=0.13, p=0.69).

Discussion: Our findings provide compelling evidence for the clinical utility of 7T rs-fMRI in localizing the seizure-onset zone in TLE by demonstrating superior SOZ lateralization with 7T compared to 3T rs-fMRI. These findings suggest that ultra-high field neuroimaging has the potential to improve patient outcomes by facilitating targeted therapy and optimizing surgical interventions in individuals with TLE.

Poster 9A | CAMB - Cancer Biology

Optimization of an in vivo CRISPR screen in CAR T

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Chimeric Antigen Receptor (CAR) T cell therapy has had success in certain hematological malignancies. This live cell product is composed of both CD4 and CD8 T cells, and it is the combination that generally leads to the greatest therapeutic efficacy. However, this therapy has faced challenges transferring to solid tumors, including inadequate *in vivo* persistence of CAR T. Recently, a subset of CD4 CAR T (CAR 4) cells were identified in patients who received CAR T therapy resulting in decade-long remissions to leukemias. Notably, these highly persistent CD4 T cells had a cytotoxic program that is typically associated with CD8 effector cells, with expression of cytolytic proteins such as granzyme and perforin. Cytotoxic CD4 T cells have previously been identified as key players in antitumor immunity for a subset of cancers expressing MHC-II. As the CAR bypasses the endogenous T cell receptor, it is possible for these cytotoxic CD4 cells to be more broadly applicable to CAR T cell therapy. However, how this cytotoxic CAR 4 program enhances the efficacy of CAR T cell therapy has not been well described. Similarly, whether there are more potent enhancers of CAR 4 persistence remains to be defined. Thus, an unbiased approach, such as a genome scale CRISPR knockout (KO) screen, can help identify other factors enhancing this program. Given that other challenges with CAR T therapy in solid tumors includes limited understanding of CAR T tumor-honing capabilities and a metabolically challenging and immunosuppressive environment, successful performance of an *in vivo* screen may better identify directly translatable perturbations. We show proof of concept of an in vivo genome-wide CRISPR KO screen performed in human CAR T cells using an osteosarcoma NSG model targeted by HER2.BBz CAR T cells. Triple viral infection of CAR T cells with CAR, Cas, and guide is feasible with an extended transduction period, and cells rapidly expand post-activation. In a small pilot experiment using 1/3 the genome-wide library, we find complete guide recovery in tumor-infiltrating CAR T cells harvested from individual mice after three weeks of treatment. Compared to pre-infusion samples, these tumor-isolated samples have correlated guide counts with relatively low variability of guide distribution, signifying high *in vivo* hit-calling capability.

Poster 10A | CAMB - Cancer Biology

N-glycosylation imposes a targetable constraint on T cell killing of cancer cells

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Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer-related death in the United States. The five-year survival rate of twelve percent is attributed mainly to a difficulty in early detection and a lack of effective treatments. Tumor-associated glycans represent a potential anti-tumor target for two reasons: (i) protein glycosylation is known to play a role in tumor progression, and (ii) alternatively glycosylated proteins may function as tumor neoantigens. The glycosyltransferase MGAT5 catalyzes the formation of β1,6-N-acetylglucosamine branched glycans, and overexpression has been implicated in tumor growth and metastasis in multiple cancers. Using a panel of clonal cell lines that recapitulate the immune heterogeneity of PDAC, we found that knockout of MGAT5 in some clones ("T cell-inflamed" tumors) allows for complete clearance of tumors while in other clones ("non-T cell-inflamed") MGAT5 deficiency led to a marked decrease in tumor growth. This phenotype was confirmed in orthotopic injection as well as subcutaneous injection into syngeneic mice. By contrast, MGAT5 loss had no impact on tumor cell growth *in vitro*.

To probe immune system involvement in this robust rejection of tumor growth, the MGAT5 KO cells were injected into NOD/SCID mice, resulting in rescue of the phenotype. Tumor eradication or growth inhibition *in vivo* was found to be dependent specifically on the presence of CD4/CD8 T cells and dendritic cells. Tumor challenge experiments, in which mice were immunized with MGAT5 KO cells and challenged with wild-type tumors four weeks later, revealed that tumor rejection was associated with a durable immunologic memory that is reliant on the interaction of live tumor cells and T cells. Overall, these findings indicate the loss of MGAT5 leads to a marked increase in anti-tumor immunity.

To delineate the mechanism underlying this robust tumor clearance, OT-I T cells targeting the strong antigen ovalbumin were cultured with both MGAT5 WT and KO tumors cells engineered to express ovalbumin. The KO tumor cells had a higher rate of killing by T cells in the co-culture. Additionally, MGAT5 KO cells were found to be exceptionally sensitive to apoptosis following stimulation by TNFα. Ongoing work focuses on determining which elements of the TNFα pathway are affected by loss of the MGAT5.

Taken together, these results are consistent with a model in which loss of MGAT5-mediated N-glycans increases the sensitivity of tumors to T cell killing through the TNFα pathway, allowing for the formation of a durable immune response. Finally, MGAT5 knockout tumors treated with immune checkpoint blockade had significantly decreased tumor size and increased survival over controls, suggesting MGAT5 has potential as a novel target for pancreatic cancer.

Poster 11A | CAMB - Cancer Biology

Investigating the role of tumor transcriptional variability in immune evasion in melanoma

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Throughout melanoma progression and immunotherapy treatment, melanoma cells often develop an ability to evade the immune system. Thus, there is a pressing need to identify and target the melanoma cell-intrinsic mechanisms underlying immune evasion. Our laboratory has pioneered the identification of transcriptional pathways that underlie melanoma progression. We have found that within even clonal melanoma cell populations, there is a tremendous amount of heterogeneity in gene expression between cells. We hypothesize that this transcriptional heterogeneity corresponds to cells that have differing abilities to evade CD8+ T cells, which are major effector cells against melanoma. To study the interactions between melanoma cells and CD8+ T cells, we engineered an ovalbumin antigen and OT-I T cell receptor system. We combined this system with DNA barcoding, which enables us to track subclones of cells undergoing immune selection. When cocultured together in vitro, ovalbumin-expressing melanoma cells are effectively killed by OT-I cells. However, we find that subclones of melanoma cells have different sensitivity to OT-I cells, and these differences are unlikely due to their ability to express and present ovalbumin. In our future experiments, we will determine the transcriptional states of melanoma cells that persist through OT-I coculture. By expanding on these preliminary results, we aim to identify novel melanoma cell targets to decrease immune evasion.

Poster 35A | CAMB - Cancer Biology

Route-specific immune surveillance during metastasis

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Distant metastasis is the primary cause of cancer-related death and is generally preceded by metastasis to the lymph node (LN), placing LN metastasis among the most important staging criteria in solid cancers. LNs simultaneously serve as a critical site for the priming of an anti-tumor immune response. LNs are comprised mostly of lymphocytes and contain all of the cell types necessary to conduct a productive anti-tumor immune response, yet they are the first and most frequent site of metastasis in most solid cancers. The ability of tumor cells to survive in the LN has been attributed to preconditioning of regional LNs by immunosuppressive secretions from the primary tumor. However, the immune system is highly compartmentalized between lymph and blood, and it has not been considered whether the LN, in its intrinsic state, is less able to mount a self-protective immune response than other commonly metastatic organs like the liver and lung which are more freely perfused by blood.

To determine whether the immunological compartmentalization between blood and lymph has implications for tumor metastasis, I compared the metastatic profile of a highly metastatic murine pancreatic cancer cell line, 6694c2 to that of the same cell line transduced to express the Ovalbumin neoantigen. I find that addition of the Ova antigen restricts growth of the primary pancreatic tumor and dramatically decreases the frequency of liver and lung metastasis, but the frequency of LN metastasis is maintained. Further, I find that the LN is an intrinsically advantageous site for the growth of tumors following direct implantation into a fully MHC-mismatched host. These studies highlight intrinsic difficulties in targeting immunotherapy towards LN metastases and help to explain the paradoxical prevalence of metastases to the lymph node.

Poster 12A | CAMB - Genetics and Epigenetics

Mapping the Cellular Biogeography of Human Bone Marrow Niches Using Single-Cell Transcriptomics and Proteomic Imaging

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The bone marrow is the organ responsible for blood production. Diverse non-hematopoietic cells contribute essentially to hematopoiesis. However, these cells and their spatial organization remain largely uncharacterized as they have been technically challenging to study in humans. Here, we used fresh femoral head samples and performed single-cell RNA sequencing (scRNA-Seq) to profile 29,325 enriched non-hematopoietic bone marrow cells and discover nine transcriptionally distinct subtypes. We next employed Co-Indexing by Epitopes (CODEX) multiplexed imaging of 18 individuals, including both healthy and acute myeloid leukemia (AML) samples, to spatially profile over one million single cells with a novel 53-antibody panel. We discovered a relatively hyperoxygenated arterio-endosteal niche for early myelopoiesis, and an adipocytic, but not endosteal or perivascular, niche for early hematopoietic stem and progenitor cells. We used our atlas to predict cell labels in new bone marrow images and used these predictions to uncover mesenchymal stromal cell (MSC) expansion and leukemic blast/MSC-enriched spatial neighborhoods in AML patient samples. Our work represents the first comprehensive, spatially-resolved multiomic atlas of human bone marrow and will serve as a reference for future investigation of cellular interactions that drive hematopoiesis.

Poster 13A | CAMB - Genetics and Epigenetics

Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma

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North America has the highest incidence of renal cancer in the world with clear cell renal cell carcinoma (ccRCC) being the most common subtype accounting for greater than 75% of cases. Metastatic ccRCC has a five-year survival rate of only ~10% and is in dire need of new therapies. One such approach is to target dysregulated metabolic pathways in this setting. Metabolic dysfunction is a hallmark of ccRCC as demonstrated by its clear cell histologic appearance due to significant cytoplasmic accumulation of lipid droplets and glycogen. Using publicly available ccRCC RNA-seq and proteomics datasets, I found reduced expression of all subunits of BCKDH, the rate-limiting enzyme in branched-chain amino acid (BCAA) metabolism, in ccRCC tumors compared to normal adjacent kidney tissue (NAT). BCKDH is a heteromeric protein complex consisting of multiple subunits called DBT, BCKDHA/B, and DLD and regulates the catabolism of all three BCAA metabolites (leucine, isoleucine, and valine). I then validated that BCKDH expression was reduced in our own cohort of ccRCC tumors and cell lines, and performed immunohistochemistry on ccRCC tumor microarrays to demonstrate that downregulation of BCKDH occurs as early as stage one and is associated with reduced overall survival in patients. Furthermore, I found that reduced expression of BCKDH was reinforced at the genome level by copy number loss of BCKDH subunits *DBT* and *BCKDHB*, underscoring the selection pressure for suppression of this pathway. Lastly, metabolomics performed with LC-MS revealed decreased abundance of BCAAs and their catabolic metabolites in ccRCC tumors. These multiple observations have led me to the hypothesis that reduced BCAA catabolism promotes ccRCC tumorigenesis. I then wanted to determine if increasing BCAA metabolism would reduce ccRCC tumor growth. I first demonstrated that ccRCC cell lines have reduced BCAA catabolism. To enhance BCAA catabolism, short hairpin RNAs were used to knockdown BCKDK, a kinase that inhibits BCAA metabolism by phosphorylating BCKDH. Excitingly, increasing BCAA metabolism genetically with short hairpin RNAs targeting BCKDK reduced the proliferation of multiple ccRCC cell lines *in vitro* by inducing apoptosis but not in control renal epithelial cells. One mechanism appears to be via increased production of reactive oxygen species (ROS). This suggests that targeting BCAA metabolism could be a novel way to selectively kill ccRCC. My next steps will be to validate these results in mouse models of ccRCC and further determine the mechanism of how increased BCAA catabolism reduces ccRCC tumor growth.

Poster 14A | CAMB - Genetics and Epigenetics

Intestinal transit amplifying cells require METTL3 for growth factor signaling, KRAS expression, and cell survival

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Intestinal epithelial transit amplifying cells are essential stem progenitors required for intestinal homeostasis, but their rapid proliferation renders them vulnerable to DNA damage from radiation and chemotherapy. Despite their critical roles in intestinal homeostasis and disease, few studies have described genes that are essential to transit amplifying cell function. We report that the RNA methyltransferase, METTL3, is required for survival of transit amplifying cells in the murine small intestine. Transit amplifying cell death after METTL3 deletion was associated with crypt and villus atrophy, loss of absorptive enterocytes, and uniform wasting and death in METTL3-depleted mice. Ribosome profiling and sequencing of methylated RNAs in enteroids and *in vivo* demonstrated decreased translation of hundreds of unique methylated transcripts after METTL3 deletion, particularly transcripts involved in growth factor signal transduction such as *Kras*. Further investigation confirmed a novel relationship between METTL3 and *Kras* methylation and protein levels *in vivo*. Our study identifies METTL3 as an essential factor supporting the homeostasis of small intestinal tissue via direct maintenance of transit amplifying cell survival. We highlight the crucial role of RNA modifications in regulating growth factor signaling in the intestine, with important implications for both homeostatic tissue renewal and epithelial regeneration.

Poster 15A | CAMB - Genetics and Epigenetics

Delineating the Developmental Trajectory of Brown Fat in the Mouse Embryo

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Obesity is a major health challenge globally and is caused by a chronic imbalance of caloric intake versus caloric expenditure. Brown adipose tissue (BAT) is a specialized tissue that promotes high caloric expenditure uncoupled from ATP production, thereby releasing heat. Although the high energy-burning capacity of BAT has the potential to combat obesity and its comorbidities, such as type II diabetes, patients suffering from these disorders are less likely to have significant amounts of metabolically active BAT. Therefore, strategies to increase the amount of active BAT are needed. Toward this end, we sought to better understand the developmental trajectory of brown adipocytes, using the mouse embryo as model. Interscapular BAT, a prominent depot in both mice and humans, derives from the same precursor population as skeletal muscle and dermis: the dermomyotome. However, the cell states traversed by BAT precursors as they gradually restrict their developmental potential remain undefined. To address this, we performed single-cell RNA sequencing (scRNA-seq) on cells from the dorsal region of mouse embryos from embryonic day 10 (E10), when the dermomyotome forms, to E15, when many brown adipocytes have formed. To gain insight into anatomic relationships between the identified cell types, we used our dataset as a reference for cell-type deconvolution analysis of the Mouse Organogenesis Spatial Transcriptomics Atlas (MOSTA), allowing us to map the cell types identified by scRNA-seq to spatial locations in the mouse embryo at high resolution. The MOSTA-facilitated analysis suggested a brown adipocyte trajectory characterized by the following cell states: (1) *En1*+ dermomyotomal cells; (2) *Cdh4*+/*Ebf2*+ cells committed to non-muscle/non-dermal trajectories; (3) transitional cells expressing many fibroblastic markers; (4) *Pparg*+ committed preadipocytes; and (5) *Adipoq*+ adipocytes. In addition, we identified a population of dermomyotome-derived *Dpp4*+ mesenchymal cells that surround developing BAT and express the brown adipogenic factor *Bmp7*, thus likely serving as niche-forming cells for brown adipocytes. Overall, these studies delineate a presumptive developmental trajectory for brown adipocytes, highlighting key branchpoints in the hierarchy of dermomyotome-derived cell types. The identification and transcriptional profiling of the relevant embryonic cell states will inform and enhance efforts to stimulate brown adipocyte development *in vitro* and *in vivo* to combat obesity and its comorbidities.

Poster 16A | CAMB - Genetics and Epigenetics

Understanding the role of STRN3 in lymphatic development

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The lymphatic vasculature is critical for tissue homeostasis and immune function. Lymphatics begin to develop in the mouse around embryonic day 10.5 when progenitors are specified in the cardinal vein and begin to migrate outward to form the primary lymph sacs. From there, vessels sprout to invest peripheral tissues. To accomplish this in a short time window, they proliferate rapidly. Lymphatic endothelial cell (LEC) migration and proliferation are known to be driven by vascular endothelial growth factor C (VEGF-C)-VEGF receptor 3 (VEGFR3) signaling. Striatin 3 (STRN3) is a ubiquitous protein that serves as a scaffold in the striatin-interacting phosphatase and kinase (STRIPAK) complex. STRIPAK is a highly conserved, multiprotein complex containing protein phosphatase 2A (PP2A) and germinal center kinase III (GCK-III) proteins. STRIPAK has been shown to regulate many cellular processes, most notably Hippo signaling, a master controller of cell proliferation. Almost all studies of mammalian STRIPAK have been conducted *in vitro* in transformed cell lines. Our lab has generated a mouse knockout of *Strn3* and found that this results in embryonic edema with LEC hyperproliferation. Strikingly, there does not appear to be a blood endothelial cell (BEC) phenotype in these animals, suggesting an LEC-specific function for STRN3. In this proposal, I aim to understand the mechanism by which STRN3 deficiency augments LEC proliferation. I hypothesize that STRN3 inhibits LEC proliferation by suppressing the action of VEGF-C-VEGFR3 signaling and/or activating the Hippo kinase cascade. In aim 1, I will assess EC-autonomy by crossing our lab's *loxP*-flanked *Strn3* allele to *TIE2-Cre* and examining embryos for edema. In aim 2, I will use an *in vitro* LEC model to assess STRN3's effect on VEGF-C-VEGFR3 signaling. I will look specifically at the level of a) receptor trafficking, expression, and dephosphorylation, and b) ERK activation and dephosphorylation. In aim 3, I will assess Hippo activity in *Strn3-/-* embryo LECs. *In vitro*, I will determine if STRN3 interacts with Hippo pathway components in LECs. These experiments will help clarify the role of STRIPAK in regulating lymphatic development. Given the lack of a BEC phenotype, these studies may highlight key differences between LECs and BECs

Poster 17A | CAMB - Genetics and Epigenetics

Mapping of neural connectome in health and disease

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In the human brain, billions of neurons connect through trillions of synapses, and electrical signals transmitted through this network form the basis of human cognition, behavior, learning, and memory. Neurons must be connected to the correct partners in the network to ensure proper electrical communication and healthy brain function. Dysregulated synaptic connectivity underlies several neurologic disorders plaguing human health, including autism, depression, schizophrenia, and Alzheimer's disease. Despite the importance of neural connectivity in maintaining healthy neurophysiology, existing methods for mapping neural connectomes are low-throughput and require technological expertise limited to a few labs worldwide. Such limitations constitute a significant barrier to understanding diseases of the brain and have contributed to decades of stalled progress in producing effective neurologic therapies. To address these limitations, I am building a new molecular technology based on cell barcoding and *in situ* sequencing to enable the scientific community to create facile, reproducible neural connectome maps called SynGram-seq. Creation of connectivity maps will empower scientists to discover transformative insights into neurological disease and inform development of novel therapeutics. My goal is to apply SynGram-seq to understand dysregulation of connectivity in models of Alzheimer's disease and fragile X syndrome, and identify molecular basis of diseased neural connectomes.

Poster 18A | CAMB - Genetics and Epigenetics

A novel mendelian neurodevelopmental disorder caused by germline variants in MAP2K4

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Our group has recently built a cohort of paediatric patients with germline mutations in Mitogen-Activated Protein Kinase Kinase 4 (*MAP2K4*). The patients' phenotypes include developmental delays and regression; structural brain differences; cranial, renal, and other variable systemic differences. MAP2K4 is an enzyme required for the phosphorylation and activation of c-Jun N-terminal kinase (JNK). JNK then phosphorylates downstream proteins critical for neurodevelopment and neurodifferentiation. Our patients' germline variants in *MAP2K4* are heterozygous, predominantly *de novo*, a combination of missense and nonsense changes, and all cluster in the kinase domain. Notably, *MAP2K4* is predicted to be highly intolerant to both loss-of-function (pLI=1) and missense mutations (z=3.23), according to the Genome Aggregation Database (gnomAD). While germline mutations in *MAP2K4* have not been previously described, somatic mutations are implicated in various cancer types with three recurrent mutations seen in the Catalogue of Somatic Mutations in Cancer (COSMIC) — Ser184Leu, Arg134Trp, Arg281*. Notably, the Arg281* nonsense mutation is also seen in our cohort. Motivated by our patients' predominantly neurological phenotypes, we leveraged open-access RNA-sequencing data sets to explore the role of *MAP2K4* in normal human and murine neurodevelopment. In the murine developing brain, we found that *MAP2K4* is expressed in the forebrain, hindbrain and midbrain, and that transcript-level expression is detectable as early as embryonic day 10.5, with stable expression through birth (P0). In the human developing brain, *MAP2K4* is expressed most highly in Cajal-Retzius cells during the 18th week of gestation. Together, our patients' phenotypes and the expression pattern of *MAP2K4* in normal neurodevelopment suggests a critical role of this gene in the maturation of the central nervous system. This informs our future functional work, where we will employ an existing mouse model of *MAP2K4* loss and isogenic human induced pluripotent stem cells (hiPSCs) with patient-specific mutations, to elucidate the pathogenic mechanism of these mutations in neurodevelopment.

Poster 19A | CAMB - Gene Therapy and Vaccines

Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease

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Chronic kidney disease (CKD) is an insidious disease characterized by the progressive decline in kidney function, low glomerular filtration rate, and additional abnormalities such as albuminuria. Histologically, this disease is marked by extensive tubular destruction, fibrosis, and inflammation, contributing to global nephron loss. Over time, this loss of function necessitates renal replacement therapy through dialysis or kidney transplantation, and will result in death if left untreated. Despite the heavy disease burden, current therapies are typically aimed at controlling blood pressure, blood glucose levels, and other mitigative strategies to slow disease progression. However, no specific therapy yet exists for targeting fibrosis, the major contributor to the underlying pathology of CKD. Thus, new therapeutic approaches are necessary to target pathologic fibroblasts and fibrosis to restore kidney function and prevent disease progression. Chimeric antigen receptor T (CAR-T) cells are one such potential therapy. CAR-T cells are engineered T cells that combine the specificity of antibodies with the killing capacity of cytotoxic T cells. The CAR can target a cell surface protein specifically expressed by a pathologic cell type, activating the T cell to induce cytotoxic killing upon ligand engagement. Notably, pathologic fibroblasts specifically and robustly express the cell surface marker fibroblast activation protein (FAP), and we have previously shown CAR-T cells targeting FAP (FAP-CAR-T cells) can eliminate cardiac fibrosis and restore cardiac function in mouse models of hypertensive heart disease. Given these results as well as the extensive fibrosis in CKD, we hope to translate these FAP-CAR-T cells to mouse models of CKD in proof-of-concept studies aiming to reduce fibrotic burden and restore kidney function. Here, we demonstrate that FAP is specifically expressed in a mouse model of CKD, correlating with histologic fibrosis, expression of fibrosis related genes, and elevation of serum nitrogen and creatinine. We also show the relevance of this protein in human disease, where FAP is upregulated in both CKD and diabetic kidney disease, correlating with histologic fibrosis. Finally, we have initiated a pilot study of injecting FAP-CAR-T cells into our mouse model of CKD to show the feasibility of this approach in preventing disease progression. Further studies are needed to understand the therapeutic potential of these FAP-CAR-T cells in this and other models of kidney disease, as well as evaluating additional therapeutic targets, including other markers of activated fibroblasts and targeting the inflammatory milieu that also contributes to kidney injury.

Poster 20A | CAMB - Microbiology, Virology, & Parasitology

The Role of TRIM72 In Regulating Neuronal Antiviral Responses

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The innate immune response to viral infection, while studied in various organ systems, is understudied in the central nervous system (CNS). Understanding immune response regulation in the brain, a major portion of the CNS, is imperative because it is chiefly comprised of post-mitotic neurons. In addition to damage caused by virus replication and dissemination, the antiviral immune response can also trigger cytotoxic damage. As such, the CNS must tightly regulate physiologic immune responses to prevent tissue pathology during infections. One cause of neurotropic infections is the LaCrosse Virus (LaCV). LaCV is an RNA virus that predominantly infects neurons. It is also a leading cause of pediatric virus-induced encephalitis. As such, it is a useful tool for studying neuronal innate immune response. To identify potential regulators of these neuron-intrinsic immune responses, we conducted bulk RNA sequencing of LaCV-infected primary murine cortical neurons. Sequencing data revealed an upregulation of genes belonging to TRIM family of proteins (Figure 1). TRIM proteins function primarily as E3 ubiquitin ligases. Additionally, many TRIM proteins have defined antiviral roles. Particularly, TRIM72, in other models of infection and neuronal injury, plays a role in suppressing the type I interferon (IFN) induction and neuronal death. However, it is currently unknown if TRIM72 has a role during RNA virus infection in neurons. I hypothesize that TRIM72 downregulates the type I IFN response to hinder neuronal death during LaCV infection.

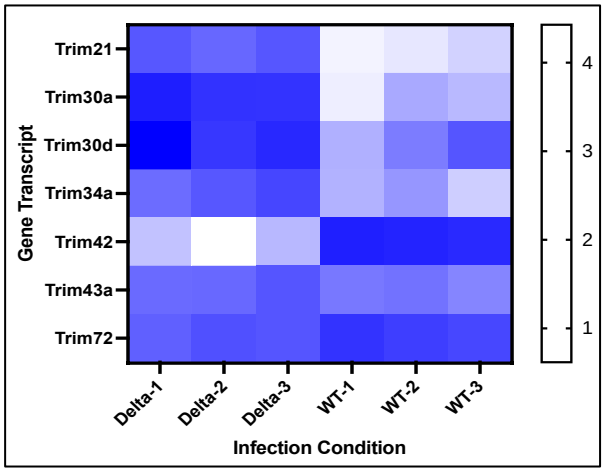

Figure 1: Trim E3 ligases bulk RNA-sequencing results of LaCV-infected primary mouse neurons. Neurons were isolated from embryonic day 16.5 C57Bl/6 mice, cultured for 9 days, then infected with LACV-ΔNSs (Delta1-3), WT-LACV (WT1-3). Heatmap displays log2FC of selected antiviral genes using unsupervised clustering; in each sample, the treatment condition (WT, or Delta) is relative to Mock-treated cells. Selected genes were derived from TRIM family.

To assess TRIM72's role in neuronal virus infection, I will use LaCV infection of primary murine cortical neurons. In **Aim One,** I will determine if TRIM72 hinders type I IFN induction by measuring secretion and transcripts of IFN-β, the predominant type I IFN. Further, I will assess if *Trim72* induction is dependent on nuclear transcription factor-κB (NFκB**)** and calcium homeostasis, two important components modeled in macrophages by *Sermersheim et al.* In **Aim Two**, I will determine if TRIM72 impacts the viability of neurons by assessing cell death during LaCV infection. I will also determine if GSK3β, a pro-apoptotic factor, is targeted by TRIM72 to promote neuron survival. Understanding the role of TRIM72 will provide novel insight into regulation of anti-viral immune responses in neurons. These aims will elucidate the potential role of TRIM72 in neuronal viral infection and may identify a therapeutic target for inflammation regulation.

Poster 21A | CAMB - Microbiology, Virology, & Parasitology

Understanding intestinal CD4+ T cell immunity using *Cryptosporidium* infection

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Many infectious and inflammatory diseases affect the gut and associated lymphoid tissue (GALT), which houses the largest number of lymphocytes in the body. It is therefore important to understand the regulation of tissue immunity to develop better therapies to manage disease. *Cryptosporidium* is a unicellular parasite that is restricted to intestinal epithelial cells (IECs) that can be used to understand immune regulation in the intestine. While infection and shedding of infectious oocysts is self-limiting in immunocompetent individuals, life-threatening chronic diarrhea and liver disease can occur in acquired and primary immunodeficiencies (AIDS, CD40L/CD40 deficiency, IL-21R deficiency). Because *Cryptosporidium* is restricted to the gut, it is a challenge to understand local T cell responses, though it has been previously established that sterilizing immunity requires CD4+ T cells and interferon gamma (IFN-γ). In order to characterize antigen-specific responses, transgenic *C. parvum* was engineered to express MHCII-restricted model antigens. Antigen-specific TCR-transgenic CD4+ T cells expanded in the gut and GALT in infected mice. These cells made IFN-γ and relied on type 1 conventional dendritic cells (cDC1s) for expansion and function. A prominent CD4+ T cell-dependent but IFN-γ-independent mechanism of control exists, as humans and mice deficient in IFN-γ are not susceptible to chronic infection. Our system revealed T cells produce IL-22 that limits infection, and therapeutic targeting using engineered cytokines suppressed infection. By using *Cryptosporidium* fecal oocyst shedding as a readout of gut immune function, this system can dissect immunity in the gut and probe therapies aiming to augment immunity within the tissue.

Poster 22A | CAMB - Microbiology, Virology, & Parasitology

The Branched Chain Amino Acids Control Macrophage Inflammation Through Translational Regulation of Cytokine Production

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Dysregulation in the levels of branched chain amino acids (BCAAs), including leucine, isoleucine, and valine, is common to a variety of disease states mediated by dysregulated inflammation, including cardiovascular disease and diabetes. Despite this, the role of BCAAs in inflammatory processes is poorly understood. Amongst their known roles, the BCAAs can 1) be catabolized to precursors of central metabolism, 2) act as signaling molecules, specifically with leucine inducing mTORC1-driven anabolic growth, or 3) be incorporated into proteins during translation. Here, we set out to test the impact of individual BCAAs on inflammatory macrophage function. Whereas much of the work on the BCAAs has focused on the BCAAs either as a unit, or with emphasis on leucine due to its role in mTORC1 signaling, little is understood how each of the BCAAs contributes to macrophage-mediated inflammation. We find that rather than acting in a uniform fashion, each BCAA has a unique capacity for regulating inflammatory macrophage cytokine production and this capacity is specific to BCAAs versus other essential amino acids. Indeed, the BCAAs regulate cytokine production at both the transcriptional and translational levels, and each BCAA regulates these steps differentially. We further show that the individual BCAAs have unique abilities to regulate both mTORC1-dependent and -independent signaling pathways within macrophages. Through genetic models, we are beginning to characterize how the balance of BCAA catabolism and signaling further contributes to macrophage-driven inflammation. Future work will uncover the impacts of BCAA catabolism and availability on macrophage-mediated inflammation *in vivo*. This work will reveal how changes to BCAA metabolites in patients may be contributing to macrophage-driven inflammation, opening the exploration of treatments targeting BCAA metabolism in a variety of disease contexts.

Poster 23A | Chemistry

Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase

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Collagen lays the foundation of bodily tissues, serving to strengthen, connect, and signal from the micro to the macro scale. The importance of collagen in cancer biology has been well-established: its functions in regulation of the tumor microenvironment—from increased stiffness of the extracellular matrix to dysregulation of cancer cell signaling—influence tumor proliferation and impenetrability. Key to these effects is the interaction between fibrillar collagens and the discoidin domain receptor type 2 (DDR2), a receptor tyrosine kinase implicated in multiple human cancers. Extracellular binding of fibrillar collagen to DDR2 transduces a cell signal that activates epithelial to mesenchymal transition, proliferation, and metastasis. This work seeks to develop synthetic collagen mimetic peptides (CMPs) for interrogation of the collagen-DDR2 interaction. As a permutated, triple helical polymer involved in biochemical signaling, collagen has potential for manipulation as a tool for modulating protein-protein interactions. However, these applications have been limited by its tripartite nature, which restrict its thermal and entropic stability. Synthetic linkage and/or cyclization of the three collagen strands may overcome these limitations. To this end, this work focuses on the synthesis, biophysical/structural characterization, and biological application of linked and cyclic CMPs targeted against DDR2 through two complementary methods. In the initial approach, we design maximally stabilized, miniaturized, double-stranded macrocyclic CMPs to target DDR2 via *in vitro* and *in cellulo* methodology. In the second approach, we develop a novel method to generate photocrosslinked, triple-stranded CMPs for targeting of DDR2 *in vitro* and *in cellulo*. Incorporation of DDR2 recognition sequences and strategic placement of unnatural amino acids within our CMPs will support studies of thermal/proteolytic stability, DDR2 binding affinity, and cell signal regulation. This work endeavors to develop chemical tools for modulating the collagen interactome via an innovative chemical biology approach, laying the foundation for new discoveries surrounding the role of collagen in cancer biology with potential for applications in drug discovery.

Poster 24A | Epidemiology & Biostatistics – Biostatistics

DeepCombat: A Statistically-Motivated, Hyperparameter-Robust, Deep Learning Approach to Harmonization of Neuroimaging Data

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Neuroimaging data acquired using multiple scanners or protocols are increasingly available. However, such data exhibit technical artifacts across batches which introduce confounding and decrease reproducibility. This is especially true when multi-batch data are analyzed using complex downstream models which are more likely to pick up on and implicitly incorporate batch-related information. Previously-proposed image harmonization methods have sought to remove these batch effects; however, batch effects remain detectable in the data after applying these methods. We present DeepComBat, a deep learning harmonization method based on a conditional variational autoencoder and the ComBat method. DeepComBat combines the strengths of statistical and deep learning methods in order to account for the multivariate relationships between features while simultaneously relaxing strong assumptions made by previous deep learning harmonization methods. As a result, DeepComBat can perform multivariate harmonization while preserving data structure and avoiding the introduction of synthetic artifacts. We apply this method to cortical thickness measurements from a cognitive-aging cohort and show DeepComBat qualitatively and quantitatively outperforms existing methods in removing batch effects while preserving biological heterogeneity. Additionally, DeepComBat provides a new perspective for statistically-motivated deep learning harmonization methods.

Poster 25A | Epidemiology & Biostatistics – Epidemiology

Predicting migraine in the pediatric emergency room

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Background: Identifying migraine in the pediatric emergency department (ED) is complicated by the wide range of other potential etiologies for headache. Currently, researchers identify patients with migraine based on either diagnostic coding or the use of certain headache treatments. However, recent work suggests that there are socioeconomic disparities contributing to the under-treatment and under-diagnosis of migraine, potentially introducing selection bias to migraine research.
Methods: We performed a retrospective chart review of patients ages 5-17 seen in the ED with a chief complaint of headache or migraine for the first time at the Children's Hospital of Philadelphia from January 2016 to February 2020 (prior to local pandemic-related shutdowns). We labeled patients as having migraine if they had a reported headache history and certain migrainous characteristics (location, severity, associated symptoms) in their provider note without having any visit diagnoses relating to injury or infection which would suggest secondary headache. Using ED visits that occurred between January 2016 and December 2018, we trained a state-of-the-art Natural Language Processing model—Bidirectional Encoder Representations from Transformers (BERT)—to predict whether a patient had migraine based on their history of present illness, past medical history, reported symptoms, and physical exam findings. We assessed the model's performance at predicting migraine using a separate dataset—visits that occurred between January 2019 and February 2020—calculating the areas under the receiver-operating characteristics curve (AUROC) and under the precision-recall curve (AUPRC). We then compared the sensitivity and specificity of the model against those from migraine diagnostic codes.
Results: We trained our BERT model on clinical notes from 703 children and adolescents seen for headache or migraine between January 2016 and December 2018. For the 133 cases in our validation set, we found that our BERT model's performance is promising with an AUROC of 0.920, and an AUPRC of 0.868. In identifying migraine, using migraine diagnostic codes has a sensitivity of 0.408 and a specificity of 0.905 whereas our model, with a cutoff of 0.03, currently has a sensitivity of 0.796 and a specificity of 0.857.
Conclusion: Our BERT classifier can identify migraine in children seen for headache based on their clinical presentation as reported in clinical notes with much greater sensitivity than migraine diagnostic codes. Our approach shows promise and we believe the model's performance will substantially improve with a larger training sample size and may be used to reliably identify children with migraine even if they did not receive the diagnosis code of migraine during their visit. Due to the high amount of under-diagnosis for migraine, our hope is that this strategy will help us to develop clinical tools to identify children in need of more aggressive long-term treatment.

Poster 26A | Genomics & Computational Biology

Characterizing ERV transcripts across healthy human tissues

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Endogenous Retroviruses (ERVs) are a group of transposable elements (TEs) that comprise roughly 8% of the human genome. ERVs often contain remnant open reading frames, allowing them to produce protein products and implicating them in neurodegeneration and cancer. However, their exact roles in health and disease remain elusive due to difficulties capturing their complex genetic structures. With insertion sizes that often span >7kb, and their frequently spliced transcripts, ERVs are challenging to study with standard next-generation sequencing approaches. Recent advances in long-read sequencing technology support more comprehensive detection and quantification of TEs. We have developed ESPRESSO-TEA (Error Statistics PRomoted Evaluator of Splice Site Options – Transposable Element Analysis), a computational pipeline to profile locus-specific TE expression from long-read RNA-seq. ESPRESSO-TEA identifies and quantifies different types of TE transcript subtypes, including those of ERVs. Our workflow generates novel ERV annotations that classifies ERV transcripts as monoexonic or multiexonic ERVs, broadly representing proviral ERVs and spliced ERVs. Here, we apply ESPRESSO-TEA to characterize ERV expression patterns across 30 healthy human tissues. We observe that ERVs are lowly expressed across all tissues, but certain tissues, like testes and skeletal muscle, express relatively higher and lower levels of ERVs compared to that of other tissues, respectively. Furthermore, we observed that every tissue expresses both monoexonic and spliced ERV transcripts, but mostly spliced ERV transcripts. Using this platform, we investigated the highest expressed ERVs across brain tissues and identified tissue-specific ERV transcripts. Our work emphasizes that widely used ERV annotations are often unreliable and highlights the importance of delineating ERV transcript expression at an isoform-specific level. Lastly, we establish the ERV transcriptional landscape across healthy human tissues, which will serve as a baseline as we move forward investigating ERV transcripts in various disease contexts.

Poster 27A | Genomics & Computational Biology

Exploiting cell cycle dynamics to interrogate YY1's role in spatiotemporal chromatin organization

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Chromatin structure and gene expression fluctuate dramatically throughout the cell cycle, yet the transcription factor binding patterns driving these precise dynamics remain understudied. Here, we comprehensively survey the roles of transcription factor YY1 via acute degradation both in asynchronous cells and in synchronized, purified cells traversing mitosis into G1 phase. YY1 shows site-specific mitotic retention and rapidly binds to many promoters prior to G1 entry. Fast recruitment of YY1 to chromatin facilitates early establishment of YY1-dependent loops. YY1 facilitates looping independently of CTCF/cohesin-mediated looping and cohesin extrusion blocking. Integrating findings from asynchronous and mitotic depletions, we uncover a subset of chromatin loops and genes which require YY1 for establishment after mitosis yet remain stable without YY1 in interphase. Thus, cell cycle stage can critically influence the permissiveness of chromatin looping and gene expression to transcription factor perturbation.

Poster 28A | History and Sociology of Science

"No one had ever told us of the human problems we should be called upon to face": The Role of Medical Students in the 1918 Influenza Pandemic in Philadelphia

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The COVID-19 pandemic created a tricky question for medical students: what is our role in responding to this burgeoning disaster? While scattered references to the 1918-1919 influenza pandemic abound, little is known or critically studied regarding medical students' roles in the 1918 flu pandemic. This project explores the experiences of medical students at Philadelphia medical schools in 1918-1919. Examining medical school yearbooks and their cartoons, poems, and writings, several themes emerge: confusion and lack of preparedness, negotiation of new roles and a sense of duty, and fear and ambiguity. Though the 1918 influenza epidemic does not seem to have enacted curriculum changes in US medical schools, students' reflections show an important case of medical students' struggle to make sense of a brief and traumatic experience on the wards, and represents a moment of professional identity crystallization. Varied reflections on the 1918 pandemic demonstrate that while pandemics can offer new autonomy to students, they are also devastating and traumatic experiences that are difficult to make sense of, especially in the immediate aftermath.

Poster 37A | Immunology

Dysregulation of the hepatic CD4 T cell compartment during childhood obesity

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Childhood obesity is a systemic inflammatory disease of epidemic proportion that affects nearly 15 million American children. Obesity strongly predisposes children to metabolic comorbidities such as non-alcoholic fatty liver disease (NAFLD) later in development, but the factors sustaining obesity-associated inflammation from childhood onwards remain unclear. The T Helper 1 (Th1) subset of liver-resident CD4 T cells is thought to drive mild fatty liver towards inflammatory nonalcoholic steatohepatitis, but these findings derive from adult patients and mouse models that cannot capture early life mechanisms of immune programming by childhood obesity. Perturbation of early life CD4 T cell development can durably shape their function later in adulthood, but the chronic effects of childhood obesity on CD4 T cell development and inflammatory function in the liver remain unknown. Alarmingly, we have no FDA-approved therapies for pediatric NAFLD that restrain liver inflammation towards fibrosis in adulthood, and lifestyle interventions have poor efficacy outside the research setting. Thus, there is an urgent need to identify mechanisms by which childhood obesity programs CD4 T cells to drive chronic fatty liver inflammation with age.

To address this question, we employed a mouse model recapitulating aspects of childhood obesity wherein progeny receive high-fat diet (HFD) from in utero development onwards through adolescence, enabling CD4 T cell pathology to develop in HFD mice before maturity. Profiling hepatic CD4 T cell subsets by flow cytometry revealed a striking expansion of Th1 cells in young HFD relative to normal control diet (NCD) mice. We observed a corresponding increase in Th1 cells in the spleen, the largest lymphoid organ that directly supplies the liver with CD4 T cells via the portal vein. Increased numbers of circulating Th1 cells are known to correlate with NAFLD diagnosis in children with obesity, suggesting that early life Th1 programming in our HFD mouse model may be clinically relevant. These data highlight dysregulation of the CD4 T cell compartment by early life HFD that may shape chronic fatty liver inflammation over time.

To identify signaling pathways by which childhood obesity programs CD4 T cell differentiation, we performed single-cell RNA-sequencing on purified hepatic leukocytes from young NCD and HFD mice. Subclustering upon CD4 T cells revealed a several-fold expansion in the proportion of cells expressing Th1-associated transcripts, corroborating our flow cytometry findings by an orthogonal approach. By performing differential gene expression testing on hepatic CD4 T cells, we found a striking enrichment in Type 1 Interferon (IFN)-stimulated genes in HFD relative to NCD. Type 1 IFN is known to promote Th1 differentiation and NAFLD in adult mouse models, raising the hypothesis that Type 1 IFN shapes the developing immune system during childhood obesity to drive NAFLD. Together, these data provide the basis for further work dissecting IFN signaling and dysregulation of CD4 T cell development in the pathogenesis of pediatric NAFLD.

Poster 29A | Immunology

Autosomal dominant C-terminal TREX1 frameshift mutants inhibit homology-directed repair and increase risk of breast cancer

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TREX1 is a 3'-5' DNA exonuclease that is normally anchored to the endoplasmic reticulum membrane and excluded from the nucleus. In humans, inherited C-terminal TREX1 variants result in nuclear localization of TREX1 and pathology resembling radiation injury. Previously, it was suggested that TREX1 truncation variants cause disease via haploinsufficiency or type I interferon. Here, we show that nuclear TREX1 triggers DNA damage in Drosophila, humans, and mice. We find that C-terminal TREX1 frameshift variants increase the risk of breast cancer before age 45, resembling the risk of BRCA1 variants. Whereas both full-length and C-terminally truncated TREX1 inhibit homology-directed repair, only the truncation mutant causes vulnerability to PARP inhibitors in primary cells and in mice. We do not observe up-regulation of type I interferon or a role for haploinsufficiency. Thus, nuclear TREX1 promotes DNA damage, a discovery that likely explains why inherited, C-terminal TREX1 variants cause pathology mimicking radiation injury.

Poster 30A | Immunology

Hepatic CD9 regulates adipose tissue function and inflammation during obesity

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Obesity is a major cause of morbidity and mortality because it increases the risk of type II diabetes, among other conditions. In response to excess caloric intake, adipose tissue (AT) undergoes extensive remodeling leading to AT expansion, inflammation, and, ultimately, fibrosis. Extracellular vesicles (EVs) are nanometer-sized lipid particles that facilitate intercellular and interorgan communication via the transport of cargo molecules. Recently, it has been suggested that EV-mediated communication between the liver and AT is critical for proper AT response in obesity. However, the mechanisms regulating this communication network are not well known. We hypothesized that the tetraspanin CD9 regulates an EV-mediated liver-AT axis that supports normal AT function and mammalian metabolism during obesity. We found that knockdown of CD9 in a hepatocyte cell line results in decreased production of small EVs. Furthermore, using liver-specific CD9-GFP reporter mice, we found that CD9+ EVs can be transferred from the liver to adipocytes and AT immune cells. To determine the impact of liver CD9 during obesity, we generated a hepatic-cell specific CD9 knock-out mouse (CD9 HKO). We found that CD9 HKO mice exposed to a model of diet-induced obesity had decreased adipocyte size, increased AT inflammation, and increased AT fibrosis compared to control mice, consistent with increased AT remodeling. Furthermore, CD9 HKO mice are more susceptible to obesity-associated sequelae including glucose intolerance, hyperlipidemia, and ectopic lipid deposition. Overall, our results suggest that hepatic CD9 supports normal AT function and acts to restrain AT inflammation, ultimately protecting against the development of obesity-associated sequelae. Future studies will aim to understand the mechanisms by which hepatic CD9 influences EV production, content, and function in AT. Understanding the details of this mechanism of interorgan communication will improve our understanding of mammalian metabolism and aid in the development of novel therapeutics for obesity and its complications.

Poster 31A | Immunology

Understanding the Role of Germinal Centers in the Generation and Durability of nAbs to mRNA-LNP vaccination

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The first few years of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were marked by an unprecedented race to develop a vaccine. Two resultant highly effective vaccines, both of which use messenger RNA (mRNA) technology, were subsequently approved by the Food and Drug Administration. These nucleoside-modified mRNA vaccines encapsulated in lipid nanoparticles (LNPs) drive potent protective immune responses, including elevated total and binding and neutralizing antibody (bAb, nAb) titers that last for several months and confer protection against subsequent viral challenge. However, the mechanism by which these mRNA-LNP vaccines induce the generation of nAbs remains yet undefined.

Traditional protein-adjuvant vaccines canonically rely on germinal center (GC) responses to select for high-affinity long-lived plasma cells (LLPCs) and memory B cells (MBCs) in secondary lymphoid organs. Our lab has recently demonstrated that SARS-CoV-2 mRNA-LNP vaccines are extremely efficient at eliciting GC responses as well as subsequent high Ab titers, LLPCs, and MBCs lasting at least a couple months post immunization. But while GCs are readily observable in response to mRNA-LNP administration, it has not been shown yet whether the GC response is absolutely necessary for nAb production for this vaccine platform, and it is unclear whether the GC reaction elicited is sufficient for the most optimal humoral response. Notably, one group has calculated that the half-life of bAbs generated by the yellow fever vaccine is around 3000 years while the half-life of bAbs generated by the BNT162b2 SARS-CoV-2 mRNA-LNP vaccine formulated by Pfizer is around 65 days in COVID-naïve individuals. Intriguingly, the Eisenbarth group has suggested infection also seems to differ from vaccination in that GC responses are not required for nAb production in the setting of COVID-19 infection.

Together, these data suggest that there is potential for improving mRNA-LNP vaccine response durability if we can better understand GC biology in response to vaccination – specifically, elucidating the relative contributions of GC versus non-GC responses to humoral immunity post-antigen exposure, and investigating differential signaling to Tfh cells in the settings of our human-made vaccinations versus natural infection. This project aims to elucidate the role of GCs in the formation of long-lived humoral responses in mRNA-LNP vaccination (MBCs, LLPCs, bAb, nAb, isotype switching, somatic hypermutation) using mouse models of attenuated GC responses.

Poster 32A | Immunology

Can microbial molecular mimics protect from type 1 diabetes?

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The early-life gut microbiota protects from type 1 diabetes (T1D), but the mechanisms of this protection remain poorly understood. Interestingly, colonization by a gut commensal that expresses an insulin-peptide molecular mimic promotes diabetes in adult non-obese diabetic (NOD) mice. This finding provokes the hypothesis that molecular mimicry protects from T1D when instead encountered during known early-life tolerogenic windows. Early-life colonization of NOD mice with PedsCom, a novel defined consortium of nine early-life commensals, significantly reduces diabetes incidence compared to both GF NOD mice and NOD mice colonized with PedsCom as adults. Bioinformatics analysis of PedsCom members identifies several potential insulin-peptide mimics. In preliminary experiments, we have observed *in vitro* recognition of one predicted mimic by an insulin-recognizing T cell hybridoma. We have also confirmed expression of this predicted mimic in the intestinal contents of PedsCom-colonized NOD mice, thus supporting its potential recognition by T cells *in vivo*.

Poster 33A | Neuroscience

Lysosomal lipid accumulation leads to macrophage dysfunction in Krabbe disease

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KD is a lysosomal storage disease that presents as a neurodegenerative condition due to mutations in *Galc* – a gene encoding a lysosomal hydrolase that breaks down galactosylceramide (galcer). Pathologically, these mutations cause extensive demyelination, the presence of lipid-laden macrophages (globoid cells), and neuroinflammation. Patients with the infantile form show limb stiffness and seizure before rapid regression in motor and cognitive function, and eventual death within 2 years of age. The mainstay treatment is bone marrow transplant (BMT), which greatly extends survival and improves neurological outcomes if given before symptom onset, typically in the first 2 months of age. A prevailing hypothesis is that BMT works by restoring functional GALC in macrophages. In support, mechanistic murine studies of macrophage-sparing *Galc* knockout mice showed that GALC-competent macrophages did not develop globoid morphology, improved myelin pathology, and reduced neuroinflammation. These data, paired with the therapeutic effects of BMT in mice and patients, suggest that lipid-laden macrophages play a key role in KD pathogenesis. Yet, how lysosomal lipid accumulation causes macrophage dysfunction is unknown.

The twitcher mouse ($Galc^{TWI}$) is a widely accepted model of KD that recapitulates many aspects of human disease, including the presence of lipid-laden macrophages in areas of injury caused by defective GALC. To understand how lipid accumulation causes macrophage dysfunction, we challenged $Galc^{WT}$ and $Galc^{TWI}$ macrophages with galcer – the lipid that cannot be metabolized in $Galc^{TWI}$ cells. We found that lipid-treated $Galc^{TWI}$ but not $Galc^{WT}$ macrophages demonstrated rounded morphology and larger lysosomes. In tandem with these gross changes, lipid-treated $Galc^{TWI}$ macrophages highly expressed genes associated with metabolic and immune activation pathways compared to lipid-treated $Galc^{WT}$ cells. Importantly, many of these transcriptional changes were also seen in a specific macrophage cluster identified by single cell sequencing of end-stage twitcher mice. Together, these data elucidate how lipid accumulation impacts macrophages and establish an in vitro culture model to study lipid-laden macrophages in KD.

Poster 34A | Neuroscience

Cell type-specific vulnerabilities in behavioral-variant Frontotemporal Dementia caused by *C9orf72* expansion mutations

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Expansion mutations in *C9orf72* are the most common genetic cause of Frontotemporal dementia (C9-FTD) and Amyotrophic Lateral Sclerosis (C9-ALS). Clinically, C9-FTD frequently presents as behavioral-variant FTD (bvFTD), in which patients display behavioral disinhibition, apathy, and loss of empathy. Pathologically, C9-FTD presents as Frontotemporal lobar degeneration due to TDP-43 inclusions (FTLD-TDP), in which neurons exhibit pathologic loss of nuclear TDP-43 and gain of phopho-TDP-43 inclusions. The medial orbitofrontal cortex (mOFC) is affected early and severely in disease pathogenesis, but the molecular identities and phenotypes of vulnerable neuron and glial cell types in the mOFC have not been studied at the cellular resolution. To assess cell type-specific vulnerabilities in C9-bvFTD due to FTLD-TDP, we performed single nucleus RNA sequencing of the mOFC from 10 C9-bvFTD patients, 3 C9-ALS patients without mOFC pathology, and 10 neurologically normal controls. We identified pathologic neuronal cell types that were selectively lost in C9-bvFTD and pathologic glial cell types that were enriched in C9-bvFTD.

Poster 36A | Neuroscience

The Aged Microbiome Drives Cognitive Decline via Intestinal Inflammation and Vagal Inhibition

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Aging is a complex process in which the body loses fitness and resiliency. Alzheimer's disease and age-associated cognitive decline are devastating diseases experienced by tens of millions of people worldwide. The collection of microorganisms living in or on the body, known as the microbiome, also changes with age, becoming less diverse and accumulating pathogenic species over time. The mammalian microbiome resides primarily in the gut, with the well-established gut-brain axis mediating bidirectional communication between the enteric and central nervous systems. While it is known that the gut microbiome can modulate brain function based on studies showing causal effects of the microbiome on psychiatric and neurological conditions including depression and autism, it remains an open question whether age-associated changes in cognition and microbiome composition are causally linked. Studies have shown that performing fecal microbiome transplant (FMT) of stool from old into young mice is sufficient to induce cognitive deficits, suggesting that age-related changes in the microbiome can impair cognition, yet the underlying mechanisms remain largely unclear. The identification and mechanistic understanding of a causal relationship would open the possibility of entirely novel therapeutics for the highly prevalent, incurable, and devastating cognitive decline associated with aging.

To investigate the effect of the aged microbiome on cognition, I developed a cohousing paradigm in which young (2-month-old) mice are cohoused with old (18-month-old) mice. After 1 month, young cohoused mice exhibit impaired performance compared to control young mice in novel object recognition (NOR) and the Barnes Maze test, two tasks of learning and memory. This effect is not seen in antibiotics-treated mice or germ-free mice and is recreated upon FMT of stool from old mice into young germ-free mice. When exposed to a novel object, the hippocampus in young and old mice with an aged microbiome show reduced neuronal activation. Thus, the aged microbiome impairs cognition and neuronal response to novelty. Using 16S rDNA sequencing, I identified a bacterial species, *Parabacteroides goldsteinii*, which is increased in aging and is sufficient to cause cognitive impairment when colonized in young mice.

I then hypothesized that the *P. goldsteinii*-induced cognitive impairment was mediated by a small molecule produced by the bacteria. Small molecules isolated from the culture supernatant of this bacteria were sufficient to induce cognitive impairment. Untargeted mass spectrometry identified 3-hydroxyoctanoic acid (3-HOA) as a candidate metabolite, and behavioral testing confirmed its ability to impair cognitive after 5 days of oral gavage. Further pharmacological experiments demonstrated that 3-HOA signaling requires the GPR84 receptor, which is necessary and sufficient for the phenotype. Finally, macrophage, but not T cell, depletion, along with an antibody targeting tumor necrosis factor alpha (TNFα) were able to attenuate cognitive impairment. Thus, the bacterium *P. goldsteinii* is increased in the microbiome with age and impairs cognition; it produces a metabolite, 3-HOA, that signals through GPR84 on macrophages increasing TNFα production. All of these effects are reversible with vagal nerve stimulation, suggesting that the downstream effect is vagal nerve inhibition.

Poster Session B

Biochemistry & Molecular Biophysics

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Presenter: Prateek Sharma | Advisors: Drs. Christoph Thaiss and Katy Wellen

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Presenter: Emily Shea | Advisor: Dr. Lewis Chodosh

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Presenter: Sweta Narayan | Advisor: Dr. Mark Kahn

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Presenter: Daniel Yen | Advisor: Dr. Nancy Speck

Genetics and Epigenetics

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Ferroptosis enhances epidermal cornification through transcriptional and metabolic reprogramming
Presenter: Napasorn (Nina) Kuprasertkul | Advisors: Drs. Brian Capell and Katy Wellen

Poster 11B
Burning in cellular memory as a mechanism for phenotypic enhancement in metastatic melanoma
Presenter: Jingxin (Jessica) Li | Advisor: Dr. Arjun Raj

Poster 12B
Novel class of pediatric neurodevelopmental "histonopathies" may be driven by shared mechanism of chromatin dysregulation
Presenter: Emily Lubin | Advisor: Dr. Elizabeth Bhoj

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Hemodynamics and KLF2/4 regulate myxomatous valve formation
Presenter: Jesse Pace | Advisor: Dr. Mark Kahn

Poster 14B
Pathogenic laminopathy mutations disrupt specific lamina-associated regions in cardiac myocytes potentially via altered mechanosensing
Presenter: Kaitlyn Shen | Advisor: Dr. Raj Jain

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Elucidating the spatially coordinated mechanisms of heterochromatinization in fragile X syndrome
Presenter: Kenneth Pham | Advisor: Dr. Jennifer Phillips-Cremins

Microbiology, Virology and Parasitology

Poster 15B
What makes a common cold virus? Respiratory viruses differentially interface with host interferon responses in the nasal epithelium
Presenter: Clayton Otter | Advisor: Dr. Susan Weiss

Poster 16B
Deficits in mitochondrial oxidative phosphorylation enhance SARS-CoV-2 replication via metabolic remodeling
Presenter: Yentli Soto Albrecht | Advisor: Dr. Doug Wallace

Epidemiology & Biostatistics

Poster 17B
Algorithmic reliability and dataset shift in a national readmission and mortality prediction algorithm
Presenter: Likhitha Kolla | Advisor: Dr. Jinbo Chen

Genomics & Computational Biology

Poster 18B
Interactions between the gut microbiome, dietary restriction, and aging in more than 900 genetically diverse mice over the lifespan
Presenter: Lev Litichevskiy | Advisor: Drs. Christoph Thaiss and Mingyao Li

Poster 19B
Single-Cell Multiomic Analysis of Pediatric High Grade Glioma Under Therapy
Presenter: Jonathan Sussman | Advisor: Dr. Kai Tan

Poster 20B
The LSV-Seq method enables machine learning-assisted recovery of low-coverage splicing events across human tissues.
Presenter: Kevin Yang | Advisors: Drs. Peter Choi and Yoseph Barash

Poster 21B
Investigating ancestry-specific genetic variation in apolipoprotein L genes associated with electronic health record phenotypes in diverse patient biobanks
Presenter: David Zhang | Advisors: Drs. Dan Rader and Marylyn Ritchie

Immunology

Poster 22B
The role of the Type III Secretion System in endogenous presentation of Salmonella enterica epitopes on MHCII
Presenter: Kathleen (Kate) Krauss | Advisor: Dr. Ike Eisenlohr

Poster 23B
Fate induction in chimeric antigen receptor T cells through asymmetric cell division
Presenter: Casey Lee | Advisor: Dr. Aimee Payne

Poster 24B
Obesity-associated long chain fatty acids regulate lung innate immune responses via the NLRP3 inflammasome.
Presenter: Samuel (Sam) McCright | Advisor: Dr. David Hill

Poster 25B
T cell homing to the small intestine during Cryptosporidium infection
Presenter: Maria Merolle | Advisor: Dr. Chris Hunter

Poster 26B
Excess IL-18 Augments Suppressor/Regulatory Cell Function to Prevent Experimental Autoimmune Encephalomyelitis
Presenter: Jeremy Morrissette | Advisor: Dr. Scott Canna

Poster 27B
Deficiency of the Pattern-recognition Receptor CD14 Protects Against LPS-induced Inhibition of Osteoclastogenesis in Vitro
Presenter: Lance Murphy | Advisors: Drs. Carla Scanzello and Rob Mauck

Poster 28B
Elicitation of V3 glycan-directed cross-neutralizing antibodies in sequentially immunized, SHIV-infected rhesus macaques
Presenter: Ashwin Skelly | Advisors: Drs. Amelia Escolano and Beatrice Hahn

Neuroscience

Poster 29B
Behavioral paradigm for investigation of the relationship between visually-evoked traveling waves and perception in mice.
Presenter: Claudia Heymach | Advisor: Dr. Alex Proekt

Poster 30B
Investigating the Role of Somatostatin-Positive Low-Threshold Spiking Interneurons in the Dorsomedial Striatum in Goal-Directed Behavior
Presenter: Evan Iliakis | Advisor: Dr. Marc Fuccillo

Poster 31B
Mapping the development of white matter structural organization and overall psychopathology
Presenter: Audrey Luo | Advisor: Dr. Theodore Satterthwaite

Poster 32B
Phosphoproteomic profiling to uncover molecular mechanisms of neurologic deficits in a mouse model of CDKL5 deficiency disorder
Presenter: Dayne Martinez | Advisor: Dr. Joe Zhou

Poster 33B
Transdiagnostic polygenic risk, psychopathology, and personalized functional brain networks in the Adolescent Brain Cognitive Development cohort
Presenter: Kevin Sun | Advisors: Dr. Aaron Block-Alexander and Theodore Satterthwaite

Poster 34B
Monosynaptic tracing defines circuit connectivity of human glioblastoma
Presenter: Yusha Sun | Advisor: Dr. Hongjun Song

Pharmacology

Poster 35B
Engineering cellular systems for biomedical imaging & diagnostics
Presenter: Jonathan Pham | Advisor: Dr. Mark Sellmyer

Poster 1B | Biochemistry & Molecular Biophysics

Structure and organization of the human centromere on purified chromosomes revealed by cryo-electron tomography

Kathryn Kixmoeller, Yi-Wei Chang, Ben Black

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The centromere is the chromosomal locus where the kinetochore protein complex assembles and binds to microtubules of the mitotic spindle in dividing cells. This interface is crucial for the proper segregation of chromosomes during cell division. This project employs cryo-electron tomography (cryo-ET) to directly visualize the 3D structure and organization of the human centromere on purified chromosomes. The centromeres of purified chromosomes can be detected by cryo-confocal imaging of a fluorescent tag attached the centromeric histone CENP-A, and correlative light and electron microscopy (CLEM) allows targeting of the same loci for cryo-ET. This approach reveals that the centromere consists of distinct chromatin clearing(s) containing kinetochore protein complexes. These clearings have a lower density of particles and higher inter-particle distance compared to surrounding chromatin. The larger protein complexes they contain are consistent with *in vitro* structural studies of the inner kinetochore complex. This chromatin architecture is not observed at non-centromeric loci. Rapid depletion of CENP-C, a key kinetochore component, leads to disruption of centromeric architecture, confirming the identification of the centromere within tomograms and supporting a key role for CENP-C in organizing centromere architecture. Characterization of centromere tomograms gives insight into the kinetochore complexes themselves and their organization along centromeric DNA. Centromere tomograms also reveal elements of the outer kinetochore including the fibrous corona, a transient fibrous mesh that contributes to microtubule capture by unattached kinetochores. This direct visualization of the centromere *in situ* within chromosomes has revealed many novel insights into the structure and organization of centromeric chromatin.

Poster 2B | Bioengineering

Cell type-specific relationship between higher-order chromatin folding and short tandem repeat instability in Huntington's Disease

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One of the most common of short tandem repeat (STR) expansion disorders is Huntington's Disease (HD), a neurodegenerative condition caused by more than 36 CAG triplets in exon 1 of the *HTT* gene. Recent studies have shown that age of HD onset correlates with increased CAG length from somatic instability. A central mystery in HD pathogenesis is how CAG tract instability arises and leads to selective degeneration of striatal and cortical projection neurons, despite ubiquitous *HTT* expression.

This study aims to understand the mechanism of cell-type specific degeneration in HD by elucidating the relationship between higher-order chromatin folding and *HTT* CAG instability across multiple cell types. Chromatin is folded into megabase (Mb)-sized topologically associating domains (TAD) and subTADs, and their boundaries are often occupied by architectural proteins such as CTCF and cohesin. Based on many studies demonstrating the close link between domain boundaries and STR instability, I hypothesize that vulnerable cell types in HD display somatic instability of the CAG tract due to its localization to domain boundaries enriched with CTCF/cohesin.

To test this hypothesis, I will first study domain boundary disruption due to a knock-in CAG tract of various lengths at *HTT*. I will differentiate isogenic human embryonic stem cell lines with various knock-in CAG lengths to different cell types, map chromatin folding with *in situ* Hi-C, and measure CAG tract length with long-read Nanopore sequencing. I hypothesize that knock-in of a disease-length CAG tract is sufficient to disrupt local domain boundaries in a cell type-specific manner. Second, I will study STR instability, domain boundary integrity, and CTCF/cohesin occupancy in HD patient-derived cortex and striatum organoids. Across various cell types, I will perform Nanopore sequencing of the *HTT* CAG tract to quantify somatic instability, single nucleus methyl 3C seq (sn-m3c-seq) to assess chromatin folding, and single cell combinatorial targeted insertion of promoters sequencing (sci-TIP-seq) to assess CTCF/cohesin occupancy. I hypothesize that vulnerable cell populations in HD will display increased CAG tract expansion and localization of the CAG tract to an intact domain boundary with dense CTCF/cohesin occupancy compared to resilient cell types. This study begins to shed light on why certain cell types are more susceptible to the same inherited *HTT* CAG tract than others through the study of higher-order chromatin folding and STR instability in a cell type-specific manner.

Poster 3B | Bioengineering

Structural and Functional Impacts of Early Type III Collagen Reduction on Tendon Healing

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Healthy tendons have a characteristic, highly aligned extracellular matrix comprised primarily of type I collagen. This hierarchical structure affords tendons their high tensile strength, allowing them to transmit large forces generated during muscle contraction into skeletal movement. In contrast, after both acute and chronic injury, tendons heal with a structurally and functionally compromised matrix, impacted by persistent fibrovascular scarring and increased type III collagen (Col3). Accordingly, patients with tendon pathology suffer from lasting functional deficits and high re-injury incidence. To guide improved therapeutic strategies for tendon injury, contributions of Col3 to structural and functional aspects of tendon healing must be better understood. We leverage the first mouse model of inducible Col3 reduction to determine the dose-dependent impacts of Col3 loss at the time of injury on structural and functional outcomes of patellar tendon healing. Tamoxifen is administered to achieve excision of both (Rosa-CreER^{T2}; *Col3a1*$^{F/F}$), one (Rosa-CreER^{T2}; *Col3a1*$^{F/+}$), or neither (Rosa-CreER^{T2}; *Col3a1*$^{+/+}$) *Col3a1* allele(s) at the time of injury. Subsequently, biopsy punch (0.75 mm diameter) injury is performed on bilateral patellar tendons of mature mice (p90). Structural and functional outcomes are assessed one, three, and six weeks after injury. Structural tendon properties are assessed with immunofluorescent staining for Col3 and second harmonic generation microscopy for collagen organization. Functional tendon properties are assessed with a custom viscoelastic mechanical testing protocol. Early results suggest that Col3 reduction at the time of injury weakens the early healing matrix but yields a more aligned late healing matrix. Continued analysis will increase sample sizes for both structural and functional assessment to further delineate the impact of Col3 on tendon healing. As modulation of Col3 is becoming more clinically realistic, results from this study will be essential for guiding their therapeutic application in the context of tendon injury.

Poster 36B | Bioengineering

Biopolymer-based Bicontinuous Hydrogels Guide Rapid 3D Cell Migration

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Cell migration is critical to tissue development and regeneration and, in 3D, requires conducive extracellular environments. Hydrogels are valuable for probing the extracellular regulators of 3D migration, but often do not recapitulate the naturally heterogeneous and anisotropic environments. For example, the presence of collagen interfaces have been shown to provide microtracks for migration *in vivo*, yet what features of these critical biophysical cues regulate migration remain largely unexplored. To better understand the role of extracellular interfaces on cell migration, we turn towards bicontinuous hydrogels, whose continuous subdomains and high surface area create inherent 3D interfaces. These interfaces guide rapid 3D cell migration across cell types and in *in vitro* and *in vivo* environments. Cell migration along these 3D interfaces interestingly does not depend on material composition or mechanical gradients but relies on continuous subdomains. Our findings suggest the importance of local interfaces on supporting migration and have implications for designing engineered tissue.

Poster 4B | CAMB – Cancer Biology

Effects of KRAS inhibition on anti-tumor immunity in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) has an estimated 5-year survival rate of only 12% and is estimated to become the second leading cause of cancer-related deaths in the US by 2030. Thus, alternative therapies are desperately needed. Despite recent advances in other tumor types, immunotherapy has yet to be effective in PDAC. One factor that limits its efficacy is the highly immunosuppressive 'cold' tumor immune microenvironment (TIME). Our lab utilizes 'cold' and 'hot' cell lines derived from single cell clones from KPCY tumors, the autochthonous PDAC mouse model. 'Hot' tumors, marked by high anti-tumor T cell infiltration, respond to immunotherapy, while 'cold' tumors, marked by an abundance of immunosuppressive cells and few T cells, are unresponsive. During my thesis, I aim to investigate tumor-intrinsic mechanisms underlying the TIME of cold PDAC tumors and reveal therapeutic approaches that remodel the TIME from cold to hot, thereby sensitizing unresponsive tumors to immunotherapy.

One such driver of immunosuppression is oncogenic KRAS signaling. As 90% of PDAC patients have activating *KRAS* mutations, KRAS is an attractive therapeutic target to turn tumors from cold to hot. In addition to promoting dramatic tumor regressions *in vivo*, treatment with a $KRAS^{G12D}$ inhibitor promotes the infiltration of T cells into cold PDAC tumors, suggesting that KRAS inhibition can augment anti-tumor immunity. Furthermore, inhibition of multiple KRAS alleles with a novel inhibitor ("$KRAS^{multi}$") increases the durability of tumor regressions. Interestingly, preliminary data shows that depletion of CD4 and CD8 T cells hinders the efficacy of $KRAS^{multi}$ inhibition, suggesting a role for T cell-mediated immunity in the anti-tumor response. Additionally, preliminary data suggests that $KRAS^{multi}$ inhibition may synergize with immunotherapy regimens. However, the mechanism through which inhibition of oncogenic KRAS regulates the immune system remains unknown. Thus, my thesis work will aim to 1) elucidate the mechanism by which KRAS inhibition influences anti-tumor immune cells in the TIME and 2) determine the role of the immune system in the anti-tumor response with KRAS inhibition. The results of this work will highlight the use of KRAS inhibition as a tool to alleviate immunosuppressive components of the TIME thereby enhancing response to immunotherapy and improving outcomes for PDAC patients.

Poster 5B | CAMB – Cancer Biology

Single-cell spatial multiomic profiling of cellular and metabolic neighborhoods in the pancreatic ductal adenocarcinoma tumor microenvironment

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Pancreatic ductal adenocarcinoma (PDAC) is a hypovascular and highly stromally-infiltrated neoplasm of the exocrine pancreas. Due to poor access to vascularly-derived nutrients and O_2, PDAC tumors are chronically hypoxic and nutritionally-stressed, rendering them critically dependent on cooperative metabolic exchanges with stromal elements for cancer cell survival and anabolism. For example, cancer-associated fibroblasts and their quiescent precursors, pancreatic stellate cells, generate amino acids and lipid species which are consumed by cancer cells to promote cell-intrinsic metabolic activities such as TCA cycle flux. However, prior studies of metabolic crosstalk in PDAC have been hampered by reliance on co-culture models, which fail to recapitulate stromal complexity and physiologic nutrient availability, and bulk/single-cell RNA sequencing studies, which require tissue dissociation that destroys spatially-encoded information. Since metabolic cooperation should occur mainly over short distances (ie the diffusion of effluxed small-molecule metabolites at bicellular metabolic synapses), we reasoned that spatial profiling of metabolically-informative protein and RNA analytes in human PDAC tumors, at single-cell resolution, would reveal novel pathways of metabolic communication which could later be interrogated mechanistically for pro- or anti-tumor function. In this preliminary work, we describe highly-multiplexed profiling of cellular lineage, state, and metabolic regulome in archival whole-slide human PDAC resections using cyclic immunofluorescence and CosMx spatial transcriptomics. With this multiomic approach, we identify several novel architectural features of the PDAC tumor microenvironment (TME). First, we find that diverse cell lineages are nonrandomly recruited to compositionally and functionally distinct tumor regions called "cellular neighborhoods." Members of a given cell lineage localizing to multiple cellular neighborhoods bear differential activation of metabolic signaling pathways in these diverse spatial contexts, suggestive of metabolic specialization dictated by neighborhood identity. Next, we focus on cellular neighborhoods containing CD66b+/MPO+ neutrophils (PMNs). PMNs are understudied in PDAC due to lack of flexible cell culture models and poor recovery from tissue for scRNAseq analysis – meanwhile, our *in situ* methods are uniquely equipped to study this highly TME-prevalent cell population. We find three cellular neighborhoods in PDAC tumors enriched for PMNs, (1) diffuse neutrophilic stroma, in which PMNs are uniformly intermixed with fibroblastic and leukocytic stromal elements, (2) intraglandular neutrophil, in which 1s-10s of PMNs localize to the lumena of malignant cancer glands, and (3) NETotic neutrophil, in which 10s-100s of PMNs undergo an unconventional form of non-apoptotic cell death called Neutrophil Extracellular Trap-osis (NETosis), intermixed with malignant cells that are more squamoid (vs. basaloid) in morphology and may be ingesting NETotic PMN cell debris via macropinocytosis. Intraglandular PMNs are highly HIF1a+ and the observation of some NETs within PDAC glands suggests this cellular neighborhood may represent an intermediary stage in PMN trajectory once it has infiltrated the PDAC tumor. Further investigation will focus on characterizing spatial expression of nutrient transporter-encoding mRNAs that may mediate metabolic cooperation within and across cellular neighborhoods as well as probing the direct transfer of biomass from PMNs to malignant cells.

Poster 6B | CAMB – Cancer Biology

Investigating the Role of Diet and Host Genetics on Colorectal Cancer Metabolism and Physiology

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Colorectal cancer (CRC) is the second leading cause of cancer-related mortality in the United States. Identifying new factors that promote or inhibit CRC is critical to mitigating cancer risk and improving patient outcomes. Alongside genetic factors, diet influences CRC by altering normal gut physiology, microbial composition, and available metabolites. These metabolites include fructose and short-chain fatty acids (SCFA) comprised of acetate, propionate, and butyrate. We recently asked whether these divergent diet-derived metabolic signals integrate to exert a net effect and showed that, in mice, a high-fiber diet leads to greater glucose tolerance compared to a low-fiber diet while supplementation of high-fructose corn syrup to a high-fiber diet mitigates this protective benefit. Metabolomics analysis revealed sets of metabolites in cecal contents that cluster with each nutritional condition. These results suggest that nutrients with opposing metabolic functions integrate to exert a net effect. However, whether central metabolites at the intersection of fructose and fiber metabolism are primarily mediating this phenotype, and how host enzymes that process these nutrients are regulated in this context is poorly understood. One major class of metabolites impacted by these nutrients is SCFAs. The enzyme acyl-Coenzyme A (CoA) synthetase short chain family member 2 (ACSS2) is responsible for metabolizing nuclear and cytosolic acetate, the most abundant SCFA, to acetyl-CoA, a central metabolite that is used in histone modifications and biosynthetic pathways. To assess correlations of ACSS2 with CRC biology, we analyzed publicly available Cancer Genome Atlas (TCGA) gene expression datasets and found that ACSS2 is underexpressed in CRC compared to normal colon tissue. Moreover, ACSS2 expression is inversely correlated with survival. We thus asked if loss of ACSS2 expression had a functional impact on CRC. TCGA analysis showed that ACSS2-low CRC patients had increased epithelial-mesenchymal transition (EMT) and inflammation gene expression signatures. Collectively, our data suggest that loss of ACSS2 expression, through currently undefined mechanisms, is correlated with de-differentiation and EMT in CRC and that dysregulation of ACSS2 may play an important role in colorectal cancer in part through the processing of dietary nutrients. Through ongoing work, we are testing the role of ACSS2 in diet-dependent CRC tumorigenesis.

Poster 7B | CAMB - Cancer Biology

GOT1, GPX4, and PNPLA2 in Breast Cancer Dormancy and Recurrence

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Lipids are involved in cellular signaling, are critical to the formation and maintenance of cellular membranes, and are a major energy source. When damaged by reactive oxygen species, lipid peroxides promote cell death via ferroptosis. We conducted a targeted CRISPR knockout screen using a library of 331 guides to find functional targets in the ferroptosis pathway regulating breast cancer dormancy and recurrence. The library was cloned into the lentiviral GFP vector (Addgene) and transduced into an inducible Her2 primary tumor model previously developed and validated in our laboratory. The transduced cells were sorted for GFP expression and injected orthotopically into mice. Primary tumors, multiple timepoints of residual lesions, and recurrent tumors were harvested and sequenced. Three of the top hits by clonal enrichment were GOT1, GPX4, and PNPLA2. PNPLA2 codes for adipose triglyceride lipase, the rate-limiting step of triglyceride lipolysis. In addition to providing free fatty acids for biosynthesis and oxidation, PNPLA2 promotes expression of PPARα and its target genes, thus also serving as a key node for cellular signaling. GOT1 encodes glutamate-oxaloacetate transaminase 1, a critical node in many cellular metabolic processes, including the citric acid cycle and urea cycle. GPX4 encodes glutathione peroxidase 4, which is the key last line of defense against ferroptotic cell death by reducing toxic lipid peroxides into non-toxic alcohols. Intriguingly, all three genes are predicted to have roles in inhibiting ferroptosis. Counterintuitively, guides knocking out each of the genes were selected for in recurrent tumors in the CRISPR screen. I have further validated in a recurrence-free survival assay that knockout of each gene accelerates recurrence. Therefore, I hypothesize that each of genes regulates recurrent tumor formation, potentially through a new role for ferroptosis in dormancy and recurrence. I am currently assaying our model system for whether and when ferroptotic death occurs, and whether knockout of these genes has predicted or unexpected effects on ferroptosis. I am further validating these hits in in vivo and in vitro competition assays to ascertain when selection occurs. Beyond ferroptotic death, further ongoing studies include assaying for reactive oxygen species and lipid accumulation to explore other mechanisms of these genes' respective roles in tumor dormancy and recurrence.

Poster 8B | CAMB - Developmental, Stem Cell, and Regenerative Biology

Angiopoietin and VEGF Signaling in Murine Spiral Artery Remodeling

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During human and mouse gestation, the uteroplacental vascular tree transports a large portion of maternal cardiac output to the uterus and placenta to facilitate the exchange of nutrients, oxygen, and waste products between the mother and her fetus. To keep pace with the rapidly increasing metabolic needs of a growing fetus, large caliber vessels called spiral arteries (SAs) within the uterine decidua must remodel into wider channels that facilitate increased maternal blood flow into the placenta. Abnormalities in SA remodeling (SAR) are associated with adverse pregnancy outcomes, including the gestational disorder preeclampsia. Currently, we know very little about how SAR occurs in normal pregnancies and in preeclampsia. Thus, the central objectives of this project are to define the molecular mechanisms underlying SAR and to characterize the decidual environment in which this remodeling process occurs. My preliminary studies implicate the Angiopoietin-Tie2 and VEGF pathways, two pathways that play important roles in blood vessel development and remodeling, in SAR. ANG-2, a molecule that promotes blood vessel destabilization by antagonizing the pro-quiescence receptor TIE2, is expressed in decidual cells and SA endothelial cells during early phases of SAR. Additionally, VEGFR3, a receptor that controls angiogenesis and arterial remodeling in response to fluid shear stress, is also expressed by SA endothelial cells. Based on these findings, I hypothesize that ANG-2 and VEGFR3 are required for proper SAR in mice. In Aim 1, I will test whether maternal ANG-2 is required for proper SAR and assess whether this requirement is autocrine or paracrine. In Aim 2, I will test whether VEGFR3 is required for proper SAR and assess whether its function is dependent upon its canonical ligand, VEGF-C. Finally, in Aim 3, I will use single cell RNA sequencing to better characterize the endothelial and non-endothelial cell types found in the decidua over the course of gestation and investigate how these cell types interact with one another during SAR. Completion of these experiments will provide insight into the role of two major vascular remodeling pathways in SAR and demystify the complex decidual environment in which SAs reside. Overall, by shedding light on the ways in which SAR is accomplished during mouse pregnancy, these studies will help us better understand how human SAR occurs and provide insight into how this process is disrupted in diseases like preeclampsia.

Poster 9B | CAMB - Developmental, Stem Cell, and Regenerative Biology

The Interaction between Inflammation and Loss of RUNX1 in Familial Platelet Disorder with Associated Myeloid Malignancies

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Individuals with inherited mutations in the RUNX1 transcription factor develop Familial Platelet Disorder with associated Myeloid Malignancies (FPDMM). FPDMM patients have a 44% risk of developing a hematopoietic malignancy, and all FPDMM patients will develop a pre-malignant state known as clonal hematopoiesis (CH). In CH, a hematopoietic stem cell(HSC) acquires a mutation that gives that cell a competitive advantage over other HSCs. The mutated HSC clonally expands under selective pressures in the bone marrow (BM) and can become cancerous after acquiring additional driver mutations. In FPDMM, the age of CH onset is earlier than that of the general population and the rate of malignant transformation is higher. It is not understood how CH is accelerated in FPDMM.

Inflammation is thought to play a critical role in the pathogenesis of CH in FPDMM. FPDMM patients show increased prevalence of inflammatory allergic conditions and possess markedly elevated levels of inflammatory cytokines in their BM. Consistent with patient phenotypes, we previously showed that loss of RUNX1 in hematopoietic cells leads to the production of neutrophils that oversecrete cytokines in response to toll-like receptor 4(TLR4) stimulation with lipopolysaccharide. We determined that loss of RUNX1 in the granulocyte-monocyte progenitors (GMPs) is sufficient to produce this hyperinflammatory neutrophil phenotype. To determine whether RUNX1 plays a direct role in regulating inflammatory pathways, we performed Cleavage Under Targets & Release Under Nuclease (CUT&RUN) in wildtype GMPs. We determined that RUNX1 binds to genes of the TLR4 and Type 1 Interferon pathways. By overlapping our previously acquired Assay for Transposase-Accessible Chromatin with Sequencing (ATAC-seq) and RNA-sequencing data sets, we found that loss of RUNX1 resulted in enhanced chromatin accessibility and increased transcription of these genes. Our results suggest that RUNX1 directly represses inflammatory pathways in the myeloid lineage.

To determine the relevance of our observed hyperinflammatory neutrophil phenotype to FPDMM patients, we performed ATAC-seq on neutrophils isolated from peripheral blood samples derived from FPDMM patients and age-matched controls. Consistent with our mouse data, we show that neutrophils from an FPDMM patient with a known pathogenic RUNX1 mutation have increased chromatin accessibility around genes related to proinflammatory immune responses. However, we did not observe increased accessibility of these genes in a second FPDMM patient with a RUNX1 mutation of unknown significance. To determine the significance of this finding, we will perform ATAC-seq on more samples from patients with known pathogenic RUNX1 variants.

Poster 10B | CAMB - Genetics and Epigenetics

Ferroptosis enhances epidermal cornification through transcriptional and metabolic reprogramming

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Epidermal cornification is a unique process that requires complex changes in lipid metabolism and a non-apoptotic cell death to form the skin barrier. Dysregulation of cornification underlies the pathogenesis of numerous common skin diseases, ranging from psoriasis to cutaneous squamous cell carcinoma (cSCC). These data highlight the need to understand the precise pathways underlying the cell death that occurs during keratinocyte differentiation. Ferroptosis is a recently described form of regulated cell death gaining significant interest for its involvement in differentiation and carcinogenesis. It is defined by iron-dependent lethal lipid peroxide accumulation and imbalance of cellular redox homeostasis. However, the role ferroptosis plays in promoting differentiation, cornification, and the potential mitigation of cSCC remains elusive. Our lab has demonstrated that loss of a major tumor suppressor, MLL4, impairs epidermal differentiation and promotes neoplasia due to impaired ferroptotic signaling (Egolf et al., *Sci. Adv.* 2021). Here, we present evidence that ferroptosis promotes cornification to govern cell fate. We demonstrate that triggering ferroptosis in primary human keratinocytes upregulates endoplasmic reticulum (ER) stress signaling, cornified layer genes, and differentiation-associated ceramides. Inhibiting ferroptosis leads to disrupted cornification and diminished expression of cornification genes, emphasizing a promising role for ferroptosis in enhancing cornification. Furthermore, cSCC cell lines are selectively vulnerable to ferroptosis induction compared to primary lines. Overall, these studies provide promising insight into metabolic mechanisms governing epidermal cornification as well as open therapeutic avenues for regulating ferroptosis in cSCC.

Poster 11B | CAMB - Genetics and Epigenetics

Burning in cellular memory as a mechanism for phenotypic enhancement in metastatic melanoma

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Decades of research have uncovered various forms of genetic resistance formation in melanoma wherein specific mutations enable the cancer to evade therapy. More recently, however, our lab and others have shown that transient fluctuations of gene expression in a genetically homogenous population can enable rare subpopulations of cells to survive treatment with targeted therapies without the need to acquire genetic mutations. Whereas genetic resistance resembles a process of pure selection, this non-genetic form of resistance allows for the possibility that certain subpopulations of cells could gradually adapt during drug exposure to become resistant. By using live-cell imaging, DNA barcode lineage tracking, and single-cell sequencing, we indeed find distinct lineages of cells within a genetically homogenous population that gradually adapt to form resistance to targeted therapy. We then wondered how these cells adapt to form this resistance; hypothesizing that this process of adaptation may result from the upregulation of transcriptionally active genes in the cell at the time of drug initiation. This hypothesis arose from the observation of several marker genes which have low but variable expression at baseline in the drug-naïve population but are highly expressed after drug exposure. Using a combination of pharmacological inhibition, fluorescent reporters of transcriptional factor activity, and RNA sequencing, we find evidence that AP-1, a general stress-responsive transcription factor family, may be a key regulatory factor in this framework of adaptation-driven resistance in which transcriptionally active genes in the cell at the time of drug initiation are upregulated during the establishment of resistance. Moving forward, we plan to evaluate changes to chromatin accessibility, histone modifications, and transcription factor binding that occur during adaptation-driven resistance with the goal of identifying potential factors that control the specificity of gene upregulation during adaptation-driven resistance. We hope that insight into the mechanisms of adaptation-driven resistance might offer a novel therapeutic angle to address targeted therapy resistance in melanoma.

Poster 12B | CAMB - Genetics and Epigenetics

Novel class of pediatric neurodevelopmental "histonopathies" may be driven by shared mechanism of chromatin dysregulation

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Histone proteins are core genome organizers that bind and wrap nuclear DNA, but their function extends beyond packaging. Histones are essential for regulating gene expression and chromatin structure. These key roles are emphasized by an emerging class of rare pediatric neurodevelopmental disorders caused by germline missense mutations in histone genes. As part of an international consortium dedicated to understanding these histonopathies, we have expanded the known cohort of individuals who harbor germline missense mutations from 102 published patients with variants in 5 of the 89 histone genes to over 200 published and unpublished patients with variants in 25 genes. Notably, these patients exhibit a striking degree of phenotypic overlap. They all present with global developmental delay, intellectual disability and craniofacial dysmorphisms. They display variable cardiac, urogenital, skeletal and dental abnormalities.

We propose that these histonopathies likely converge on a shared pathogenic mechanism of chromatin dysregulation mediated by nucleosomal destabilization as well as local and global splicing perturbations, leading to premature senescence. Published RNA-sequencing data from patient fibroblasts, which harbor germline Histone H4 or H3.3 mutations, indicate altered expression of genes regulating chromatin packaging and cell cycle pathways. Patient fibroblasts also demonstrate hyperproliferative phenotypes. Our group is employing a multifaceted approach to further dissect the underlying causative mechanism, with a focus on histonopathies driven by mutations in Histones H3.3 and MACROH2A1. We are using 1) isogenic hiPSCs to model patient mutations in disease-relevant cell types; 2) transcriptomic data to guide our hiPSC lineage differentiation; and 3) *in silico* modeling to predict the impact of these patient mutations on chromatin architecture. This comprehensive and highly-collaborative approach will enable us to delineate the conserved mechanism of pathogenesis, establishing a foundation for therapeutic development for all patients affected by histonopathies. This work will also advance our understanding of the neurodevelopmental function of histones.

Poster 13B | CAMB - Genetics and Epigenetics

Hemodynamics and KLF2/4 regulate myxomatous valve formation

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Valvular heart disease affects up to 2% of people worldwide and often requires surgical intervention since effective medical therapies do not exist. Myxomatous valve disease (MVD) is among the most common forms of valvular heart disease and is characterized by pathologic thickening and degeneration of valve leaflets, leading to regurgitation and valve prolapse. MVD can be syndromic, occurring due to single gene mutations such as in Marfan's Syndrome, but often presents in the absence of known mutations suggesting that environmental factors influence MVD formation. We have previously shown that shear-responsive transcription factors KLF2 and KLF4 (KLF2/4) are required for cardiac valve formation by transducing hemodynamic forces. However, the role of these hemodynamic forces and Klf2/4 in adult cardiac valves remains unknown. Here we show that inducible genetic loss of Klf2 and Klf4 in valve endothelial cells of adult mice (Klf2/4$^{\Delta VEC}$) using the *Prox1*^{CreERT2} leads to rapid myxomatous valve formation, characterized by increased valve leaflet size, pathologic ECM accumulation, and increased valve endothelial and interstitial cell proliferation. Interestingly, similar myxomatous valve pathology is conferred by loss of blood flow across the mitral valve in transplanted hearts. Together, these data support a model in which hemodynamic forces maintain cardiac valve homeostasis through KLF2/4 and loss of flow or KLF2/4 drives acquired myxomatous valve formation.

Poster 14B | CAMB - Genetics and Epigenetics

Pathogenic laminopathy mutations disrupt specific lamina-associated regions in cardiac myocytes potentially via altered mechanosensing

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The mammalian genome is organized into various regions at different scales as one mechanism regulating gene expression and mediating cellular identity. One type of well-characterized region is the lamina-associated domain (LAD), which contains chromatin regions that directly interact with the nuclear lamina (NL) at the nuclear periphery. Found across all chromosomes, LADs dynamically interact with the NL to release or attach genes and regulatory elements in accordance with cell-type and differentiation state-specific gene expression programs. Patients with mutations in *LMNA*, encoding the A and C type lamins in the NL, develop a heterogenous group of diseases, known as laminopathies. We used induced pluripotent stem cells (iPSC) to determine the impact of a DCM patient-modeled *LMNA* T10I mutation on peripheral heterochromatin organization. T10I iPSC-derived cardiomyocytes exhibited gross nuclear abnormalities and demonstrated loss of lamina-bound chromatin enriched in genes and lower lamin B1 contact frequency. These regions are also enriched in genes related to non-myocyte identity and mutant myocytes expressed these genes associated with non-myocyte lineages. These effects were myocyte-specific, as the *LMNA* variants did not disrupt lamina-chromatin interactions in iPSC-derived hepatocytes or adipocytes. Additionally, evidence from mouse models and human genetic studies have also suggested a potential role for the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex in mediating genome organization. However, while a LINC-LMNA-gene positioning axis has been suggested, the mechanism of how this may occur remains elusive. Using a combination of population-based genomics analyses and single-cell microscopy, we will test the hypothesis that cytoskeletal-lamina interactions destabilize LADs in lamin mutant cells. These studies will provide mechanistic insights into how the nuclear lamina and LINC complex are involved in LAD organization in cardiomyocytes, which will begin to provide novel understanding of the molecular basis of laminopathy phenotypes.

Poster 37B | CAMB - Genetics and Epigenetics

Elucidating the spatially coordinated mechanisms of heterochromatinization in fragile X syndrome

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Fragile X syndrome (FXS) is associated with an expansion of the CGG short tandem repeat (STR) located in the 5' UTR of the *FMR1* gene. Upon expansion to mutation-length, local DNA methylation at the *FMR1* promoter leads to silenced transcription which is thought to drive the pathophysiology of FXS. However, *Fmr1* knock-out mice do not reproducibly recapitulate the range of FXS clinical presentations, suggesting that *FMR1* dysregulation alone cannot explain the pathophysiology of FXS. Recently, our lab uncovered Megabase-scale domains of the H3K9me3 at the *FMR1* locus on chromosome X and multiple autosomes that form inter-chromosomal contacts. These domains encompass silenced genes encoding synaptic plasticity and epithelial integrity, raising the possibility that these heterochromatin domains contribute to the pathophysiology of FXS. However, the causal contribution of the mutation-length STR in establishing the heterochromatin domains and the mediators of their inter-chromosomal contacts remain unknown. I describe strategies for the synthesis of mutation-length CGG STRs to enable scarless knock-in of the mutation-length CGG STR using CRISPR/Cas9 genome engineering. Then, using single-cell imaging, I found that H3K9me3 domain on chromosome X forms ectopic *trans* interactions with autosomal H3K9me3 domains interactions in FXS genome in FXS patient-derived cells. Moreover, using multimodal imaging, I demonstrate a correlation between H3K9me3 signal and frequency of *trans* interactions at the FXS domains at single-nucleus resolution, suggesting that H3K9me3 contributes to the inter-chromosomal contacts. Elucidating the mechanisms through the mutation-length CGG STR are causally linked to epigenetic dysregulation genome-wide in the FXS will be of relevance not only to FXS but also more broadly to other repeat expansion disorders.

Poster 15B | CAMB - Microbiology, Virology and Parasitology

What makes a common cold virus? Respiratory viruses differentially interface with host interferon responses in the nasal epithelium

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All respiratory viruses establish a primary infection in the nasal epithelium, where efficient induction of antiviral innate immune responses may result in local control of viral replication, limited spread to the lower airway, and minimal pathogenesis. Using a primary epithelial culture system in which patient-derived sinonasal epithelial cells are grown at an air-liquid interface (ALI), we compare a panel of respiratory viruses in order to identify features that differentiate pathogenic viruses from those typically associated with a common cold phenotype. Importantly, these primary nasal ALI cultures recapitulate the polarized heterogenous cellular population and mucociliary functions of the *in vivo* airway, and thus are an optimal system in which to study virus-host interactions. Four human coronaviruses (HCoVs) – two lethal betacoronaviruses (SARS-CoV-2 and MERS-CoV) and two seasonal or common cold-associated alphacoronaviruses (HCoV-229E and -NL63), human rhinovirus-16 (HRV-16), and a seasonal H1N1 influenza A strain are able to replicate productively in nasal ALI cultures. Common cold-associated viruses (HCoV-229E, HCoV-NL63, and HRV-16) replicate more efficiently at 33ºC, the temperature reflective of the nasal cavity, than at 37ºC, the temperature of the lung. We model long-term infections (terminal time point of 192 hours or 8 days post infection) and find that nasal epithelial cells are able to clear common cold-associated viruses (229E, NL63, and HRV-16) as observed by diminishing titers to the limit of detection. Clearance does not occur during SARS-CoV-2, MERS-CoV, or H1N1-Influenza infection. Bulk RNA sequencing of infected nasal ALI cultures revealed that these respiratory viruses diverge significantly in the degree to which they induce antiviral interferon (IFN) signaling. While pathogenic HCoVs are associated with near complete shutdown (MERS-CoV) or a significant delay (SARS-CoV-2) in IFN signaling, the seasonal or common-cold associated HCoVs, as well as HRV-16 and H1N1-Influenza, induce robust IFN pathway activation. Given this gradient of IFN induction, we carried out infections in the presence of ruxolitinib (RUX), a Janus kinase inhibitor which abrogates IFN signaling. While RUX treatment had minimal impact on viral titers at early times during infection with all of the respiratory viruses, nasal cells treated with RUX were unable to clear common cold-associated viruses. Finally, we use a panel of SARS-CoV-2 and MERS-CoV recombinant viruses to show that inactivation of the IFN antagonists encoded by these viruses results in a phenotype similar to that of common cold-associated HCoVs and HRV-16. The mutant viruses show more robust IFN signatures and are attenuated in viral replication compared to their wild-type counterparts (consistent with the relative clearance of common cold viruses by nasal cells). These experiments illustrate unique features of common cold-associated respiratory virus infections in the nasal epithelium – optimal replication at the temperature of the nasal airway (33ºC), robust IFN induction, and early clearance by host cells. Induction of antiviral IFN signaling in the nasal epithelium is likely pivotal in clearance and resolution of respiratory viruses, and this pathway should be leveraged as an antiviral strategy against current and future emerging respiratory pathogens.

Poster 16B | CAMB - Microbiology, Virology and Parasitology

Deficits in mitochondrial oxidative phosphorylation enhance SARS-CoV-2 replication via metabolic remodeling

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SARS-CoV-2 rewires host metabolism to optimize virus production. While glycolysis is necessary for virus production, whether mitochondrial oxidative phosphorylation (OXPHOS) is required for SARS-CoV-2 replication is unknown. Mitochondrial DNA (mtDNA) codes for 13 critical oxidative phosphorylation (OXPHOS) polypeptides of the electron transport chain (ETC) as well as the mitochondrial translation machinery necessary for their production. We discovered ~5 to 100-fold greater SARS-CoV-2 virus production in infected human ACE2-expressing A549 cells deficient in OXPHOS due to mtDNA depletion (ρ^0 cells). A similar infection enhancement effect was observed upon blocking mitochondrial translation and chemically inhibiting ETC complexes. Cells deficient in OXPHOS demonstrated increased size and distribution of viral replication centers and promoted both infectious particle production and release two hours earlier than WT cells following infection. Notably, the interferon-stimulated gene response at the transcript and protein levels remained intact throughout infection, and enhanced glycolysis was a pro-viral correlate. Reintroduction of mtDNA in the ρ^0 cells reinstated OXPHOS and impaired SARS-CoV-2 viral replication compared to parental ρ^0 cells. In summary, our findings support that metabolic balance regulates SARS-CoV-2 replication, whereby OXPHOS exerts an antiviral effect on SARS-CoV-2 infection. Work is ongoing to explore how mitochondrial OXPHOS regulates the SARS-CoV-2 life cycle and the cytosolic mediators of this relationship. We hope to inform future approaches to modulating mitochondrial function in infected cells, animal models, and humans to mitigate severe sequelae of COVID-19.

Poster 17B | Epidemiology & Biostatistics

Algorithmic reliability and dataset shift in a national readmission and mortality prediction algorithm

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Risk prediction algorithms trained on electronic health record (EHR) data, such as patient characteristics, administrative codes, and lab values, are increasingly being used in healthcare to direct resources to high-risk patients. However, the long-term use of these tools faces challenges due to dataset shift, a mechanism that can cause algorithm inaccuracies over time. Changes in healthcare practices can lead to shift by altering predictor distributions (covariate shift) or changing the relationship between input predictors and outcome (concept shift). Despite the increasing use of predictive tools in high stakes clinical settings and growing recognition of shift, a comprehensive framework for quantifying performance decline and dataset shift in EHR-based risk prediction models is lacking, as well as effective mitigation strategies. To this end, we study a nationally deployed risk prediction algorithm, the Veteran Affairs Care Assessment Needs (VA CAN) model, to examine gradual and sudden changes in model performance and dataset distributions between 2014-2020. The VA CAN algorithm is a set of logistic regression models used to calculate 90-day and 1-year readmission and/or mortality risk for over 5 million primary care patients each year. Our findings indicate a consistent decline in model performance metrics, with a 12.8% decrease in true positive rates for high-risk patients. Performance decline was most significant during the COVID-19 period (2019-2020). Changes in the proportion of most covariates (covariate shift) and in a limited number of predictor-outcome associations (concept shift), such as the relationship between white blood cell count and mortality, were strongly linked with the decline in model performance. Model revision with machine learning approaches can reduce the decline in performance and is currently being further explored.

Poster 18B | Genomics and Computational Biology

Interactions between the gut microbiome, dietary restriction, and aging in more than 900 genetically diverse mice over the lifespan

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Dietary restriction (DR) extends lifespan and improves health in diverse organisms. However, the factors governing the efficacy of DR in a given individual remain poorly defined. Recently, the gastrointestinal microbiome has been suggested to modulate the process of aging as well as individual responses to DR. To investigate the interactions between the gut microbiome, aging, and dietary restriction, we profiled the gut microbiomes of 913 genetically diverse mice over the lifespan. These mice were randomized at six months of age to one of five dietary groups: *ad libitum* feeding (control group), 20% fewer calories, 40% fewer calories, fasting one day per week, and fasting two consecutive days per week. After extensive quality-control, we generated 2997 shotgun metagenomic profiles, resulting in the largest mouse microbiome study to date. We integrated this dataset with host genetics and hundreds of other longitudinally collected aging-associated phenotypes to make the following discoveries.

First, we found that aging markedly shapes the microbiome. One notable aging-associated change is an increase in "uniqueness," suggesting that an individual's microbiome becomes more distinct from other microbiomes over time. Furthermore, while we could train a machine learning classifier to predict a mouse's age, cross-dataset prediction was poor, suggesting that microbiome aging does not follow a universal and deterministic process. Second, dietary restriction induced large microbiome changes, and the changes induced by fasting were distinct from those induced by caloric restriction. Third, we found that in our controlled laboratory environment, host genetics exerted a surprisingly large effect on the microbiome. In addition, through genetic association analyses, we identified an interaction between individual microbes and the *Reg3* locus, which contains numerous antimicrobial genes. Finally, we performed association and mediation analysis to nominate causal microbiome-phenotype interactions. We recapitulated known associations, such as multiple microbes influencing body weight, and uncovered several previously unknown associations, such as the genus *Romboutsia* influencing red blood cell distribution width (RDW), a biomarker of mortality.

In summary, we have generated the largest-to-date mouse microbiome dataset; we found that aging strongly affects the microbiome, but there does not exist a conserved "signature" of aging; we observed that host genetics have a larger-than-expected influence on the microbiome; and we discovered a large number of known and previously unknown microbiome-phenotype interactions, such as with RDW, which is itself a strong predictor of mortality.

Poster 19B | Genomics and Computational Biology

Single-Cell Multiomic Analysis of Pediatric High Grade Glioma Under Therapy

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Pediatric high grade glioma (pHGG) is an incurable central nervous system malignancy that is the leading cause of pediatric cancer death. While pHGG shares many similarities to adult glioma, it is increasingly recognized as a distinct, yet highly heterogeneous entity. In this study, we profile a molecularly diverse cohort 16 longitudinal pHGG cases before and after standard therapy through single-nucleus RNA and open-chromatin sequencing, whole genome sequencing, and spatial proteomics to capture the evolution of the tumor microenvironment during progression and treatment. We find that the canonical neoplastic cell phenotypes are insufficient to capture the range of tumor cell states in a pediatric cohort, and we characterize the unique heterogeneity in the immune microenvironment. We identify key transcriptional regulators of pHGG cell states and notably, do not observe the marked proneural to mesenchymal transition characteristic of adult glioblastoma. We show that key neuromodulators and the interferon response are upregulated post-therapy along with an increase in non-neoplastic oligodendrocytes. Through in vitro pharmacological perturbation, we demonstrate novel tumor cell-intrinsic targets across multiple pHGG subtypes. This multiomics atlas of longitudinal pHGG captures the key features that support its distinction from adult glioblastoma and proposes therapeutic strategies which are targeted to pediatric gliomas.

Poster 20B | Genomics and Computational Biology

The LSV-Seq method enables machine learning-assisted recovery of low-coverage splicing events across human tissues.

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Alternative splicing (AS) has been primarily studied on a high-throughput scale via RNA sequencing (RNA-Seq). However, most RNA-Seq reads do not span splice junctions, preventing consistent capture of less abundant isoforms. For instance, analysis of RNA-Seq data from the GTEx project failed to quantify ~30% of detectable AS events, leading us to hypothesize that coverage deficiencies can conceal biologically relevant splicing changes. To address this, we developed Local Splicing Variation Sequencing (LSV-Seq). LSV-Seq enriches junction-spanning reads by anchoring a few thousand reverse transcription primers proximal to known 3' splice sites. High-yield and specific primers were designed using a deep learning Transformer model trained on genomic sequence, combined with decision trees over manually constructed features such as expression and simulated binding. To benchmark our method, we sequenced unstimulated and stimulated Jurkat T-cells with both LSV-Seq and RNA-Seq. For AS events which were well-covered by both technologies, we found highly consistent quantifications for both individual samples ($R = 0.98$) and difference between conditions ($R = 0.86$). However, LSV-Seq vastly increased overall coverage (mean 800 reads per event) compared to RNA-Seq (mean 40 reads) and detected hundreds of splice junctions missing in RNA-Seq.

Based on these results, we reasoned LSV-Seq could recover previously unquantifiable AS events from GTEx RNA-Seq analyses. We targeted 1400 events with < 20 mean reads across 3 tissues (brain cortex, liver, and heart atrial appendage) which were predicted to be tissue-specific based on either ENCODE RNA-binding protein CLIP-Seq data or an in-house splicing code model. With only one-tenth the read depth of the RNA-Seq data, LSV-Seq enriched coverage by a median of 30-fold after UMI deduplication, allowing us to achieve > 50 reads for ~1,000 events per tissue. These events were over 5-fold enriched for differential splicing as a result of the initial selection criteria. Numerous tissue-specific events occurred in genes classified as functionally relevant and/or adjacent to binding sites of known regulatory proteins.

Our results demonstrate that LSV-Seq greatly improves accuracy, sensitivity, and cost. We foresee our method being applied to numerous tasks such as AS quantification across large patient cohorts or highly multiplexed experimental conditions, detection of transient splice intermediates as in recursive splicing, target enrichment for RNA structure studies, and targeted single-cell RNA-Seq.

Poster 21B | Genomics and Computational Biology

Investigating ancestry-specific genetic variation in apolipoprotein L genes associated with electronic health record phenotypes in diverse patient biobanks

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Health care disparities between people of different ancestries and ethnicities are well-documented in every field of medicine. As a poignant example, African Americans are more than 3 times as likely to have kidney failure compared to White Americans per the National Kidney Foundation. A major contributor to this disparity is the sample bias underlying existing genomic studies. Of the ~6,401 studies compiled in the genome-wide association studies (GWAS) catalog, ~95% of all GWAS participants are of European (EUR) ancestry with less than 1% of participants being of African American (AFR) ancestry. Using the Penn Medicine Biobank (PMBB) and adopting a genome-first approach, we investigated 837 protein-altering variants in the apolipoprotein L gene family, including 100 predicted loss-of-function (pLOF) and 737 missense variants, with a specific interest in those more common in non-European populations. We performed phenome-wide association studies (PheWAS) on 62 variants with a MAF > 0.1% in the PMBB AFR population (n = 11,198) against 1,236 binary phenotypes derived from electronic health records data with at least 20 cases. In addition to confirming the known AFR-specific rs73885319 and rs60910145 *APOL1* variants as strong positive controls for their association with end-stage renal disease (ESRD), our results identified a stop-gain variant rs11089781 (p.Gln58*) in the *APOL3* gene also found to be significantly associated with increased risk for ESRD (OR = 1.38, p = 3.64e-08). This variant has a gnomAD minor allele frequency of 0.22 in AFR compared to 3.97e-04 in EUR. It is also in linkage equilibrium (r^2 < 0.05) with the *APOL1* G1 and G2 known risk alleles for renal disease. Furthermore, the association between rs11089781 under a recessive model and ESRD is strongest in individuals who have low *APOL1* risk and carry either 0 or 1 copy of the risk alleles (OR = 1.79, p = 2.48e-03) compared to those who carry 2 copies of the risk alleles (OR = 0.98, p = 0.914). Using laboratory values for creatinine and estimated glomerular filtration rate (eGFR), we found that rs11089781 is also significantly associated with increased levels of max creatinine (beta = 0.07, p = 2.96e-05) and decreased levels of max eGFR (beta = -0.08, p = 2.36e-06). Replication of this association in up to 121,790 AFR individuals from the Million Veterans Program also yielded significant associations, both with ESRD (OR = 1.16, p = 1.01e-08) and mean eGFR (beta = -0.04, p = 2.42e-13). Initial hypotheses suggest that *APOL3* may play a protective role against *APOL1* and loss-of-function in *APOL3* increases susceptibility to *APOL1*-induced kidney dysfunction, though further functional characterization is still required.

Poster 22B | Immunology

The role of the Type III Secretion System in endogenous presentation of Salmonella enterica epitopes on MHCII

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Salmonella enterica is a pathogen with an ingenious niche: a phagosome reprogrammed via bacterial effector proteins secreted into the host cytosol. CD4 T cells form the backbone of an efficient response to *S enterica* infections. However, the role of the intracellular niche of *S enterica* on formation of CD4 T cell responses remains relatively unexplored. We have performed a screen of predicted Salmonella CD4 epitopes in mice and found that CD4 responses are highly skewed toward the secreted effector proteins at the expense of other bacterial proteins sequestered in the vacuole. This suggests that secreted effectors in the cytosol of infected antigen-presenting cells (APCs) are an abundant source of peptide to present to CD4 T cells, running counter to the classical paradigm of CD4 antigens originating from material outside the presenting cell. To further probe this skew, we have used a T hybridoma reporter system to quantify the presentation of ectopic antigens from different compartments of the bacterium or host cell. We also plan to use an mRNA vaccine system pioneered by the Eisenlohr lab to generate epitope-specific CD4 T cell responses to test the differential protection from infection offered by CD4 T cells directed towards individual epitopes. Understanding this skew could not only improve our understanding of the immune response to *S enterica* but could also illuminate a novel pathway for antigen presentation in bacterial infections.

Poster 23B | Immunology

Fate induction in chimeric antigen receptor T cells through asymmetric cell division

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Early expansion and long-term persistence predict efficacy of chimeric antigen receptor (CAR) T cells. This is thought to reflect the induction of both effector and memory T cell populations to provide short-term target clearance and long-lasting remission. Despite these subsets' roles in therapeutic success, the cellular mechanisms of fate induction after CAR T cell activation are unknown.

Here, we show that activated human CAR T cells undergo asymmetric cell division to impose distinct fates upon first-division daughter cells. We use a novel protein-protein interaction dependent molecular labeling technique to label target-engaged CAR molecules and sort first division proximal and distal daughter CAR T cells for single-cell surface proteomic and transcriptional profiling; metabolic profiling; and assessment of *in vitro* and *in vivo* cytotoxic function. Target-engaged CAR molecules aggregate on proximal first-division daughter cells and induce asymmetry between proximal and distal daughter cells in surface proteome, transcriptional profile, and metabolic program. Proximal daughter cells enrich in the surface protein levels for CD25 and Notch1; upregulate *MYC* and *MTORC1* target genes; upregulate a core transcription factor set promoting proliferation (E2F7), apoptosis (TP73), and effector differentiation (YBX1); and demonstrate increased metabolic activity largely supported by glycolysis, consistent with proximal daughter cell activation and differentiation toward a terminal effector cell fate. In contrast, distal daughter cells enrich in the surface protein levels for CD45RA and CD5; upregulate genes such as *CCR7*, *IL7R*, and *KLF2*; upregulate a core transcription factor set promoting quiescence (STAT1) and restraining effector cell expansion (FLI1); and employ an oxidative phosphorylation-predominant metabolic profile indicative of distal daughter cell differentiation toward a memory cell fate. RNA velocity analysis reveals that proximal and distal daughter cells demonstrate diverging cell fate trajectories and that both uneven distribution of pre-existing transcripts and changes in transcriptional regulation establish transcriptional asymmetry between proximal and distal daughter cells. In line with their memory T cell fate, distal daughter T cells exhibit greater *in vivo* persistence and clearance of target tumor cells compared to proximal daughter T cells ($p < 0.05$). Surprisingly, despite their memory phenotypes and *in vivo* functional longevity, first-division distal daughter cells demonstrate potent cytolytic activity similar to proximal daughter cells for up to 48 hours after first cell division, resulting in superior long-term leukemic control ($p < 0.01$). This period of 'target readiness' is followed by a substantial decrease in cytotoxicity in distal, but not proximal, daughter cells. Together, these phenotypes and transcriptional profiles reveal that distal daughter cells assume a transient effector-like state along their differentiation trajectory towards becoming memory T cells. These studies establish asymmetric cell division as a framework for studying mechanisms of human CAR T cell differentiation and improving therapeutic outcomes.

Poster 24B | Immunology

Obesity-associated long chain fatty acids regulate lung innate immune responses via the NLRP3 inflammasome.

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Obesity is a risk factor for asthma, and obesity-associated asthma (OAA) is more severe and more difficult to treat than allergic asthma. Macrophages regulate the lung innate immune response to allergic stimuli, and obesity influences macrophage functional states outside the lung, implicating obesity as an immune pressure that may modify macrophage-dependent lung inflammation. However, the mechanisms by which obesity alters lung macrophage function, and the consequences of these effects on lung inflammation, are not well understood. Improving our understanding of these processes will facilitate the development of targeted therapies for OAA and other obesity-associated inflammatory lung conditions.

We first compared the cellular phenotype of lung macrophages from lean and obese mice and found that obesity expands lung macrophage populations with features of obesity-associated activation including surface CD9 and increased intracellular lipid. Additionally, obesity increases IRE1a endonuclease activity in lung macrophages, and increases production of IL-1b, a cytokine implicated in the development of OAA. Lipidomics analysis revealed increased stearate (SA), a saturated long chain fatty acid, in obese lung macrophages. *In vitro*, SA induces IRE1a-dependent priming of the macrophage NLRP3 inflammasome. *In vivo*, SA causes expansion of IL-1b producing, CD9+ lung macrophages, and increases the severity of inflammation in a model of neutrophil-predominant asthma. Finally, we identified a population of obesity- and asthma-associated CD9+ lipid-laden lung monocytes in humans, suggesting that obesity-associated activation of lung macrophages may be conserved across species.

Our work has identified an axis by which high fat diet-associated fatty acids prime lung macrophages for exaggerated, inflammasome-mediated inflammation in response to an allergic stimulus, with broad implications for understanding the etiology and refractory nature of OAA. Ongoing studies will investigate the molecular and metabolic mechanisms by which stearate alters macrophage activation and will inform future treatment efforts through dietary modification or targeted therapeutics.

Poster 25B | Immunology

T cell homing to the small intestine during *Cryptosporidium* infection

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LPAM-1 (integrin $\alpha_4\beta_7$) and chemokine receptor CCR9 are the homing receptors required for effector T cells to traffic to the mucosa of the small intestine. Mora et al. demonstrated in 2003 that dendritic cells (DCs) isolated from the mesenteric lymph nodes (mLN) or Peyer's Patches (PP) are uniquely capable of inducing LPAM-1 expression on T cells in co-culture. Subsequent studies indicated that retinoic acid (RA) secreted by DCs is the factor responsible for the expression of LPAM-1 on T cells. Since these seminal studies, the role of LPAM-1 expression on T cells in various disease states has been studied as have the DCs that possess the ability to induce this important integrin. We utilized *in vitro* DC:T cell co-culture system to study the signals important for upregulating LPAM-1. Although it has been reported that RA is sufficient for LPAM-1 expression, we found that exogenous RA was not sufficient for T cells cultured with splenic DCs to express the same level of LPAM-1 as the T cells cultured with DCs isolated from the mesenteric lymph node (mLN) or Peyer's Patches (PP). These data indicate that there is a second factor expressed specifically by gut-associated DCs that promotes LPAM-1 expression. Identifying the secondary signals required for LPAM-1 upregulation will improve our understanding of the generation of intestinal T cell responses. Our laboratory utilizes a murine model of *Cryptosporidium* infection to study T cells in the intestine. We have found that LPAM-1 is upregulated on T cells primed in the mesenteric lymph nodes during *Cryptosporidium* infection and that antibody blockade of LPAM-1 leads to an increased susceptibility to infection at early timepoints. Therefore, LPAM-1 contributes to protection against this important pathogen, but the data implies the existence of LPAM-1-independent mechanisms of gut-homing at later timepoints in infection. The rules governing LPAM-1 expression and its role in the context of infection remain to be elucidated.

Poster 26B | Immunology

Excess IL-18 Augments Suppressor/Regulatory Cell Function to Prevent Experimental Autoimmune Encephalomyelitis

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Interleukin 18 (IL-18) is an inflammasome-activated cytokine that canonically amplifies interferon-gamma (IFNg) production and cytotoxicity by CD8 T-cells (CD8Ts) and Th1 CD4 T-cells (CD4Ts). It is inhibited by the circulating high affinity antagonist, IL-18 binding protein (Il18bp). In certain contexts, IL-18 amplifies non-Th1 responses including Th2, Th17, and Treg. Despite its likely pathogenic role in autoinflammation, clinical observations suggest a possible immunoregulatory role in CD4T-mediated autoimmunity. We sought to understand the role of excess IL-18 in the mixed Th1/17 experimental autoimmune encephalomyelitis (EAE) model of central nervous system autoimmunity.

EAE was induced via immunization with a complete Freund's adjuvant/Myelin Oligodendrocyte Glycoprotein peptide (MOG^{35-55}) emulsion and pertussis toxin in the following mice: mice deficient in Il-18bp (*Il18bp*$^{-/-}$), with transgenic overproduction of mature IL-18 (*Il18tg*), transgenic for a T-cell receptor recognizing MOG^{35-55} (2D2 mice), and relevant controls. EAE was monitored by daily clinical scoring of ascending paralysis. Cell number and protein phenotype were determined by flow cytometry.

We hypothesized that systemic excess IL-18, would amplify autoreactive T-cell activation and lead to more severe EAE, but both Il18bp$^{-/-}$ and Il18tg mice were profoundly protected from EAE. Protection from EAE was dependent on IFNg in Il18bp$^{-/-}$ mice. Analysis of draining lymph nodes and spleens from Il18bp$^{-/-}$ mice showed a modest increase in IFNg-producing CD4Ts and equivalent IL-17-producing CD4Ts. Spinal cords from IL18bp$^{-/-}$ mice demonstrated fewer CD4Ts but comparable markers of Th function/polarization on a per-cell basis. There was increased infiltration of CD8Ts relative to CD4Ts in spinal cords of Il18bp$^{-/-}$ mice. 2D2 mice have an abundance of MOG autoreactive CD4Ts and very few CD8Ts (due to allelic exclusion). Paradoxically, Il18bp$^{-/-}$;2D2 mice developed more severe EAE than control 2D2 mice, suggesting that an increase in precursor autoreactive CD4Ts and/or loss of CD8Ts switches the effect of excess IL-18 from protective to pathogenic. Sensitivity to EAE was restored in Il18bp$^{-/-}$ mice only after transfer of >5 million autoreactive 2D2 CD4Ts. Further, ex vivo culture with IL-18 improved CD8Ts ability to inhibit EAE while CD8-depletion substantially, but incompletely, diminished protection in Il18bp$^{-/-}$ mice. These data suggest the IL-18 can amplify both autoreactive CD4T and previously described CD8 T suppressor cells but the latter dominate with a physiologic T cell repertoire. Excess IL-18 may function to preferentially augment CD8T suppressor function to clear autoreactive CD4T and mediate protection in EAE. This may inform novel strategies to amplify endogenous suppressive T cells or enhance cellular therapeutics in the treatment of autoimmune diseases.

Poster 27B | Immunology

Deficiency of the Pattern-recognition Receptor CD14 Protects Against LPS-induced Inhibition of Osteoclastogenesis *in Vitro*

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Osteoarthritis is associated with bone changes such as subchondral sclerosis, bone marrow lesions, and osteophyte formation.[1] Prior work has demonstrated that CD14-deficient mice show significantly less OA-associated subchondral bone.[2] CD14 is a GPI-anchored surface protein and co-receptor for several inflammatory TLRs, and is highly expressed in myeloid cells including osteoclast precursors.[3,4] TLR activation can both activate and inhibit osteoclastogenic potential. Using a CD14-deficient mouse model we **hypothesized that inhibitory effects of TLR-stimuli on RANKL-mediated osteoclast differentiation would be ameliorated in CD14 deficient cells.** Bone Marrow was isolated from femurs and tibiae C57BL/6 and CD14-deficient mice. Cells were cultured in complete DMEM with 30 ng/mL M-CSF, for 5 days to expand osteoclast precursors (macrophages). Cells were passaged on day 6 and cultured in the presence or absence of RANKL (100 ng/mL). In a separate study, cells were stimulated with a TLR4-stimulus (LPS, 1 ng/mL), soluble CD14 (1 µg/mL), and a TLR4-inhibitor (CLI-095, 1 µg/mL). Cells were stained for Tartrate-resistant acid phosphatase (TRAP) on days 3 and 4 after addition of RANKL. Cells were imaged at 10x (3 images/well over 4 wells per timepoint) and quantified (% area covered) using ImageJ (NIH) and CellProfiler. Multiple unpaired t-test were performed with Holm-Sidak correction. With LPS stimulation, CD14-deficient cells differentiated more quickly compared to WT at day 3 ($p<0.001$) (**Fig. 2G**). LPS stimulation led to a 77% decrease of osteoclastogenesis in the WT cells, but only a 7.5% decrease in osteoclastogenesis on the CD14-deficient cells **(Figure 1G, 2G)** The addition of sCD14 inhibited osteoclastogenesis in the CD14-deficient group, both with and without LPS. Our results show that **CD14-deficient cells were protected from LPS-TLR4 mediated inhibition of osteoclastogenesis, compared to WT cells.** Further work will investigate further effects of OA-relevant TLR ligands on osteoclastogenesis in the setting of CD14 deficiency or blockade. References: [1] Donel S, 2019 [2] Sambamurthy N, 2018 [3] Zanoni, I 2013 [4] Xue, J 2020)

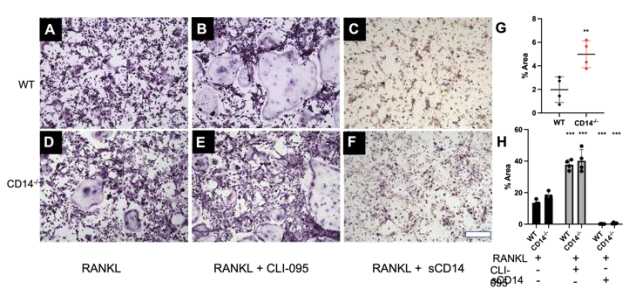

Figure 1: TRAP staining of Osteoclasts treated with TLR4 inhibitor and soluble CD14 at Day 4: Osteoclasts derived from WT (A,B,C) and CD14-deficient precursors (D,E,F) treated with RANKL, RANKL + CLI-095, and RANKL+ sCD14, respectively. Quantification of RANKL treatment at day 3 (G) and all groups at day 4 (H).**p < .01, ***p < .001 compared to RANKL within mouse strain. Scale bar 100 µM

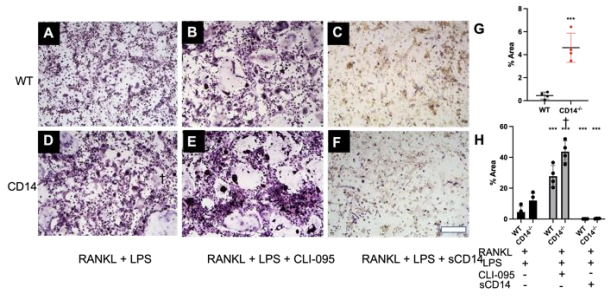

Figure 3: TRAP staining of osteoclasts treated with LPS at day 4: Osteoclasts derived from WT (A,B,C) and CD14-deficient cells (D,E,F) treated with RANKL + LPS, RANKL + LPS + CLI-095, and RANKL + LPS + sCD14, respectively. Quantification of RANKL + LPS treatment at day 3 (G, *** p < .001 compared to WT) and all treatments at day 4 (H). ***p < .001 compared to RANKL + LPS within mouse strain. † p<.001 compared to WT within treatment. Scale bar is 100 µM

Poster 28B | Immunology

Elicitation of V3 glycan-directed cross-neutralizing antibodies in sequentially immunized, SHIV-infected rhesus macaques

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Background: Sequential immunization strategies using HIV Env trimers multimerized on nanoparticles have shown promise at eliciting V3 glycan-directed broadly neutralizing antibody (bNAb) responses. Priming with RC1 (a V3-glycan patch-targeting BG505-based trimer) has been shown to stimulate V3 glycan bNAb-like precursors in mice and rhesus macaques (RMs), and subsequent boosting with more native-like Env immunogens such as 11MUTB induced antibodies that neutralized some primary HIV-1 strains, albeit with limited potency and breadth. Here, we compared this immunization strategy with nucleoside-modified mRNA versions of these immunogens, followed by a lineage-based SHIV.5MUT infection to affinity mature primed responses.

Methods: Membrane-anchored mRNA counterparts of RC1 and 11MUTB SOSIPs were created by adding 1) a non-native signal peptide, 2) a flexible linker replacing the furin cleavage site, 3) various stabilization mutations, and 4) a truncated SIVmac gp41 cytoplasmic domain. RMs were immunized at weeks 0 and 8 with protein-nanoparticle (n=12) or mRNA (n=11) versions of RC1 and 11MUTB, and then infected at week 16 with SHIV.5MUT. Additionally, four naïve (unvaccinated) RMs were infected with SHIV.5MUT. RMs were followed longitudinally for serologic responses and analysis of Env-antibody coevolution.

Results: All mRNA constructs expressed well *in vitro*, displaying robust binding to representative bNAbs including PGDM1400, indicating that intact trimers were formed on the cell surface. There was no detectable binding to CD4i antibodies, though there was binding to some antibodies recognizing linear V3 epitopes. We observed binding to V3 glycan bNAb precursor antibodies DH270_UCA and PGT121_iGL, suggesting that RC1 and 11MUTB mRNA immunogens may stimulate germline B cells related to these bNAb lineages.

Vaccinated animals developed high-titer (ID50 >1:10,000) autologous neutralization responses against RC1 and 11MUTB that were highly immunofocused to the V3 glycan region. Overall, autologous neutralization titers were 5-10-fold higher in protein-nanoparticle- versus mRNA-immunized animals. Intravenous inoculation with SHIV.5MUT led to productive infection in all vaccinated animals, which are now in follow-up for breadth analysis. Surprisingly, in all four naïve RMs infected with SHIV.5MUT, we observed a highly focused NAb response targeting N332, GDIR, and V1 residues 134-139 corresponding to germline-targeted substitutions. One of these RMs developed remarkably broad and potent bNAbs, neutralizing 11 of 19 heterologous tier 2 viruses by 24 weeks post-infection.

Conclusions: We show here that BG505-derived RC1/11MUTB/5MUT immunogens, presented as protein nanoparticles, mRNA-LNPs, replicating SHIVs, or a combination of these platforms, consistently elicited immunofocused B-cell responses to the V3 region of HIV-1 Env. Some of these responses resulted in broad and potent neutralizing responses, supporting this platform for further development as an HIV-1 vaccine.

Poster 29B | Neuroscience

Behavioral paradigm for investigation of the relationship between visually-evoked traveling waves and perception in mice.

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Introduction: Traveling waves of neural activity have been observed across diverse scales and techniques, from EEG to voltage sensitive dyes. Recently, in mice, we have identified wavelike patterns evoked by simple visual stimuli: a fast (30-50 Hz) feedforward wave and a slow (3-6 Hz) feedback wave that are coupled into a single spatiotemporal pattern. Intriguingly, for weaker visual stimuli, the spatial spread of the slow feedback wave varies with stimulus intensity: it is primarily restricted to V1 with the lowest intensity stimuli and spreads to much of the cortex with higher stimulus intensity. This result begs the question: could the spatial extent of the feedback wave be associated with perception of the visual stimulus? We propose a behavioral paradigm for mice in order to address this question, aiming to isolate visual perception-related neural activity.
Experimental design: Our behavioral paradigm consists of two phases. In the first phase, we train mice on a go/no go task in which they lick if they perceive a visual stimulus (a light flash on a CRT monitor). Once they perform well on this task, we will vary the stimulus intensity (luminance) to determine the perceptual threshold. Next, we will conduct neurophysiological recordings simultaneously with running the task, ensuring adequate coverage of trials at the perceptual threshold.
Then, in the second phase, we will implement an inverted design in which we retrain mice on the task, but this time we train them not to lick if they perceive a visual stimulus and lick if they do not perceive a visual stimulus (the opposite of the first task). The rest of the training and recording proceeds as described above. By using an inverted design, we aim to control for the neural signatures of the motor report (licking) in downstream analyses so that we can isolate perception-related neural activity.
Conclusions: We have observed that simple visual stimuli evoke wave-like spatiotemporal neural signatures, and that for weaker visual stimuli, the spatial extent of the feedback wave component depends upon stimulus intensity and is thus consistent with psychophysics. To test whether the feedback wave is actually associated with perception, a behavioral read-out is required; here, we propose using an inverted go/no go visual behavioral paradigm to decouple perception from motor activity related to the report.

Poster 30B | Neuroscience

Investigating the Role of Somatostatin-Positive Low-Threshold Spiking Interneurons in the Dorsomedial Striatum in Goal-Directed Behavior

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Goal-directed behavior aims to maximize rewards across changing environments. The dorsomedial striatum (DMS) is critical for the execution of goal-directed behavior, and serves as a hub for cortical, midbrain, and thalamic inputs that guide value-based choice and motor vigor. Within the DMS, somatostatin-positive low-threshold spiking interneurons (LTSIs) have been found to regulate early operant learning. They receive afferent projections from multiple prefrontal cortical areas such as prelimbic, anterior cingulate, and orbitofrontal cortices that guide value-based choice and encode reward prediction errors, positioning them optimally to regulate goal-directed behavior. However, their role in more complex goal-directed behaviors is not known. I have designed a head-fixed two-alternative forced choice behavioral paradigm in mice that assays the effect of varying reward values, reward probabilities, and net reward environment on goal-directed value-based choice and motor vigor. Using this task, I aim to (1) elucidate LTSI activity patterns during value-based behavior, (2) causally manipulate their activity, and (3) record from striatal principal neurons during LTSI manipulations to assess the impact of LTSIs on behaviorally-relevant striatal circuit processing. My preliminary fiber photometry data suggest that LTSIs encode trial outcomes and net reward environment, hinting at a role in regulating goal-directed choice and motor vigor. My preliminary data from LTSI constitutive inhibition show increases in motor vigor relative to control mice. These novel findings will contribute to a growing evidence base on the role of striatal microcircuitry in striatal function and goal-directed behavior, with potential translational relevance suggested by evidence implicating striatal interneurons in a range of neuropsychiatric disease presentations.

Poster 31B | Neuroscience

Mapping the development of white matter structural organization and overall psychopathology

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Neuropsychiatric disorders are increasingly understood to be disorders of neurodevelopment and are linked to aberrant maturation of brain connectivity. During adolescence, the human brain undergoes profound refinements in white matter structural connectivity to support rapid cognitive and behavioral development. Aberrant developmental changes in white matter connectivity have been linked to diverse psychopathology, including mood disorders and psychosis. Thus, it is imperative to characterize normative white matter structural network development and identify developmental deviations that predict risk for transdiagnostic psychopathology. We apply advanced statistical and neuroimaging techniques to three cross-sectional, large-scale datasets that include harmonized clinical phenotyping and diffusion MRI data from typically developing and clinical youth populations: the Human Connectome Project: Development (HCP-D, N=1,300), the Philadelphia Neurodevelopmental Cohort (PNC, N=1,601), and the Healthy Brain Network (HBN, N=2,748). This work maps the maturation of white matter structural network organization using a framework of hierarchical cortical organization, the sensorimotor-association axis and identifies deviations of white matter structural connectivity from normative development that are associated with transdiagnostic overall psychopathology. This work tests the overarching hypothesis that white matter structural connectivity matures along the sensorimotor-association axis, and further, aberrant white matter maturation is associated with a transdiagnostic measure of psychopathology. Findings may help define dimensional biomarkers of psychopathology and lead to early identification of individuals at risk for developing diverse neuropsychiatric disorders.

Poster 32B | Neuroscience

Phosphoproteomic profiling to uncover molecular mechanisms of neurologic deficits in a mouse model of CDKL5 deficiency disorder

Dayne Martinez and Zhaolan (Joe) Zhou

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CDKL5 deficiency disorder (CDD) is one of the most common forms of genetic epilepsy and is a debilitating childhood disorder characterized by infantile-onset seizures, global neurodevelopmental delay, motor and intellectual impairments, and autistic features. CDD is caused by loss-of-function mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene, which encodes a serine-threonine kinase that is highly expressed in neurons of the brain. Currently, there are no effective treatments for CDD.

Mouse models of CDD reproducibly exhibit disease-related behavioral phenotypes such as learning and memory impairments and social interaction deficits. Notably, most of these behavioral phenotypes can be recapitulated or ameliorated in mice by adult knockout or rescue of CDKL5, respectively, suggesting that CDKL5 has important roles both during and after development. Loss of CDKL5 in either forebrain excitatory or inhibitory neurons results in microcircuit dysfunction characterized by increased excitatory synaptic activity. CDKL5 interacts with synaptic proteins as well as proteins that regulate the cytoskeleton, indicating that CDKL5 likely plays a role in the development and maintenance of synapses.

I hypothesize that losing CDKL5 disrupts biochemical signaling pathways that are critical for proper synapse formation and functioning, and that these synaptic perturbations underly the circuit and behavioral phenotypes observed in CDD mouse models. To determine how signaling pathways are altered upon loss of CDKL5 in mouse brain, I have taken two parallel approaches, one by phosphoproteomic profiling of isolated cortical synaptosomes and whole hippocampi from mice during and after early development and one by single-nucleus RNA-seq in heterozygous females across the development of epileptic seizures. The goal is to identify proteins and signaling pathways that contribute substantially to the development of neurologic deficits in CDD, which could provide opportunities for the development of novel therapeutics.

Poster 33B | Neuroscience

Transdiagnostic polygenic risk, psychopathology, and personalized functional brain networks in the Adolescent Brain Cognitive Development cohort

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A critical question in developmental neuroscience is how genetic risk influences functional brain networks and psychopathology in early adolescence. A recent multivariate genome-wide association study found two genetic factors, F1 and F2, that capture the majority of genetic variability associated with transdiagnostic psychopathology. F1 was found to be associated with more common psychiatric symptoms and F2 with rarer, more severe disease. Additionally, emerging evidence has suggested the biological importance of a latent overall factor, or p-factor, that quantifies an individual's generalized vulnerability to psychiatric symptoms. However, it is unclear how F1 and F2 are related to p-factor, and how these variables are reflected in functional brain networks. We used personalized functional networks (PFNs)—which capture individual variation in functional network topography that is otherwise ignored by standard analyses based on group atlases—to elucidate the associations between polygenic risk, functional brain networks, and p-factor in the Adolescent Brain Cognitive Development (ABCD) cohort (N=7,045, ages 9-10). The F1 polygenic risk score was found to be significantly correlated to p-factor, whereas the F2 polygenic risk score was not. Furthermore, PFN topography was robustly related to interindividual differences in F1, F2, and p-factor. Notably, the prediction accuracy of p-factor was consistent with prior PFN findings in the Philadelphia Neurodevelopmental Cohort (N=790, ages 8-23). The association of PFN topography with F2 implies that although F2 risk has yet to clinically manifest in preadolescence, it is captured by functional brain network topography.

Poster 34B | Neuroscience

Monosynaptic tracing defines circuit connectivity of human glioblastoma

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Glioblastoma is a universally fatal brain cancer that functionally integrates into neural circuitry. Neuronal activity drives tumor progression via glutamatergic synapses, and in parallel glioblastoma cells alter neuronal excitability, remodel neural circuits, and impair cognitive function. However, little is known regarding the cellular diversity and circuit architecture of neurons actively connected with tumor cells. Here we sought to define the glioblastoma connectome by a monosynaptic rabies virus retrograde tracing approach. We transplanted patient-derived glioblastoma organoids (GBOs) into distinct cortical and subcortical areas in adult immunodeficient mice and found that they undergo rapid integration into brain-wide networks. We identified recurrent patterns of both short- and long-range connectivity to a highly migratory glioma population. While inputs to tumor cells consist of primarily glutamatergic neurons, we observed many other inputs, including neuromodulatory inputs across different brain regions. Current efforts are focused on exploring the functional role of these neuromodulatory projections in tumor biology. Together, these data demonstrate a strikingly robust integration of glioma into anatomically and molecularly diverse neuronal networks in the brain.

Poster 35B | Pharmacology

Engineering cellular systems for biomedical imaging & diagnostics

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Despite advances in the sensitivity of biomedical imaging, the detection of micrometastatic disease remains a significant challenge. Because current imaging methods (e.g. FDG-PET, CT, MRI) rely on intrinsic tumor features (e.g. metabolic activity, tissue density, etc.) as a source of signal, the ability of these methods to identify micrometastases is fundamentally limited. Here, we aim to engineer cells which amplify a detectable signal upon sensing cancer, improving our ability to diagnose and monitor micrometastatic disease. The sensing component of the system will be derived from a synthetic intramembrane proteolysis receptor (SNIPR) with an scFv extracellular domain and a TEV protease as the intracellular domain. Upon antigen binding, proteolysis will liberate the intracellular protease domain. The reporter will consist of the fluorescent protein mCherry fused to the murine ornithine decarboxylase degron, which will target the protein for degradation by the proteasome. In this system, scFv-antigen binding will result in liberation of the TEV protease, which will cleave the degron from mCherry and increase the abundance of the reporter protein. We aim to demonstrate the generalizability and translational potential of this approach using luciferase, E. coli dihydrofolate reductase, and secreted embryonic alkaline phosphatase for detection by bioluminescence imaging, PET imaging, and serum ELISA in vitro and in a murine model of hematogenous metastasis. If successful, this work will provide the foundation for the development of cell-based diagnostic agents which enable the diagnosis and monitoring of micrometastatic disease.

Poster Session List
Alphabetically by Student

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Bannerman, Carl	A20	Mentor: Dr. Kellie Jurado *The Role of TRIM72 In Regulating Neuronal Antiviral Responses*
Barnett Dubensky, Thomas (Sam)	A37	Mentor: Dr. Jorge Henao-Mejia *Dysregulation of the hepatic CD4 T cell compartment during childhood obesity*
Chaluvadi, Venkata (Sai)	A33	Mentor: Dr. F. Chris Bennett *Lysosomal lipid accumulation leads to macrophage dysfunction in Krabbe disease*
Chauvin, Samuel (Sam)	A29	Mentor: Dr. Jonathan Miner *Autosomal dominant C-terminal TREX1 frameshift mutants inhibit homology-directed repair and increase risk of breast cancer*
Chen, Christina	A4	Mentor: Dr. Russell T. Shinohara *Subject-level weights for detecting brain volume differences*
Chini, Julia	A30	Mentor: Dr. David Hill *Hepatic CD9 regulates adipose tissue function and inflammation during obesity*
Chiu, Joy	A31	Mentor: Dr. Michaela Locci *Understanding the Role of Germinal Centers in the Generation and Durability of nAbs to mRNA-LNP vaccination*
Coffey, Nathan (Nate)	A13	Mentors: Drs. Arany Zolt and Celeste Simon *Increasing branched-chain amino acid metabolism reduces growth of clear cell renal cell carcinoma*
Cohn, Ian	A21	Mentors: Drs. Christopher A. Hunter and Boris Striepen *Understanding intestinal CD4+ T cell immunity using Cryptosporidium infection*
Cox, Timothy (Tim)	A36	Mentors: Drs. Christoph A. Thaiss and Virginia Lee *The Aged Microbiome Drives Cognitive Decline via Intestinal Inflammation and Vagal Inhibition*
Creekmore, Benjamin (Ben)	A1	Mentors: Drs. Eddie Lee and Yi-Wei Chang *Small Molecule Activation of Valosin-containing protein (VCP)*
Dai, David	A34	Mentor: Dr. Eddie Lee *Cell type-specific vulnerabilities in behavioral-variant Frontotemporal Dementia caused by C9orf72 expansion mutations*

Danan, Charles	A14	Mentor: Dr. Kate Hamilton *Intestinal transit amplifying cells require METTL3 for growth factor signaling, KRAS expression, and cell survival*
Deschaine, B. (John)	A32	Mentor: Dr. Michael Silverman *Can microbial molecular mimics protect from type 1 diabetes?*
Deshpande, Rajiv	A5	Mentor: Dr. Felix W. Wehrli *MRI-based quantification of renal oxygen consumption*
Dong, Royce	A6	Mentors: Drs. Brian Litt and Flavia Vitale *Microfabrication of a microLED array based on SU-8 for implantable optogenetics*
Erlitzki, Noa	A2	Mentor: Dr. Rahul Kohli *Signatures of 5-methylcytosine modification in DNA by the tumor suppressor TET2*
Fein, Ethan	A15	Mentor: Dr. Patrick Seale *Delineating the Developmental Trajectory of Brown Fat in the Mouse Embryo*
Fernandez del Castillo, Andres	A3	Mentor: Dr. Mark Sellmyer *Towards the Targeted Degradation of a-Synuclein Fibrils in Models of Oxidative Neurodegeneration*
Frankfurter, Maxwell (Max)	A16	Mentor: Dr. Mark Kahn *Understanding the role of STRN3 in lymphatic development*
Franklin, Brian	A17	Mentor: Dr. Jennifer Phillips-Cremins *Mapping of neural connectome in health and disease*
Goldspiel, Brian	A22	Mentor: Dr. Will Bailis *The Branched Chain Amino Acids Control Macrophage Inflammation Through Translational Regulation of Cytokine Production*
Gonzalez, Elizabeth (Eli)	A18	Mentor: Dr. Elizabeth Bhoj *A novel mendelian neurodevelopmental disorder caused by germline variants in MAP2K4*
Hapke, Robert (Rob)	A9	Mentors: Drs. Andy Minn and Evan Weber *Optimization of an in vivo CRISPR screen in CAR T*
Heymach, Claudia	B29	Mentor: Dr. Alex Proekt *Behavioral paradigm for investigation of the relationship between visually-evoked traveling waves and perception in mice*
Hollander, Erin	A10	Mentor: Dr. Ben Stanger *N-glycosylation imposes a targetable constraint on T cell killing of cancer cells*
Hu, Fengling (Feng)	A24	Mentor: Dr. Russell T. Shinohara *DeepCombat: A Statistically-Motivated, Hyperparameter-Robust, Deep Learning Approach to Harmonization of Neuroimaging Data*
Hunter, Emerson	A26	Mentor: Dr. Yi Xing *Characterizing ERV transcripts across healthy human tissues*
Iliakis, Evan	B30	Mentor: Dr. Marc Fuccillo

		Investigating the Role of Somatostatin-Positive Low-Threshold Spiking Interneurons in the Dorsomedial Striatum in Goal-Directed Behavior
Jardin, Blake	A19	Mentor: Dr. Jonathan A. Epstein *Reducing Kidney Fibrosis by FAP-CAR-T Cells in Mouse Models of Kidney Disease*
Kahn, Benjamin (Ben)	A35	Mentor: Dr. Ben Stanger *Route-specific immune surveillance during metastasis*
Kellier, Danielle	A25	Mentors: Drs. John T. Farrar and Christina L. Szperka *Predicting migraine in the pediatric emergency room*
Kixmoeller, Kathryn (Katie)	B1	Mentors: Drs. Ben Black and Yi-Wei Chang *Structure and organization of the human centromere on purified chromosomes revealed by cryo-electron tomography*
Kolla, Likhitha	B17	Mentors: Drs. Jinbo Chen and Ravi B. Parikh *Algorithmic reliability and dataset shift in a national readmission and mortality prediction algorithm*
Krauss, Kathleen (Kate)	B22	Mentor: Dr. Lawrence Eisenlohr *The role of the Type III Secretion System in endogenous presentation of Salmonella enterica epitopes on MHCII*
Kuprasertkul, Napasorn (Nina)	B10	Mentors: Drs. Brian C. Capell and Kathryn E. Wellen *Ferroptosis enhances epidermal cornification through transcriptional and metabolic reprogramming*
Lam, Jessica	A27	Mentor: Dr. Gerd Blobel *Exploiting cell cycle dynamics to interrogate YY1's role in spatiotemporal chromatin organization*
Lee, Casey	B23	Mentors: Drs. Christoph T. Ellebrecht and Aimee S. Payne *Fate induction in chimeric antigen receptor T cells through asymmetric cell division*
Li, Jingxin (Jessica)	B11	Mentor: Dr. Arjun Raj *Burning in cellular memory as a mechanism for phenotypic enhancement in metastatic melanoma*
Lin, Andrew	A7	Mentor: Dr. Dave Issadore *Engineering Two-Dimensional and Three-Dimensional Nanopore Devices for Immunomagnetic Extracellular Vesicle Sorting in Multiple Disease Contexts*
Litichevskiy, Lev	B18	Mentor: Dr. Christoph A. Thaiss and Mingyao Li *Interactions between the gut microbiome, dietary restriction, and aging in more than 900 genetically diverse mice over the lifespan*
Lubin, Emily	B12	Mentor: Dr. Elizabeth Bhoj *Novel class of pediatric neurodevelopmental "histonopathies" may be driven by shared mechanism of chromatin dysregulation*
Lucas, Alfredo	A8	Mentor: Dr. Kathryn A. Davis *Improved Seizure Onset-Zone Lateralization in Temporal Lobe Epilepsy using 7T Resting-State fMRI: A Direct Comparison with 3T*

Luo, Audrey	B31	Mentor: Dr. Theodore D. Satterthwaite *Mapping the development of white matter structural organization and overall psychopathology*
Martinez, Dayne	B32	Mentor: Dr. Zhaolan (Joe) Zhou *Phosphoproteomic profiling to uncover molecular mechanisms of neurologic deficits in a mouse model of CDKL5 deficiency disorder*
McCright, Samuel (Sam)	B24	Mentor: Dr. David Hill *Obesity-associated long chain fatty acids regulate lung innate immune responses via the NLRP3 inflammasom*
Merolle, Maria	B25	Mentor: Dr. Christopher A. Hunter *T cell homing to the small intestine during Cryptosporidium infection*
Morrissette, Jeremy	B26	Mentor: Dr. Scott Canna *Excess IL-18 Augments Suppressor/Regulatory Cell Function to Prevent Experimental Autoimmune Encephalomyelitis*
Murphy, Lance	B27	Mentors: Drs. Carla Scanzello and Robert L. Mauck *Deficiency of the Pattern-recognition Receptor CD14 Protects Against LPS-induced Inhibition of Osteoclastogenesis in Vitro*
Narayan, Sweta	B8	Mentor: Dr. Mark Kahn *Angiopoietin and VEGF Signaling in Murine Spiral Artery Remodeling*
Ng, Raymond	A11	Mentor: Dr. Sydney Shaffer *Investigating the role of tumor transcriptional variability in immune evasion in melanoma*
Orlen, Margo	B4	Mentor: Dr. Ben Stanger *Effects of KRAS inhibition on anti-tumor immunity in pancreatic cancer*
Otter, Clayton	B15	Mentor: Dr. Susan Weiss *What makes a common cold virus? Respiratory viruses differentially interface with host interferon responses in the nasal epithelium*
Pace, Jesse	B13	Mentor: Dr. Mark Kahn *Hemodynamics and KLF2/4 regulate myxomatous valve formation*
Pham, Jonathan	B35	Mentor: Dr. Mark Sellmyer *Engineering cellular systems for biomedical imaging & diagnostics*
Pham, Kenneth	B37	Mentor: Dr. Jennifer Phillips-Cremins *Elucidating the spatially coordinated mechanisms of heterochromatinization in fragile X syndrome*
Poltorack, Carson	B5	Mentors: Drs. Sydney Shaffer and Celeste Simon *Single-cell spatial multiomic profiling of cellular and metabolic neighborhoods in the pancreatic ductal adenocarcinoma tumor microenvironment*
Rafizadeh, Diane	A23	Mentor: Dr. Dave Chenoweth

		Synthesis and design of macrocyclic collagen mimetic peptides for targeting the cancer-implicated DDR2 kinase
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Sharma, Prateek	B6	Mentors: Dr. Christoph A. Thaiss and Kathryn E. Wellen *Investigating the Role of Diet and Host Genetics on Colorectal Cancer Metabolism and Physiology*
Shea, Emily	B7	Mentor: Dr. Lewis Chodosh *GOT1, GPX4, and PNPLA2 in Breast Cancer Dormancy and Recurrence*
Shen, Kaitlyn	B14	Mentor: Dr. Rajan Jain *Pathogenic laminopathy mutations disrupt specific lamina-associated regions in cardiac myocytes potentially via altered mechanosensing*
Skelly, Ashwin	B28	Mentor: Dr. Amelia Escolano and Beatrice Hahn *Elicitation of V3 glycan-directed cross-neutralizing antibodies in sequentially immunized, SHIV-infected rhesus macaques*
Soto Albrecht, Yentli	B16	Mentor: Dr. Douglas C. Wallace *Deficits in mitochondrial oxidative phosphorylation enhance SARS-CoV-2 replication via metabolic remodeling*
Sun, Kevin	B33	Mentors: Drs. Aaron Alexander-Block and Theodore D. Satterthwaite *Transdiagnostic polygenic risk, psychopathology, and personalized functional brain networks in the Adolescent Brain Cognitive Development cohort*
Sun, Yusha	B34	Mentor: Dr. Hongjun Song *Monosynaptic tracing defines circuit connectivity of human glioblastoma*
Sussman, Jonathan	B19	Mentor: Dr. Kai Tan *Single-Cell Multiomic Analysis of Pediatric High Grade Glioma Under Therapy*
Tamburro, Margaret (Maggie)	B3	Mentor: Dr. Louis Soslowsky *Structural and Functional Impacts of Early Type III Collagen Reduction on Tendon Healing*
Wechsler, Caroline	A28	Mentor: Dr. Robert Aronowitz *No one had ever told us of the human problems we should be called upon to face: The Role of Medical Students in the 1918 Influenza Pandemic in Philadelphia*
Xu, Karen	B36	Mentors: Drs. Robert L. Mauck and Jason A. Burdick *Biopolymer-based Bicontinuous Hydrogels Guide Rapid 3D Cell Migration*
Yang, Kevin	B20	Mentors: Drs. Peter Choi and Yoseph Barash

		The LSV-Seq method enables machine learning-assisted recovery of low-coverage splicing events across human tissues
Yen, Daniel	B9	Mentor: Dr. Nancy Speck *The Interaction between Inflammation and Loss of RUNX1 in Familial Platelet Disorder with Associated Myeloid Malignancies*
Zhang, David	B21	Mentors: Drs. Marylyn Ritchie and Daniel Rader *Investigating ancestry-specific genetic variation in apolipoprotein L genes associated with electronic health record phenotypes in diverse patient biobanks*