Student posters, March 27, 2024, Full Abstracts

Poster number	Session	Topic area	First name	Last name	GSBSE student?	Organization /Affiliation	Abstract Title	Poster abstract Text (not to exceed 500 words). Abstract must state the purpose, significant results and main conclusion of work.
200	Basic and Applied Research in Biological Sciences	Biochemistry	**Peter**	**Swanson**	☐	University of New England	Phase Behavior of Multi-Stimuli Responsive Biopolymers	Presented herein is the design, production, and characterization of several stimuli responsive elastin-like biopolymers. We use dynamic light scattering, and zeta potential measurements, to show our biopolymers are responsive to solution environment, buffer type, and temperature.
201	Basic and Applied Research in Biological Sciences	Biochemistry/ Microbiology/Virology/ Molecular Biology	**Lucas**	**Bennett**	☐	University of Maine	Probing the Role of Serotonin Receptors in the JC Polyomavirus Infectious Cycle	JC polyomavirus (JCPyV) is the causative agent of the debilitating demyelinating disease known as progressive multifocal leukoencephalopathy (PML). Although PML is a rare disease, JCPyV infects 50-80% of the human population, persisting as an asymptomatic infection in the kidney. In immunocompromised individuals, JCPyV can migrate to the brain to cause PML. During infection, viruses utilize a specific subset of receptors on the cell surface to hijack normal cell processes and enter the host cell. JCPyV requires the serotonin (5-hydroxytryptamine) subtype-2 receptors (5-HT2Rs) to mediate entry and infection of host cells. All three 5-HT2R subtypes, 5-HT2AR, 5-HT2BR, and 5-HT2CR, support JCPyV infection. JCPyV localizes with 5-HT2Rs during entry and induces receptor clustering, which likely drives viral endocytosis. However, the contribution of each 5-HT2R subtype during viral entry and cellular signaling remains unknown. We have probed the role of 5-HT2Rs in various combinations in JCPyV infection and for their role in activation of the mitogen-activated protein kinase pathway during the initial phase of infection. Understanding how these receptors support both entry and priming the cellular machinery for viral infection are key to defining potential cellular targets for treatments to prevent PML.
202	Basic and Applied Research in Biological Sciences	Biochemistry/ Microbiology/Virology/ Molecular Biology	**Jessica**	**Walsh**	☐	University of Maine, Orono	Modeling Acute Kidney Injury Following Influenza Virus Infection Using Zebrafish	On average, 27 million individuals become infected by influenza virus annually with 300,000 requiring hospitalization from severe infections. During the 2009 influenza A virus (IAV) pandemic, acute kidney injury (AKI) was reported in individuals with severe infections. Impaired kidney function increases the risk of developing cardiovascular diseases and can lead to kidney failure. The zebrafish is a model organism to study AKI where kidney function can be studied in vivo to research possible treatments. In developing larvae, the pronephros has two nephrons with glomeruli, tubules and ducts. The glomerulus acts as a filter and will allow water and other small molecules to pass into the tubules. The tubules absorb solutes and nutrients that pass into them and excrete waste into the ducts that then exits the kidney. These components of the pronephros are similar to those in human kidneys and make the zebrafish pronephros a useful model. Currently, how IAV infection results in AKI and reduced kidney function is not well characterized. To investigate potential mechanisms, we will model the response to IAV infection by microinjecting IAV into zebrafish larvae where any tissue damage, including the kidney pronephros, can be monitored using in vivo fluorescent confocal imaging. We hypothesize that a hyperinflammatory response following IAV infection leads to tissue damage in the pronephros that leads to decreased kidney function. We'll examine this hypothesis by analyzing gene expression data of the response to IAV infection in wild-type (AB) larvae and comparing that response to a zebrafish mutant with a defective neutrophil function (WHIM; Tg(-8mpx:cxcr4b-EGFP)), and a myeloid-specific peroxidase (mpx) mutant (Spotless; mpxNL144-/-) with an improved response to IAV infection . In particular, we'll assay the gene expression of several genes associated with AKI from previous studies, including wt1b, il1b, tnfa, tgfb1a and cxcl8a, following IAV infection using qRT-PCR. These genes were chosen due to previous studies showing their involvement with gene expression in immune response, indicating they might be useful in our research. Understanding the expression profiles of these genes will enable us to create studies of genes that become targets of future AKI therapies.

204	Basic and Applied Research in Biological Sciences	Biology	**Tayo**	**Adekeye**	☐	University of Maine	Mylpf dosage controls myofibril growth in fast-twitch skeletal muscle	Author: Tayo Adekeye1, Emily Teets2, Emily Tomak1, Sadie Waterman1, Sabrina Varga1, Carla Rodriguez-Medio, Sharon Amacher2, Joshua Kelley1, and Jared Talbot1 1University of Maine, 2The Ohio state university Muscle fibers contain strings of contractile proteins called myofibrils which are made of fundamental repeats called sarcomeres. Although the key myofibrillar proteins have been long understood, it remains unclear which components regulate muscle growth. Here we show that a sarcomeric protein, Myosin-Light-Chain-Phosporylatable-Fast (Mylpf), is both necessary and sufficient for myofibril growth in zebrafish muscle. Mylpf is encoded by a single gene (MYLPF) in humans and two genes (mylpfa and mylpfb) in zebrafish, which are solely expressed in fast-twitch skeletal muscle. Overexpression of zebrafish Mylpfa-GFP or human MYLPF-GFP in fast-twitch skeletal muscle can increase myofibril width beyond typical ranges to a degree that is correlated with the amount of GFP expression. Furthermore, graded loss of Mylpf function causes graded loss of myofibril. Loss of the high-abundance gene mylpfa causes severe myofibril defects, but animals mutant for the low-abundance gene mylpfb have normal muscle fibers. In the mylpfa-/-;mylpfb-/- double mutant, the fast-twitch muscle cytosol contains a patchwork of unassembled sarcomeric components and myofibril loss is total. The severe mylpfa-/- mutant can be rescued equally well by Mylpfa-GFP, Mylpfb-GFP, and MYLPF-GFP when expressed at the same dosage. However, a version of MYLPF that causes recessive Distal Arthrogryposis (DA1) (p.Cys157Phe) can only partially rescue the mylpfa-/- mutant. This pCys157Phe MYLPF localizes poorly to the myofibril, and cannot improve growth in the wild type zebrafish, suggesting that its effective dosage is reduced. Likewise, a version of MYLPF found in dominantly inherited DA (p.Gly163Ser), completely fails to rescue the mylpfa-/- mutant and is sometimes sufficient to destroy muscle fibers in the wild-type. Together, these findings suggest that Mylpf dosage is among the few known determinants of muscle growth, vital to muscle development and human health.
205	Basic and Applied Research in Biological Sciences	Biology	**Ahmed**	**Almaghasilah**	☑	University of Maine	Assessing nuclear architecture in zebrafish Duchenne muscular dystrophy model	Duchenne muscular dystrophy (DMD) is a genetic disorder that results in progressive muscle fiber weakness, leading to early death in children. DMD is caused by a mutation in the dystrophin gene that provides a mechanical link from actin, an intracellular component, to glycoprotein complex located at muscle cell membrane. We have previously found that sapje zebrafish, a dmd animal model, have deformed nuclei in muscle cells. Abnormal nuclear shapes are linked to laminopathies, rare genetic diseases caused by mutations in the nuclear lamina that lead to a variety of pathologies, including some muscular dystrophies. Some laminopathies involve a loss of heterochromatin, regions of dense chromatin layers that are transcriptionally inactive. Recently, it has been reported that heterochromatin increases in mdx mice, a popular mouse model for studying DMD. However, it is not clear whether this increase of heterochromatin is correlated and colocalized with the dystrophic muscle fibers. This has motivated us to investigate the state of chromatin in sapje zebrafish. We plan to image and quantify the heterochromatin in dystrophic muscle in sapje embryos using confocal microscopy and ImageJ. We will examine heterochromatin levels in injured and uninjured muscle in the DMD fish model. Despite the whole organism expressing the mutant gene, only a subset of muscle is damaged in DMD. If heterochromatin levels change, as they are reported to do in mice, we will be looking for whether they are different throughout the fish, or whether the changes track with damaged muscle specifically. This will enhance our understanding of the mechanisms underlying muscle defects in DMD, which will influence research in designing therapeutic approaches and drug interventions.
206	Basic and Applied Research in Biological Sciences	Biology	**Amanda**	**Ignacz**	☑	University of Maine	DPM3 functions in both dystroglycan-dependent and independent roles in neuromuscular disease progression	Skeletal muscle development, growth, and homeostasis relies on post-translational modifications, such as glycosylation. Healthy skeletal muscle function also requires the development of neuromuscular junctions (NMJs) and myotendinous junctions (MTJs) in addition to skeletal muscle structure. Muscle, NMJs, and MTJs are all connected via cell-matrix adhesion complexes that are replete with glycosylated proteins. Glycosylation is disrupted in two main groups of incurable diseases: the congenital disorders of glycosylation (CDGs), and the dystroglycanopathies. CDGs affect glycan biosynthesis and metabolism. Dystroglycanopathies are identified by their role in glycosylation of dystroglycan (DG), a critical transmembrane receptor that anchors the intracellular cytoskeleton to the extracellular matrix. Both CDGs and dystroglycanopathies are clinically heterogeneous, multisystem disorders that can lead to severe progressive neuromuscular degeneration, which deleteriously impacts quality of life and is frequently lethal. Interestingly, mutations in some genes, such as dolichylphosphate mannose synthase 3 (dpm3), can lead to either CDGs or dystroglycanopathy, bridging CDGs and dystroglycanopathies. An important question for both CDGs and dystroglycanopathies is regarding what aspects of neuromusculoskeletal degeneration are specifically due to disruption of DG glycosylation versus disruption of the myriad of other glycosylated proteins that promote adhesion to the extracellular matrix. We developed a zebrafish model of DPM3-dystroglycanopathy through CRISPR/Cas9 technology that recapitulates the phenotypic variation and disease progression observed in human DPM3 patients. Through use of dpm3 mutants, as well as dpm3;dg double mutants, we have also developed a model to elucidate what aspects of DPM3 function are DG-dependent and DG-independent in disease progression. While dg -/- mutants do harbor dystrophy and muscle fiber detachments, the loss of even one copy of wild-type dpm3 dramatically exacerbates NMJ, MTJ, and skeletal muscle degeneration. Interestingly, we have also observed heterogeneity within the dpm3 +/-;dg -/- genotype, ranging from mild posterior muscle degeneration to severe multi-segment degeneration soon after the development of segmentation in the fish body. These findings indicate that DPM3 likely contributes to neuromuscular disease progression via both DG-dependent and DG-independent pathways, and possibly acts in a gene-dose dependent manner. Ultimately, we aim to elucidate DG-independent roles of dystroglycanopathy genes to gain fundamental knowledge for future therapeutic strategies in treating neuromuscular diseases.

207	Basic and Applied Research in Biological Sciences	Biology	**Hannah**	**Megathlin**	☑	University of Maine	Removal of mouse FcγRI in NSG mouse strain increased IgG1 survival in serum	Monoclonal antibodies (mAbs) are a widely used therapeutic tool for the treatment of a diverse set of diseases, yet optima mouse models to test these drugs are lacking. The successful therapeutic use of mAbs rely on their function in the context of a human immune system, so to accurately test how they work in humans, mice that have functional human immune systems are needed. This can be accomplished using humanized mice. Humanized mice are immunodeficient mice that have been engrafted with human hematopoietic stem cells (HSCs) and subsequently develop a functional human immune system. The most frequently used immunodeficient mouse strain used for hyman HSC engraftment is NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG). However, NSG mice have a gain of function mutation at the Fcgr1 gene and clear human immunoglobulins (IgGs) very quickly. The serum half-life for IgG1 in NSG mice is much shorter than in humans. Therefore, a mouse model that can be engrafted with human HSC and has a more human-like physiological rate of human IgG clearance rate is needed. IgG clearance in mammals is largely controlled by the Fc receptors, namely the Fcγ receptors (FcγRs) and neonatal Fc receptor (FcRn). We created three different mouse models to try and extend the half-life of human IgG in NSG mice, an NSG Fcγ Receptor I knock out (NSG-FcγRI KO), an NSG human FcRn transgenic combined with a mouse FcRn knock out (NSG-hFcRn Tg, mFcRn KO), and a model combining all three alleles designated (NSG-hFcRn Tg mFcRn KO FcγRI KO). Mice from each model, along with NSG controls, were given an intravenous dose of Rituximab, a human IgG1 mAb used to treat B cell lymphomas. Sera were collected from mice at regular intervals from 2 days to eight weeks post injection. IgG1 levels were measured by enzyme linked immunosorbent assay (ELISA). We found that the NSG-FcγRI KO model had the longest IgG1 survival. The NSG, NSG-hFcRN Tg mFcRn KO, and NSG-hFcRN Tg mFcRn KO FcγRI mice all had cleared the Rituximab completely by 4 weeks, while the NSG-FcγRI KO mice retained about 15% of the initial dose remaining at the end of 8 weeks. Thus, the NSG-FcγRI KO mouse supports an extended half-life of therapeutic human IgG1 mAbs similar to the half life of IgG1 in humans.
208	Basic and Applied Research in Biological Sciences	Biology	**Courtney**	**Willey**	☑	JAX	NBL1 Correlates with Kidney Disease in Alport Syndrome	Chronic kidney disease (CKD) affects about 10% of people worldwide with most people unaware that they even have the disease until symptoms start to appear in the very late stages. With millions of people dying from kidney-related illnesses every day and limited treatment options available, uncovering potential new treatments is necessary. Alport syndrome (AS) is a rare genetic condition that often results in progressive loss of kidney function and end-stage kidney disease and although causal genes for AS are well characterized, individuals with AS that have similar genetic mutations still display a wide range of variation in kidney function and age of onset. Our collaborators at the Joslin Diabetes Center, identified plasma neuroblastoma suppressor of tumorigenicity 1 (NBL1) to be strongly and independently associated with more severe progression into kidney disease during a 10-year follow up in diabetic patients. Curious about these results we decided to investigate if we see similar outcomes in a genetically diverse population of Diversity Outbred (DO) mice with X-linked Alport Syndrome (DO-XLAS). We generated a cohort of 200 DO-XLAS mice and measured albumin to creatinine ratio (ACR) at 6, 10, and 15 weeks of age, and glomerular filtration rate (GFR) at 14 weeks of age. We then correlated these values with serum NBL1 levels at 10 weeks of age and found a significant correlation between NBL1 and kidney function. However, it is still unclear whether NBL1 is causal or consequential to kidney disease. To investigate this, we created an NBL1 knockout (KO) mouse model and confirmed that heterozygous (HET) animals have significantly lower serum NBL1 compared to wildtype (WT) (homozygous KO animals were not viable). Then, we induced CKD by breeding a mutated Col4a5 allele into our NBL1 KO mice to induce AS. Measuring GFR revealed no significant difference in kidney damage between HET and WT animals, suggesting that NBL1 is not causal for decreased kidney function in our model. Overall, much remains to be understood regarding NBL1 and its relationship to kidney disease and function, including what is driving NBL1 expression and what the source of increased NBL1 is. Preliminary Bulk-RNA sequencing (RNAseq) data has revealed ~130 differentially expressed genes between HET and WT mice, yet additional RNAseq data needs to be obtained to confirm our previous expression data. By analyzing NBL1 levels in a larger cohort of 600 DO-XLAS mice we hope to uncover its role in individuals with different genetic backgrounds, allowing us to perform a genetic analysis to determine how NBL1 levels behave with different genetic predispositions. These experiments will help elucidate why NBL1 levels increase with kidney disease.

209	Basic and Applied Research in Biological Sciences	Biology	**Caryl**	**Young**	☑	University of Main	Mybl2 regulates cell cycle progression and genomic stability in sensory progenitors.	The cochlea is the auditory half of the inner ear with an asymmetric sensory epithelium, the organ of Corti (OC). The OC consists of one row of sound-detecting inner hair cells (IHCs) and three rows of sound-amplifying outer hair cells (OHCs). The hair cells (HCs) are intercalated by support cells that provide structural and trophic support to the HCs. The sensory cells are highly susceptible to damage and do not have regenerative capacities. Therefore, the auditory sensory cells that arise from progenitors during early development must survive throughout adulthood. Thus, the progenitors have mechanisms in place to maintain the genomic integrity and repair DNA damage. When repair is disrupted, hearing loss ensues due to the accumulation of errors and genomic instability. However, little is known about the DNA repair pathways in cochlear progenitors. Mybl2 is an important regulator of the cell cycle and DNA repair in embryonic stem cells (ESCs). Mybl2 is also expressed in the cochlear progenitors between embryonic day (E)12.5 and E14.5 in cycling cochlear progenitors and is no longer expressed by E15.0. We hypothesize Mybl2 is necessary to guide proper cell cycle progression and maintain the genomic integrity in the cochlea. To test this hypothesis, we generated Sox2CreER; Mybl2 conditional knockout (cKO) mice to drive Mybl2 deletion in cochlear sensory progenitors between E10.5-E14.5 upon tamoxifen administration. Using immunohistochemistry, we analyzed the effects of Mybl2 cKO on cell cycle progression, DNA damage, and sensory cell differentiation. Mybl2 cKO cochleas show a decrease in Ki67, a mitotic (M) phase proliferation marker on E14.5. Our data suggest that the progenitors fail to progress past the synthesis (S) phase. In addition, when we culture cochleas in BrdU, a thymidine analog that is incorporated into the DNA of replicating cells during S phase, Mybl2 cKO cochleas show increased BrdU incorporation per cell, supporting the idea that progenitors are in an extended S-phase in Mybl2 cKO cochleas, experiencing replicative stress. In addition, the increased DNA replication, and an inability to divide during M phase may be responsible for the enlarged nuclei, compromising genomic integrity. To assess DNA damage, we immunolabeled double strand breaks using a gH2AX antibody in control and Mybl2 cKO cochleas. Mybl2 cKOs show increased gH2AX labeling on E14.5; thus, there is an increase in DNA damage. We also find that differentiated sensory hair cells are abnormally shaped and binucleated, and sensory support cells are swollen. This data supports the hypothesis that Mybl2 is an important regulator of cell cycle progression and maintenance of genomic integrity in cochlear progenitors. Dysregulation of this important pathway will lead to unfit sensory cells, leading to cell death or organ dysfunction. Thus, this study will help advance our understanding of the important role progenitors have on organ development and maintenance.
210	Basic and Applied Research in Biological Sciences	Biology, Molecular Biolog	**Vijayishwer**	**Jamwal**	☐	Mount Desert Island Biological Laboratory	Cellular mechanisms of muscle formation during axolotl tail Development and regeneration	In mammals, muscle repair upon injury exclusively depends upon PAX7+ satellite cells to form new muscle fibers. However, in salamanders, two modes of muscle regeneration have been reported. One that depends upon the differentiation of PAX7+ muscle progenitors and another that depends upon the dedifferentiation of muscle fibers to generate MHC+ myocytes. Over the last decade, many conflicting studies have emerged that propose the role of muscle fiber dedifferentiation as a plausible mechanism for cellular source in a regenerating appendage of diverse salamanders. To make the matter more complicated, one study even proposes that during neoteny muscle fiber dedifferentiation does not happen whereas upon metamorphosis muscle fiber dedifferentiation can occur. All these studies were conducted using less reliable technical tools for the purpose of lineage tracing such as histology, electroporation of Cre constructs, or F0 transgenic animals that are not uniformly labeled or thoroughly characterized. To resolve some of these issues, we have developed transgenic axolotls with the powerful tamoxifen inducible Cre-LoxP system that provides indelible labeling along with spatiotemporal control. We have generated germline transmitted muscle fiber-specific (MCK) inducible Cre line, thus avoiding the problem of using F0 animals. Lineage tracing of neotenic axolotl tail using MCK and PAX7 inducible Cre line showed complete absence of muscle fiber dedifferentiation and dependence on differentiation of PAX7+ satellite cells. We are expanding our study to understand if muscle fiber dedifferentiation is a metamorphosis-dependent phenomena.

211	Basic and Applied Research in Biological Sciences	Biology, Molecular Biology	**Samantha**	**Costa**	☑	UMaine/MHIR	PD-L1 Expressing Myeloid Cells Promote Bone Marrow Immunosuppression and Osteoclastogenesis with Diet-induced Obesity	Programmed death ligand 1 (PD-L1) when combined with PD-1 inhibits immune responses and promotes self-tolerance by modulating T-cell function. Previous studies have investigated aberrant PD-L1 expression in tumor-bearing models. Recently, we identified a novel population of PD-L1 expressing myeloid cells within the bone marrow of obese, non-tumor bearing mice. However, the role of PD-1/PD-L1 signaling in osteoclast differentiation resulting in obesity-related bone loss has yet to be explored. We hypothesized obesity-induced bone loss is linked to PD-1/PD-L1 signaling through a pro-osteoclastogenic mechanism. Starting at 8 weeks of age, C57BL/6J mice were fed a high-fat diet (HFD, 60% kcal) or sucrose-matched low-fat diet (LFD, 10% kcal) for 12 weeks. After 12 weeks of HFD-feeding, pre-determined parameters based on body measurements (i.e., body weight >40g and percent body fat >32%) were used to classify obese mice. Compared to LFD-fed mice, obese mice had an increase in MHCIInegPD-L1+ myeloid cells (fully defined as CD11bhighCD11chighMHCIInegF4/80+PD-L1+, p<0.0001). The increase of PD-L1 coupled with the loss of MHCII expression negatively affected T-cell activation and increased immunosuppressive CD39 and CD73 expression (p=0.0019 and p=0.0012). In vitro PD-L1 blockade suppressed osteoclast differentiation (p=0.014), suggesting the PD-1/PD-L1 axis mediates osteoclastogenesis. In addition, PD-1 expressing osteoclast precursors (defined as [B220/CD3/NK1.1]negc-kit+CD115+CD11blowPD-1+) were increased in obese mice compared to LFD-fed mice (1.34-fold higher than LFD, n=10 mice/group, p=0.0042). TRAP staining and histomorphometric analyses revealed obese mice had an increase in osteoclasts (1.82-fold higher than LFD, p=0.0070) and enhanced eroded surface, while no significant changes in osteoblast number and mineralizing surface were observed. As a result, obese mice had decreased femoral (Tb.BV/TV, obese 10.76% vs LFD 12.13%, n=7 mice/group, p=0.020) and tibial (Tb.BV/TV, obese 13.76% vs LFD 20.34%, n=8-10 mice/group, p<0.0001) trabecular bone mass as well as decreased cortical area (Ct.Ar/T.Ar, obese 56.59% vs LFD 58.61%, n=8-10 mice/group, p=0.0245). In summary, obesity creates an immunosuppressive bone marrow microenvironment through increased PD-1 and PD-L1 expression in myeloid and myeloid lineage cells that results in osteoclast-driven bone loss. The impact obesity-induced PD-1/PD-L1 signaling has on osteoclast over-activation remains to be elucidated.
212	Basic and Applied Research in Biological Sciences	Biology/ Computer Data	**John**	**Butts**	☑	GSBSE	PRIORITIZATION OF NON-CODING CANCER DRIVERS USING MPRA	The non-coding portion of the genome accounts for 98% of the total genome and houses numerous classes of regulatory elements responsible for the precise spatiotemporal expression of genes. While a great deal of effort has gone into the study of promoters, cis-regulatory elements (CREs), and silencers, a comprehensive understanding of the sequence basis of regulatory activity is outstanding.Unlike coding mutations, the impacts of non-coding mutations are often obscure. For example, if a mutation lies in a cis-regulatory element (CRE) one must determine the gene it regulates and even then, gene expression changes may be small between alleles and operate through cryptic mechanisms. This makes it challenging to interpret the impact of regulatory elements, and the variation within regulatory elements on biological phenomena including human health. Massively Parallel Reporter Assays (MPRA) allow researchers to test thousands of sequences for their regulatory potential and are sensitive enough to quantify the impact of single nucleotide changes. Our group has generated a model, Malinois, from dozens of MPRA experiments, testing hundreds of thousands of sequences, capable of predicting MPRA activity with high accuracy. Here we present the performance of Malinois on a number of criteria including experimental MPRA data, DNase I Assays, and other regulatory criteria. We then provide examples of how Malinois can be used to overcome experimental limitations and explore outstanding problems in regulatory biology, namely non-coding contributions to cancer. Somatic mutations accumulate as cells age through several means. These mutations are predominantly benign, “passenger mutations”, however when mutations confer selective advantages they are known as“drivers” and contribute to tumorigenesis. Given the high background of passenger mutations, often thousands per driver, the prioritization of drivers for validation is challenging. Consequently, studies of driver mutations have largely focused on coding sequences, where the impact of a mutation is readily assigned. Advancements in whole-genome sequencing (WGS) have expanded the search for drivers to the non-coding genome but this comes with significant challenges. Using Malinois we generated predictions for all WGS derived non-coding mutations in the Catalog of Somatic Mutations in Cancer (COSMIC) and find enrichments for active elements in promoters, enrichment of expression modulating variants in recurrent promoter mutations and more highlighting the power of reporter assay models to prioritize non-coding cancer drivers in-silico.

213	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology	**Olaleye**	**Olajuyin**	☑	University of Maine	Exploring Inflammatory Stimulation for Neo-Nephron Regeneration in Adult Zebrafish Kidneys	The human kidney is a vital organ needed to maintain body homeostasis and health, it is susceptible to damage from various underlying causes, with dialysis and transplantation as the main treatments. Kidney disease affects over 20 million Americans, it has become a global health challenge. The shortage of organ donors and the increasing number of patients waiting for transplants has increased the search for alternative therapies. Kidney regeneration holds immense promise in reducing mortality among patients on long transplant waiting lists, yet the mechanisms governing this process remain unclear. Previous research in our lab discovered similarities between adult zebrafish kidney stem cells and mammalian nephrogenic stem cells, particularly mice and humans based on their transcriptomes. This led us to investigate why these stem cells stay quiescent in adult kidneys and how they activate and differentiate into nephron structures. We used single-cell RNA sequencing to analyze these kidney stem cells, revealing their close resemblance to mammalian nephrogenic stem cells, which was intriguing. We studied the potential mechanisms of communication between these cells and the external environment and surprisingly found cell surface receptors, including growth hormone (GH) and interleukin 6 (IL-6) signal transducing complexes. The presence of IL-6 signaling components like gp130 and CNTFR was unexpected and surprising. To understand the role of these receptors, we explored their involvement in inflammatory responses during nephron regeneration. We hypothesized that inflammation-induced cytokine signaling, mediated by Toll-like receptors (TLR) and Damage-Associated Molecular Patterns (DAMP), activates kidney stem cells. To investigate this, we conducted Bulk RNA sequencing of injured tubules and identified a cluster of differentially expressed cells 7 days post-injury, all linked to the inflammatory response. This led us to examine the role of inflammatory expression in stem cell activation. We injected gentamycin, a nephrotoxin, into zebrafish. At 4 days post-injection, we measured pro-inflammatory markers (IL-6, IL-1b, TNFa) using RT-PCR and observed significant upregulation. Additionally, we detected increased expression of lhx1a, wnt9b, and frzd9b, key regulators of kidney stem cell aggregation and regeneration, at 7 days post-injection, confirmed with in-situ hybridization, providing direct evidence of stem cell activation in response to inflammation. To determine if inflammatory responses alone, without injury, stimulate stem cell activation and nephrogenesis, we administered lipopolysaccharide, an endotoxin known to induce inflammation. At 7 days post-injection, we detected upregulation of stem cell activation markers, though less pronounced than in the gentamycin group. In conclusion, our findings highlight the crucial role of inflammatory cytokines in kidney stem cell activation and nephron regeneration in zebrafish.
214	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology	**Mary**	**Astumian**	☑	GSBSE, Univesity of Maine	The Localization of Dystroglycan and Integrin Proteins Within Muscle Cell Membranes	Muscular dystrophy and dystroglycanopathies are progressive diseases, varying in severity, affecting both muscle and neurological health. In the diseased state, muscles do not adhere correctly to the extracellular matrix (ECM) and fibers detach. In healthy muscle, the transmembrane proteins integrin and dystroglycan bind to ECM laminin protein. In zebrafish dystroglycan mutants and integrin mutants, laminin deposition and muscle health improved after oxidized nicotinamide adenine dinucleotide (NAD+) treatment. But in some dystroglycanopathy mutants, where dystroglycan is present but not correctly glycosylated, muscles health was not improved. To explain this, one hypothesis is that hypoglycosylated dystroglycan protein physically interferes with integrin clustering in the muscle membrane, preventing normal integrin-ECM binding activity. Therefore, the localization of dystroglycan and integrin proteins relative to each other at the muscle cell membrane is hypothesized to be important for muscle health. Dystroglycanopathy mutants that model human dystroglycanopathies are made. Using super resolution microscopy, the cluster size and average distance between the molecules of dystroglycan at the zebrafish myofiber membranes and myotendinous junction has been assessed. In the future, localization of integrin and dystroglycan in NAD+ treated mutants and untreated mutants and controls will be compared.
215	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology	**Cory**	**Johnson**	☐	MDI Biological Laboratory	Methods to Improve The Vascularization of iPSC-Derived Kidney Organoids	Kidney organoids have enormous potential in disease modeling and therapeutic development. However, current kidney organoids are immature and do not accurately recapitulate the adult kidney. One major contributor to organoid immaturity is the lack functional vasculature. Recently several groups have attempted to solve this problem using genetic tools, microfluidics, and xenograft models. Our laboratory has identified that indirect co-culture of human iPSC-derived kidney organoids with blood-derived monocytes improves vascular development in kidney organoid cultures. Some vessels also form luminal compartments, indicating significant maturation of the vascular tissue though they lack perfusion. Additionally, the crosstalk between monocytes and the kidney organoids modulates the gene expression profiles of blood-derived monocytes toward an anti-inflammatory state. Our results demonstrate for the first time that vascular development in kidney organoids can be accomplished in vitro without the use of transgenics or perfusion. We also found that the application of exogenous glucocorticoids during mesodermal differentiation also significantly influences vascular development in kidney organoids and promotes the formation of capillary-like structures within podocyte-rich regions. These results provide the critical basis for investigating the cellular and molecular mechanisms involved in vascular development in kidney organoids.

216	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology	**Michayla**	**Moore**	☑	MHIR	ALK1 Coordinates Pro-Angiogenic Sclerostin, CD105, and IGFBP3 Secretion Following Preconditioning with BMP9 in Human Cardiac Progenitor Cells	Myocardial infarction (MI) is the number one cause of cardiovascular disease mortality. Recent evidence has highlighted the potential protective role of transplanted cardiac progenitor cells (CPCs) in the improving of cardiac recovery after MI, with an emerging role of CPC paracrine response and secreted proteins in this process. However, the molecular mechanisms for CPC paracrine effects on cardiac repair are not understood. Our lab isolated CPCs from human epicardium (hHiPCs) during cardiac surgery. hHiPC clonal isolates are characterized by their high proliferation rate, and expression of mesenchymal stem cell markers such as CD90 and CD105 (Endoglin). We found that Activin receptor-like kinase 1 (ACVRL1, ALK1) is expressed in hHiPC. Bone morphogenetic protein-9 (BMP9) is a ligand for ALK1/CD105 and regulates key regenerative processes in endothelial cells and throughout development including cell migration, proliferation, and angiogenesis. We pre-treated hHiPC with BMP9 and using SWATH LC-MS/MS analysis of conditioned media (CM) found enhanced hHiPC secretion of pro-angiogenic and BMP-regulated secreted proteins, including Sclerostin (SOST), Meflin (ISLR), IGFBP3, and CD105, in vitro. Transcriptional regulation by BMP9 stimulation of SOST, ISLR, IGFBP3, and CD105 was confirmed by RT-qPCR. To investigate this pathway's role in angiogenesis we examined hHiPCs in the Matrigel tube formation assay and found increased tube formation in BMP9-treated CM compared to vehicle control. To further evaluate the role of ALK1 in hHiPC we used a lentiviral vector and pharmacological inhibitor, LDN-193189, to reduce and inhibit ALK1 signaling. We found that suppressing or inhibiting ALK1 significantly decreased RNA expression of pro-angiogenic factors CD105, SOST and IGFBP3 following BMP9 treatment compared to controls. We also found decreased secretion of BMP9-induced IGFBP3 in the conditioned media following ALK1 inhibition using LC-MS/MS analysis. These findings implicate BMP9/ALK1 signaling in cardiac progenitor cell mediated repair via secreted SOST and IGFBP3, and support further investigation using in vivo models.
217	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology	**Madeleine**	**Nowak**	☑	MaineHealth Institute for Research/GSBSE	Potential Liver-Adipose Crosstalk in the Regulation of Adipose Mest and Obesity	Obesity adversely influences an individuals' risk for heart disease, type 2 diabetes, cancer and other aspects of metabolic health. Known factors which contribute to obesity, such as the interplay of an individual's genetic background and demographics, only account for approximately 10% of its variability in the human population. Thus, studies designed to further identify factors that impact the development of obesity are critical. Epigenetic mechanisms, which alter DNA function without changing DNA sequence, were unaccounted for in initial assessments of factors that drive obesity. Using an isogenic population of mice, we identified mesoderm specific transcript (Mest), a gene regulated by epigenetics, to show inter-individual variability in expression in white adipose tissue (WAT) which positively correlates with increased risk for development of obesity. Although mechanisms causing inter-individual differences in WAT Mest expression are unknown, its expression is consistent across multiple WAT depots within an individual. This implies that a systemic mechanism is involved in the regulation of WAT Mest. Re-analysis of liver microarray data from earlier studies suggests that circulating hepatokines correlate with WAT Mest expression and diet-induced obesity (DIO) in mice and could be involved in this systemic mechanism. Here, we focused on the hepatokine adropin due to the body of literature demonstrating its association with increased lipid metabolism and mitigation of DIO. Hepatic mRNA levels of energy homeostasis associated (Enho), the gene that encodes adropin, also show inter-individual variability with a negative correlation to WAT Mest expression. We hypothesized that circulating plasma adropin would be consistent with hepatic Enho mRNA and show a negative correlation with WAT Mest. To test this, we fed 40 mice western diet for 4 weeks starting at 8 weeks of age. We measured bodyweight and body composition prior to and at the end of the dietary regime, collected plasma at 2-week intervals from the start to finish of the study, and then harvested visceral and subcutaneous WAT and liver from the mice. RNA isolated from these tissues was assayed by RT-qPCR to test WAT Mest and liver Enho expression. Based on these results, we chose plasma samples from the upper and lower quartiles of mice with the highest or lowest levels of Mest or Enho expression to measure circulating adropin levels by ELISA. Our results concur with earlier studies showing a negative correlation between hepatic Enho and WAT Mest expression. Additionally, we anticipate that circulating levels of adropin will correlate with hepatic Enho expression and show a reciprocal association with WAT Mest expression. These results suggest that adropin could act as a circulating marker for the development of obesity and may also indicate hepatic-WAT 'crosstalk' involving the regulation of liver Enho, WAT Mest, and susceptibility for development of obesity.
219	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology/ Other/ Regeneration	**Gabriela**	**Johnson**	☑	University of Maine and M.D.I. Biological Laboratory	Development of two mechanistically distinct transgenic in vivo axolotl cell-ablation systems at various life stages	Unlike humans, salamanders have a high regenerative capacity that enables them to undergo scar-free wound repair and functionally replace a range of clinically relevant adult structures following injury, including parts of the brain, heart, spinal cord, and limbs. The axolotl is a type of salamander that is supported by a growing catalog of tissue and cell-specific transgenic lines, where the translucent skin facilitates high quality imaging and cell tracking. The ability to assign function to specific cell types in regeneration has been obstructed by the lack of effective cell-specific ablation systems. To solve this problem, we developed two independent transgenic cell-ablation platforms in parallel that genetically sensitize target cells to drug induced cell death in vivo. The well-established metronidazole prodrug-inducible enhanced-nitroreductase (NTR 2.0) system in zebrafish, and the small molecule-inducible Caspase 9 (ihCasp9) ablation system commonly used in mice, were compared in transgenic axolotls to evaluate ablation efficiency in different stages of axolotl development. Drug concentrations capable of effectively inducing death in genetically sensitized cells were determined in vitro. In vivo grafting studies were used to optimize drug dose, carrier vehicle, route of administration, and ablation kinetics. These robust genetic systems are the first to be implemented in axolotl and provide researchers with the tools required to conduct detailed studies aimed at understanding cell-specific functions during regenerative or developmental processes. Investigating the cellular mechanisms required for scar-free wound repair can provide insights for improving the regenerative capabilities of humans.

220	Basic and Applied Research in Biological Sciences	Biology/ Molecular Biology/Physiology/Pathophysiology	**Rebecca**	**Peters**	☑	University of Maine	Osteoclast-specific Deletion of β2-Adrenergic Receptor Limits Trabecular and Cortical Bone Acquisition in Male but not Female Mice	The sympathetic nervous system (SNS) is important for maintenance of bone homeostasis through β-adrenergic signaling. Our lab and others found that osteoclasts are directly targeted by SNS activity, and that β -blockers limit osteoclast differentiation and activity. Osteoclasts express both β1- and β2-adrenergic receptor (β2AR), with β2AR being more highly expressed. To directly examine the effects of β2AR on osteoclasts, we developed an osteoclast-specific β2AR knockout mouse model. Adrb2fl/fl mice were crossed with the myeloid lineage specific Lyz2Cre/Cre (a.k.a. LysM-Cre) mice. We tested the efficiency of deletion with Adrb2fl/flLyz2Cre/+ (Cre/+) and Adrb2fl/flLyz2Cre/Cre (Cre/Cre) mice and found deletion of Adrb2 was comparable in both using gene expression and in situ hybridization in the distal femur. Using μCT, we measured the bone microarchitecture of wildtype (+/+) and Cre/+ male and female mice at 8 and 26 weeks of age (N=7-10). In 8-week-old male mice, trabecular and cortical bone were significantly lower in both the femur and L5 vertebrae in Cre/+ compared to +/+ ($p<0.05$). Even though there was no change in osteoclast-specific gene expression, several osteoblast-specific genes were significantly reduced in tibia. Although no changes in serum CTX-1 or P1NP were observed in untreated mice, β2AR deletion in osteoclasts attenuated the elevated CTX-1 caused by the β2AR agonist salbutamol (N=3, $p=0.031$). The low bone mass phenotype subsided by 26 weeks, suggesting age-related bone loss is unaffected by Adrb2 deletion in osteoclasts in male mice. Female mice had no significant changes in bone microarchitecture or serum bone remodeling markers at 8 or 26 weeks of age. Although global deletion of Adrb2 has been reported to cause a high bone mass phenotype, our work indicates that β2AR function in bone is sex-, age-, cell-type, and stress-dependent. Osteoclast-specific β2AR deletion may have indirect effects on osteoblast function, indicated by lower osteoblast marker gene expression. Future studies will examine this using osteoblast culture and co-culture experiments with osteoclasts. These studies will aid in our understanding of mechanisms through which stress and β-blockers influence bone density, which may inform osteoporosis prevention and treatment strategies.
221	Basic and Applied Research in Biological Sciences	Biology/ Neuroscience/ Physiology/Pathophysiology	**Giovanna M**	**Crystal Novi**	☐	Colby College	Effect of Aluminum in Associative Learning in Drosophila	Heavy metal agents are well-established to impact the development of the brain. Heavy metals are Alzheimerogenic chemicals, known to induce cognitive impairment and several diseases, including neurodegenerative disorders. Even though aluminum does not meet the density requirements to be considered a heavy metal, it is sometimes included in lists of heavy metals due to its toxicity. According to the Center for Disease Control and Prevention, everyone is exposed to low levels of aluminum, but exposure to high levels may result in neurological problems. Studies in rats suggest that aluminum exposure is associated with oxidative stress, gliosis, loss of neurons, and higher expression of hyperphosphorylated tau and amyloidogenic proteins. Drosophila is an excellent system to model various categories of diseases, especially because of the possibility of genetic manipulation and their inexpensive maintenance. By being short-lived, Drosophila allows for fast data collection, which would take years in other models. The advantages of using this model are even more profound in the context of neurodegenerative diseases and dementia. However, to the best of our knowledge, the effects of aluminum on cognition in flies are not well known. The purpose of our work is to understand the role of aluminum in inducing cognitive decline in Drosophila. Drosophila is already used to model associative learning, so our aim was to adapt a cost and time-effective associative learning assay that could be used as a proxy for cognitive decline. We used quinine as an aversive stimulus to suppress the phototactic tendencies of Drosophila; that is, their tendency to go toward light. Wildtype flies (10 to 15) are placed in two connected vials: one open to light and one completely dark. During the training phase, a filter paper with quinine was placed in the light chamber, and 16 training trials were performed, enabling the flies to associate light with the aversive stimulus. Flies trained with quinine went less toward the light, that is, the natural behavior of going toward the light was suppressed, suggesting that the flies learned the association between light and the aversive stimulus. Another aim of our work is to understand the effect of aluminum on cognitive decline. Flies exposed to aluminum and trained with quinine did not show a significant difference in performance compared to flies trained with water. That is, the suppression of phototactic tendencies was reduced, suggesting that aluminum disrupts associative learning. With a model that establishes that aluminum is disruptive to at least one cognitive function, we will be able to explore the mechanisms causing the disruption and get insight into the Alzheimerogenic role of aluminum.

222	Basic and Applied Research in Biological Sciences	Microbiology/Virology	**Nnamdi**	**Baker**	☐		Using intravital imaging in zebrafish to understand signaling underlying innate immune response to C. albicans infection.	Candida albicans is a commensal fungus affecting immunocompromised patients due to their impaired innate immune response which is integral in preventing lethal invasive candidiasis. Neutrophils maintain immunity by being recruited to the site of infection and clearing it through phagocytosis or production of extracellular traps. However, defects in recruitment lead to human disorders like WAS (Wiskott-Aldrich Syndrome), LAD (Leukocyte Adhesion Deficiency) or WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) which all promote increased susceptibility to recurrent infection. Although we understand the molecular defects of each disease, it is unclear how those defects translate to altered phagocyte recruitment, phagocytosis, and fungal killing. Intravital imaging of mutant neutrophils in the context of infection could shed some light into how each defect affects distinct aspects of the neutrophil's functional response. To quantify defects in neutrophil recruitment and clearance, we have monitored neutrophil recruitment in larval zebrafish during hindbrain injection of C. albicans. This route of infection models a systemic infection. Our preliminary results modeling loss of gradient sensing indicate that the CXCR2 receptor is important for immunity in this infection route, as expected. However, blockade of this receptor does not significantly diminish neutrophil recruitment to the infection site, suggesting that other functions of CXCR2 signaling are important for controlling candidemia. Future work will continue to examine these neutrophil immune pathways in control of wildtype as well as evasion-deficient strains of C. albicans. A cellular understanding of the roles of these pathways in candidiasis may lead to targeted treatments for increasing survival in immunosuppressed patients.
224	Basic and Applied Research in Biological Sciences	Microbiology/Virology	**Sophie**	**Craig**	☑	GSBSE/University of Maine	Cell-type specific mechanisms of JC polyomavirus infection	JC polyomavirus (JCPyV) is a ubiquitous human pathogen that infects kidney and brain cells. In most people the virus will go unnoticed as a lifelong, asymptomatic infection of the kidney; in certain severely immunocompromised individuals, the virus can traffic to the brain and cause a lytic infection of glial cells. This destruction of glial cells leads to the development of a neurodegenerative disease called progressive multifocal leukoencephalopathy (PML), a debilitating and often fatal infection for which there is no cure or approved treatment. JCPyV is highly species- and tissue-specific, limiting models with which it can be studied. Primary cells provide an accurate in vitro model with which to study JCPyV and are a novel tool to study JCPyV entry and infection. JCPyV has a double-stranded DNA genome with a hypervariable noncoding control region (NCCR) that contains transcription factor binding sites (TFBS) and is rearranged based on the disease state of the patient and the type of infected tissue from which the virus was isolated. NCCR rearrangement is associated with PML, and an analysis of JCPyV sequences identified potentially novel TFBS that are associated with JCPyV infection of specific tissues. Through RNA sequencing of primary cells infected with JCPyV NCCR variants followed by differential gene expression analysis, the cell- and JCPyV variant-specific mechanisms of JCPyV infection will provide insight into JCPyV tissue tropism and pathogenesis. Ultimately, this work will help uncover antiviral targets to reduce the spread of JCPyV and the impact of PML.
225	Basic and Applied Research in Biological Sciences	Microbiology/Virology	**Anjanadevi**	**Govindaraj**	☐	University of New England	Pyrogallol-hydrocarbon hybrid compounds increase linezolid efficacy against staphylococci	Staphylococcus aureus and Staphylococcus epidermidis are common bacterial pathogens and are leading causes of hospital- and community-acquired infections. Given the global emergence of drug-resistant Staphylococcal strains and their contribution to human morbidity and mortality, there is an urgent need for novel therapeutic strategies to treat these serious infections. One potential strategy for treating drug-resistant infections is the use of antimicrobial adjuvants, which are compounds that enhance the antimicrobial activity of existing antibiotics. Our lab previously found that the phenolic compound, pyrogallol, increased staphylococcal susceptibility to the oxazolidinone antibiotic linezolid, in a mechanism involving bacterial oxidative stress. Here, we expanded upon that work by testing the effect of chemically modified versions of pyrogallol on linezolid efficacy. Hydrocarbon side chains of varying lengths were added to the pyrogallol molecule to create pyrogallol-hydrocarbon hybrid compounds. Using broth microdilution antibiotic susceptibility assays, we compared the effect of pyrogallol versus the hybrid compounds on linezolid efficacy in S. aureus USA300 and S. epidermidis NRRL-41021. In all cases, the pyrogallol-based hybrid compounds improved linezolid activity against S. aureus and S. epidermidis as well as or better than pyrogallol alone. Ongoing studies will further probe the potential mechanism by which hybrid compounds and pyrogallol alone alter staphylococcal antibiotic susceptibility by examining the effect of the adjuvant compounds on staphylococcal oxidative stress and intracellular linezolid accumulation.
226	Basic and Applied Research in Biological Sciences	Microbiology/Virology	**Lola**	**Holcomb**	☑	University of Maine	Early life exposure to broccoli sprouts confers stronger protection against enterocolitis development in an immunological mouse model of inflammatory bowel disease.	Inflammatory Bowel Diseases (IBD) are chronic conditions characterized by inflammation of the gastrointestinal tract that heavily burden daily life, result in surgery or other complications, and disrupt the gut microbiome. How IBD influences gut microbial ecology, especially biogeographic patterns of microbial location, and how the gut microbiota can use diet components and microbial metabolites to mediate disease, are still poorly understood. Many studies on diet and IBD in mice use a chemically induced ulcerative colitis model, despite the availability of an immune-modulated Crohn's Disease model. Interleukin-10-knockout (IL-10-ko) mice on a C57BL/6 background, beginning at age 4 or 7 weeks, were fed either a control diet or one containing 10% (w/w) raw broccoli sprouts which was high in the sprout-sourced anti-inflammatory sulforaphane. Diets began 7 days prior to inoculation with Helicobacter hepaticus, which triggers Crohn's-like symptoms in these immune-impaired mice, and ran for two additional weeks. Key findings of this study suggest that the broccoli sprout diet increases sulforaphane concentration in plasma; decreases weight stagnation, fecal blood, and diarrhea associated with enterocolitis; and increases microbiota richness in the gut, especially in younger mice. Sprout diets resulted in some anatomically specific bacterial communities in younger mice, and reduced the prevalence and abundance of potentially pathogenic or otherwise-commensal bacteria which trigger inflammation in the IL-10 deficient mouse, for example, Escherichia coli and Helicobacter. Overall, the IL-10-ko mouse model is responsive to a raw broccoli sprout diet and represents an opportunity for more diet-host-microbiome research.

227	Basic and Applied Research in Biological Sciences	Microbiology/Virology	**Amanda**	**Sandberg**	☐	University of Maine	GPCR Agonists and Antagonists Identified to Reduce JC Polyomavirus Infection in Human Brain Cells	JC polyomavirus (JCPyV) is a common virus that infects up to 80% of the population. The virus establishes an asymptomatic but persistent and life-long infection in the kidneys, which remains controlled by the host immune system. However, for individuals who become immunocompromised, often as a result of uncontrolled HIV infection or immunomodulatory treatments, JCPyV infection can spread to the brain where it targets the glial cells astrocytes and oligodendrocytes. Infection of these cells causes neuron demyelination and results in the fatal disease progressive multifocal leukoencephalopathy (PML). Most individuals with PML suffer rapid cognitive deterioration, muscle weakness, and loss of vision. Despite the often fatal outcome of PML there are currently no approved treatments available, which highlights the critical need for additional research of JCPyV infection and ultimately the discovery of novel therapeutic targets. In an effort to identify novel inhibitors of JCPyV infection the Maginnis Laboratory conducted a high-throughput drug screen of >700 drugs that are currently FDA-approved or in clinical trials. The drug screen yielded numerous hits categorized as G-protein coupled receptor (GPCR) agonists and antagonists. This drug category is of interest as JCPyV is known to utilize the GPCR serotonin receptors to internalize, and JCPyV infection activates GPCR-mediated signaling pathways. Additional validation of specific agonists and antagonists support initial findings and present the serotonin receptor and components of the signaling pathway as a possible target to prevent JCPyV infection. Due to the devastating nature of PML, evaluating hits in the drug screen is an important next step to identify potential anti-viral treatment options that could effectively reduce JCPyV infection and prevent this fatal disease.
230	Basic and Applied Research in Biological Sciences	Molecular Biology	**Omodasola**	**Adekeye**	☑	University of Maine	Protein Tyrosine Phosphatase Receptor Type Q (Ptprq) is involved in the maintenance of glomerular filtration barrier integrity	The podocyte is the key unit of the kidney glomerular filtration barrier comprised of interdigitating foot processes, bridged by slit diaphragms (SD). Dysfunction of the podocyte is a major cause of proteinuria and a leading cause of end-stage kidney disease. Using a transcriptomic approach to discovering novel genes important for podocyte development, we identified protein tyrosine phosphatase receptor type Q (ptprq) to be highly enriched in the developing pronephric glomeruli of zebrafish. Ptprq is a receptor tyrosine phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-triphosphate(PIP3) to phosphatidylinositol (4,5)-biphosphate(PIP2). This process induces and regulates PIP2-dependent signaling, promoting the binding of receptors to the plasma membrane, which is essential for SD formation. However, the relevance of Ptprq in the formation and regulation of podocyte structure and function remains unknown. We hypothesize that Ptprq results in proper interdigitation of the podocyte foot processes and podocyte morphogenesis. To better understand the morphogenesis of podocytes, we studied the function of Ptprq in podocytes. Here we confirmed that zebrafish larvae glomeruli express ptprq. Whole-mount in situ hybridization and immunohistochemistry demonstrated that ptprq is expressed in the zebrafish larval glomerulus and Ptprq protein localizes to the membrane of the podocytes, colocalizing with ZO-1, a podocyte SD marker. Additionally, we used the zebrafish CRISPR G0 screen to test Ptprq function in glomerular development. Our CRISPR efficiency was validated using fluorescent polymerase chain reaction (PCR) and fragment analysis. Knockout of ptprq in zebrafish larvae led to whole-body edema, a phenotype associated with primary kidney failure. Permeability analysis of the glomerular filtration barrier of this zebrafish crisprant showed a disruption of the selective glomerular permeability filter resulting in proteinuria. In conclusion, these data demonstrate that Ptprq promotes the normal function of the podocyte, suggesting that Ptprq may play a crucial role in the development of the podocyte. Our research would identify novel causes of genetic glomerular disease and help inform further analysis of human kidney disease.
231	Basic and Applied Research in Biological Sciences	Molecular Biology	**Arad**	**Bustan**	☑	JAX	Investigating the Role of KRAB Zinc Finger Proteins (KZFPs) in Mitigating Non-syndromic Cleft Lip and Palate (CLP) in A/WySn Mice	Purpose: Non-syndromic cleft lip and palate (CLP) in the A/WySn mouse strain is attributed to reduced Wnt9b expression, stemming from the insertion of an intracisternal A particle (IAP). Previous research indicates that a gene on chromosome 13 may regulate IAP activity through methylation of its 5' long terminal repeat (LTR), thereby facilitating Wnt9b expression. This chromosomal region contains a family of genes that encode KRAB zinc finger proteins, known to establish repressive heterochromatin. We hypothesized that KRAB zinc finger proteins (KZFP) on chromosome 13 play a role in mitigating CLP in mice through suppressing IAP elements and restoring normal development. Methods: We will employee a novel method to quantify the expression of IAP levels using fluorescence-based cell sorting (termed HCR flow FISH). We will introduce KZFPs from the non-CLP strain into mouse embryonic stem cells from A/WySn mice using lentiviral transduction. HCR probes were designed to identify IAP transcripts, and flow cytometry will be utilized to collect cells with low IAP expression, suggesting they contain the KZFP repressor. mESCs will undergo RNA-seq to identify which KZFP they express to identify the modifier of CLP. Expected Results: In A/WySn mice without CLP, we anticipate observing diminished levels of IAP LTR expression coupled with heightened levels of KRAB zinc finger protein expression in comparison to mice with CLP. These findings would suggest that KRAB zinc finger proteins exert a suppressive effect on IAP activity, thus permitting proper Wnt9b expression and preventing the development of CLP. Preliminary Results: We cloned two KZFPs and transduced them into HEK293 cells. By conducting a western blot, we confirmed their successful transduction and subsequent protein presence. Furthermore, we transduced these KZFPs into B6 mESCs, which contained plasmids expressing GFP. Transduced cells were then sorted using flow cytometry, and these cells are now ready for assessment via western blot to examine whether they exhibit the desired expression of these two KZFPs.

232	Basic and Applied Research in Biological Sciences	Molecular Biology	**Carolina**	**Cora**	☑	Graduate School Biomedical Sciences and Engineering	Brown adipose tissue activation for post-cardiac arrest syndrome attenuation in patients receiving targeted temperature management	Background: Cardiac arrest (CA) is a public health crisis affecting 600,000 people annually in the US with an overall mortality rate of 90%, where catastrophic brain injury to the cerebral cortex and deep gray matter structures is the most common cause of death. Among resuscitated patients, whole-body ischemia-reperfusion injury often leads to multi-organ damage. During the immediate post-resuscitation period, alterations to systemic metabolism that include changes in glycemia, potentiate and exacerbate organ injury. Brown adipose tissue (BAT) contributes to whole-body glucose homeostasis, which can be activated by cold temperature via the sympathetic nervous system to regulate body temperature. Targeted temperature management (TTM), an intensive care therapy, is employed for the first 24 hours after ROSC to mitigate the effects of CA on brain injury through systemic cooling. Hypothesis: Here, we test the hypothesis that cardiac arrest alters brown adipose tissue metabolism and function following cold exposure. Approach: BAT produces distinct sets of lipid hormones that regulate metabolism. We measured the concentration of a panel of 110 oxidized lipid metabolites using LC-MS on serum from patients at six different time points post-ROSC both during and after treatment with TTM. One of these lipids is 12,13-diHOME, an oxidized derivative of linoleic acid, which activates BAT glucose uptake and is associated with glycemic control. Measuring 12,13-diHOME, we identify patients with and without BAT and characterize the tissue activation. Results: Our findings indicate that at 24 hours after ROSC, 12,13-diHOME levels are increased in cardiac arrest patients that survive compared to patients that do not survive to hospital discharge. Conclusion: These results suggest that brown adipose tissue is affected by cardiac arrest and that brown adipose tissue activation may be a therapeutic target to mitigate the effects of hyperglycemia after cardiac arrest.
234	Basic and Applied Research in Biological Sciences	Molecular Biology	**Remi**	**Geohegan**	☑	Graduate School of Biomedical Sciences UMaine Orono	Characterizing the Effects of Aging on Nuclear Transport in Yeast	The population of Americans 85 years old and over will triple by 2050 increasing the need for medical interventions and the study of age associated diseases. The age associated diseases Alzheimer's disease, Amyotrophic Lateral sclerosis (ALS) and frontotemporal dementia display defects in the primary regulator of nuclear transport, the small G-protein Ran. Regulation of transport of molecules across the nucleus is necessary for diverse processes, such as cell cycle progression, signal transduction and gene expression. Nuclear transport is also disrupted in the premature aging disorder, Hutchinson Gilford Progeria Syndrome (HGPS). Cells from HGPS patients display reduced heterochromatin associated with loss of Ran regulation. Baker's yeast serves as a model for studying aging due to the presence of highly conserved molecular pathways with humans. We have previously found that loss of heterochromatin in yeast results in dysregulation of Ran. The loss of gene silencing and compaction of heterochromatin have been observed in yeast as they age suggesting dysregulation of transport and subsequent gene transcription. This project aims to characterize the effects of aging on nuclear transport in yeast using a microfluidic yeast aging chamber, determining if yeast can serve as a model to study nuclear aging. The chamber traps individual yeast cells that have a fluorescent label on Ran to quantify both nuclear and cytosolic levels as the yeast age over the course of three days. Optimization of this chamber and the monitoring of the Ran gradient in aging yeast were observed with notable changes in cell morphology.
235	Basic and Applied Research in Biological Sciences	Molecular Biology	**Audrie**	**Langlais**	☑	MaineHealth Institute for Research	Morphine-induced Bone Loss is Associated with altered Bone miRNA Expression in Male C57BL/6J Mice	Opioids pose a serious risk to bone health by reducing bone mineral density and increasing fracture risk. Previously, we identified morphine-induced bone loss was due to reduced bone formation in male but not female mice. This was associated with differentially expressed circulating micro RNAs (miRNA) in serum and altered target gene expression in bone. However, miRNA changes in bone have not yet been assessed. We delivered morphine (17 mg/kg) or vehicle (0.9% saline) subcutaneously via osmotic minipumps to 8-week-old C57BL/6J male mice (N = 12/group) for four weeks. Femur and vertebrae were isolated for micro-computed tomography and mRNA/miRNA was extracted from tibia for qPCR. Consistent with our previous study, trabecular bone volume fraction was reduced by 10% in the L5 vertebrae ($p < 0.05$) and cortical area fraction was reduced by 5% in the femur midshaft. We confirmed that the top differentially expressed miRNAs from serum were also expressed in tibia bone marrow and cortical bone. miR-223-3p was significantly elevated in serum but downregulated in bone marrow and not altered in cortical bone. Interestingly, miR-223-3p has been previously associated with opioid use in humans and shown to regulate osteogenesis through its targets, including Foxo3 and Igf1r. Alternately, miR-28a-3p was downregulated in serum and cortical bone. Although the function of this miRNA has not been previously investigated within bone, several predicted and validated targets (e.g., Tgif1, Sox2) suggest an influence on osteoblast function. Furthermore, within bone marrow, expression of all six miRNAs examined were significantly lower, which is consistent with morphine causing a negative skew in miRNA expression in serum in our previous study. Due to the robust suppression of miRNA levels in marrow, we assessed expression of miRNA biogenesis genes (Dicer1, Drosha, Ago2 and Xpo5), but none were altered by morphine. Future studies will test whether the nervous system, the major target of morphine, is the source of suppressed miRNA levels in marrow and serum. Furthermore, we will test how opioids and miRNA gene targets collectively impact osteoblast function to cause bone loss. This work will critically expand our understanding of how opioids impair bone and may influence future clinical treatment and prevention strategies.

236	Basic and Applied Research in Biological Sciences	Molecular Biology	**Marissa**	**McGilvrey**	☑	MaineHealth	Essential for cell survival: optimizing an in vitro model of methionine restriction	Methionine is an essential amino acid and is a precursor to important metabolic intermediates including other amino acids; cysteine and cystine. Without these amino acids, cell proliferation and maturation will be inhibited because methionine is the initiating amino acid in synthesis for most proteins. Restricting methionine in the diet of animals has been shown to counteract obesity-related changes in glucose metabolism and adipose function. We have found that this benefit occurs rapidly, within a few days, and extends to the perivascular adipose tissue (PVAT). This metabolically active adipose tissue surrounds blood vessels and can provide protection or cause disease in the vessel. PVAT does this by sending molecular signals that can adjust the structure and function of the vessel wall, thus altering cardiovascular disease risk. This collective knowledge suggests that obesity-related vascular dysfunction has potential to be improved or prevented by a methionine restricted diet. To distinguish molecular pathways that mediate the beneficial effects induced by methionine restriction, we have developed an in vitro model of methionine restriction on PVAT-derived pre-adipocytes. To do this, we isolated pre-adipocytes from PVAT of C57Bl/6J mice and cultured them in nutrient media that has various levels of methionine. To measure the impact on cell proliferation, we employed the EdU incorporation assay, to measure DNA synthesis, and the puromycin incorporation assay to measure protein synthesis. We found that completely removing methionine, cysteine, and cystine under nourishes our cultured pre-adipocytes and induces rapid cell death. We also found that supplementing only methionine into deprived media, halts protein synthesis, which still leads to cell death. Optimization of cell survival was achieved by supplementing cystine back into the deprived culture media to improve cell survival and proliferation. These improvements in cell survival have allowed us to measure the effects induced by methionine restriction on pre-adipocyte differentiation into mature adipocytes. Methionine restriction significantly reduces the number of PVAT-derived adipocytes that differentiate and the amount of lipid that is accumulated in them. These observations indicate that methionine restriction impacts the ability of PVAT-derived pre-adipocytes to become functional mature adipocytes. These effects of methionine restriction on cell proliferation and differentiation in vitro are meaningful because they represent ways in which PVAT can expand in size, thus changing whether PVAT can have protective or pathological effects on the nearby vessel. These findings are consistent with observations of PVAT from our in vivo study, and this model will be further utilized to test molecular pathways that mediate these effects.
237	Basic and Applied Research in Biological Sciences	Molecular Biology	**Monique**	**Mills**	☑		The Role and Regulation of TRP53 in the oocyte's response to radiation-induced damage	Genotoxic treatments, including radiation, can deplete the ovarian reserve of primordial follicles (PFs) leading to infertility and endocrine dysfunction. This project aims to elucidate the DNA damage response (DRR) in PFs to understand the mechanisms that eliminate PFs during genotoxic treatment. CHEK2 kinase and its target TAp63 are key mediators of DDR in oocytes. TRP53, a target of CHEK2 in all cell types has been previously considered as non-essential for oocyte elimination. To identify factors involved in radiation-induced oocyte elimination, we conducted bulk and single-cell RNA-sequencing of irradiated and non-irradiated ovaries from wild-type and Chek2-/- mice. Bulk RNA-sequencing identified 83 Differentially Expressed Genes (DEGs) (FC≥2, FDR<0.05) in wild-type but not Chek2 deficient ovaries. According to functional characterization, many DEGs are known to be involved in the p53 signaling pathway, cell cycle, and apoptosis. Moreover, Gene Set Enrichment Analysis (GSEA) revealed activation of the p53 pathway, interferon alpha and gamma response, apoptosis, and TNF alpha signaling via NFkB in irradiated ovaries. Using transcriptome analysis at the single-cell level, we identified 125 CHEK2-dependent DEGs (FC≥1.5, FDR<0.05) including novel oocyte- specific candidates and known genes involved in DDR and TRP53 pathway. To determine if TRP53 can contribute to PF elimination, TAp63A/ATrp53-/- ovaries were treated with a higher dose of IR, a dose at which loss of TAp63 activity does not prevent oocyte death. TAp63A/ATrp53-/- ovaries had improved PF survival (83.99% survival ±13.38) compared to PF-depleted controls (3.67% survival ±4.15), confirming that upon higher load of DNA damage, TRP53 is activated and participates in PF elimination. Analysis of TRP53 activation in the ovary after a higher dose of radiation revealed a larger form of TRP53 (~64 kDa) expressed in irradiated purified oocytes but not in somatic cells. This suggests that an oocyte-specific mechanism regulates TRP53 pro-apoptotic activity in response to IR-induced damage. Follow-up studies will further define mechanisms regulating TAp63 and TRP53 activity in ovaries in response to DNA damage. Thus, improving our understanding of how genotoxic treatments lead to PF loss and facilitating the development of fertility preservation strategies. This research is supported by a V Foundation grant and NIH F31 1F31HD108973-01.

238	Basic and Applied Research in Biological Sciences	Molecular Biology	**Connor**	**Murphy**	☑	University of Maine/MaineHealth Institute for Research	Pharmacological Inhibition of the Long-chain Acyl-CoA Synthetase Isozymes with Triacsin C Impairs Multiple Myeloma Cell Proliferation, Survival and Mitochondrial Function	Multiple myeloma (MM) is defined by the clonal expansion of malignant plasma cells in the bone marrow and has a 5-year survival rate of 57.9%. Obesity is a metabolic disease characterized by dysregulated glucose and fatty acid (FA) metabolism. Interestingly, obesity correlates with a poor treatment response in MM patients and an increased incidence of MM. However, how dysfunctional fatty acid metabolism contributes to MM is unknown. Therefore, it is imperative to understand how FA metabolism contributes to support MM. FA metabolism alterations have been shown to support cell proliferation, migration, and drug resistance in other blood cancers and solid tumors. Thus, we hypothesized that FA metabolism is important to supporting MM cell proliferation or survival. Candidate FA metabolism genes that support MM cells were identified in the Hallmark FA Metabolism gene set within the Cancer Dependency Map, a genome-wide CRISPR screen of essential human genes. We found that the long-chain acyl-CoA synthetase (ACSL) family members, which contribute to both catabolic and anabolic FA metabolism, supported MM cell line fitness. We therefore hypothesized that the ACSL family supports MM cell survival or proliferation. To test this hypothesis, we measured human MM cell proliferation (through cell counting and Ki67 staining), apoptosis (DAPI and Annexin V staining), mitochondrial number and membrane potential (MitoTracker and TMRE staining), cell cycle (DAPI), respiration parameters (Seahorse XF Mitostress), gene expression changes (qRT-PCR) and proteomic changes with sequential window acquisition of all theoretical fragments mass spectrometry (SWATH-MS) in the presence of triacsin C (triC), a small molecule inhibitor of 4 of the 5 human ACSLs (ACSL1, 3, 4 and 5). Treatment of 5 distinct human MM cell lines with triC decreased MM cell proliferation ($p<0.0001$, two-way ANOVA Tukey's multiple comparisons test is used throughout) mitochondrial number and membrane potential and increased apoptosis ($p<0.001$) in a dose-dependent manner starting at 48 hrs after triC exposure. SWATH-MS of MM.1S cells treated with 1.0 or 2.0 μM triC for 48 hrs showed a significant decrease in proteins associated with oxidative phosphorylation and a significant increase with those involved in mitochondrial dysfunction. Consistent with the proteomics data, a metabolic flux assay of MM.1S cells treated with 1.0 μM TriC for 24 hours showed significantly decreased basal, maximal, ATP-dependent respiration, and mitochondrial ATP production rate ($p<0.001$). Taken together, our data suggest that ACSLs support MM cell proliferation, survival, respiration, and mitochondrial function. Our work contributes additional evidence of the importance of FA metabolism in MM. Future studies will investigate the role of the ACSL family to chemotherapeutic resistance and the mechanisms of how the ACSLs modulate key cellular functions linked to proliferation, survival, and mitochondrial function.
239	Basic and Applied Research in Biological Sciences	Molecular Biology	**Hilda**	**Opoku Frempong**	☑	The university of Maine and the Jackson Laboratory	Karyotyping 6 Strains of Wild-derived Inbred Mice	Inbred mouse strains have been used for nearly a century to empower mechanistic investigations into gene and pathway function. However, the reliance on inbred strains with a static genetic background has limited the translatability of research findings from mouse to human, underscoring the need for next generation mouse models that more accurately capture human genetic complexity. Wild mice harbor much greater genomic diversity than their laboratory counterparts and present an opportunity to bring genomic complexity into the fold of biomedical research. To this end, we have been pursuing a course of phenotypic and genomic investigations on a panel of wild-derived inbred strains developed from wild-caught house mice from North and South America. Our ultimate goal is to develop a novel high diversity outbred mouse population from a subset of strains in this panel. As an initial step toward this mission, we sought to karyotype 4 strains - GAIC/NachJ, MANB/NachJ, EDMB/NachJ, and SARA/NachJ – to assure the absence of large-scale structural rearrangements that could lead to breeding challenges and infertility. To this end, I generated metaphase cell spreads from tail-tip fibroblasts and visualized DAPI-stained chromosomes using high-resolution fluorescent microscopy. I showed that GAIC/NachJ and SARA/NachJ exhibited karyotypes with the expected 40 acrocentric chromosomes. However, EDMB/NachJ, MANB/NachJ had chromosome counts greater than 40. These unexpected findings may be an artifact of prolonged culturing or our protocol for preparing metaphase cells. To confirm these results and assess additional strains (TUCB/NachJ and SARB/NachJ), we generated spermatocyte spreads. Visualization of meiotic chromosomes revealed that all tested strains, including EDMB/NachJ and MANB/NachJ, exhibited the standard karyotype with 20 homologous chromosomes. My findings point to a conserved karyotype in wild mice from North and South America and suggest that genomic incompatibilities due to large-scale chromosome rearrangements are unlikely to impede future breeding efforts with these strains.

240	Basic and Applied Research in Biological Sciences	Molecular Biology	**Kodey**	**Silknitter**	☑	Graduate School of Biomedical Science and Engineering, University of Maine	Characterizing dystroglycanopathy in novel zebrafish models; the need of glycosylation for muscle and motor neuron development	By confining the context of a disease to a single tissue instead of multiple, the potential of understanding the molecular mechanisms of the pathology is lost. The dystroglycan complex is a glycosylated, transmembrane receptor that binds to extracellular proteins and is critical for muscle development. Dystroglycanopathy is a subset of muscular dystrophies in which one of the 19 proteins responsible for alpha-dystroglycan glycosylation (αDG) is non-functional. Clinical presentation includes brain and eye abnormalities, congenital muscular dystrophy, and shortened lifespan. Although dystroglycan is expressed in several tissues, skeletal muscle has dominated much of the research space. Recently, the role of dystroglycan in brain tissue and motor neurons have been investigated, but independently of other tissues. Thus, our lab seeks to characterize the role αDG plays in the relationship between the skeletal muscle and motor neurons. CRPPA is a protein required for αDG. CRPPA-associated dystroglycanopathy is the second most common form of the disease. The function of CRPPA in αDG occurs earlier than most of the other glycosylation proteins. Animal models with a nonfunctional form of CRPPA fail to or poorly perform αDG and have muscle defects. B4GAT1 is another αDG protein that functions later in the glycosylation process. Previous studies have found that when b4gat1 is knocked-out in zebrafish, there is little to no glycosylation of α-dystroglycan. Additionally, when B4gat1 is mutated in mice, they display muscular dystrophy and disrupted axon guidance. Preliminary data from our lab suggests that primary motor neuron axon guidance and subsequent muscle development are variably disrupted in forms of dystroglycanopathy. We have generated multiple, novel, dystroglycanopathy zebrafish models using CRISPR/Cas9, including zebrafish harboring a mutation in b4gat1 or crppa. Both mutant lines display muscle fiber disruption and disorganization in early development. Currently, we are characterizing these mutants via immunohistochemistry staining and time-lapse microscopy and expect to find that the development of the motor neurons has a direct impact on muscle health. Ultimately, these findings will offer a clearer understanding of how b4gat1's and crppa's independent roles. Specifically, their roles as necessary intermediate components of muscle and motor neuron development.
241	Basic and Applied Research in Biological Sciences	Neuroscience	**Felix Gersho**	**Anim**	☑	University of Maine/The Jackson Laboratory	Investigation of the expression pattern of eEF1A and its role in early onset motor neuron disease	The translation elongation factor eEF1A as part of a complex channel charged tRNA to the ribosomes to facilitate polypeptide chain formation during protein synthesis. It has two independently encoded isoforms, eEF1A1 and eEF1A2. Although eEF1A1 and eEF1A2 in humans and mice are 92% identical and 98% similar in terms of amino acid composition, the well-conserved developmental switch between the two isoforms in tissues, such as muscles and neurons, shows that there may be significant functional differences. In wasting mice (wst/wst), the gene encoding eEF1A2 has a spontaneous 15.8 kb deletion that affects the first noncoding exon and all promoter regions. Neuromuscular abnormalities are caused by this genetic change in mice that are homozygous for the mutant gene, and mice survive about 5 weeks. Interestingly, transgenic expression of eEF1A2 in muscle tissue failed to correct the phenotypic defects of the wst/wst mice. However, transgenic expression in neurons and muscles rescued the phenotype and increased the lifespan. This outcome suggests the critical role of eEF1A2 expression in neurons and the potential primary role of neurons in the observed neuromuscular phenotype in these mice. Furthermore, the wst/wst mouse model presents a valuable opportunity to study early-onset motor neuron disease, given the aggressive nature and early onset of the phenotypic abnormalities. Nevertheless, a crucial aspect that is inadequately defined is the expression patterns of eEF1A1 and eEF1A2 in motor neurons versus other neuronal cell types. This gap is particularly significant due to the abnormalities observed at the neuromuscular junction in wst/wst mice and the known function of elongation factors in ensuring translation fidelity and efficiency in motor neurons as seen in other motor neuron disease models. To address this gap, our study employed RNAscope and Basescope in situ hybridization techniques to determine the mRNA levels of Eef1a1 and Eef1a2 in motor neurons of wildtype mice. While our analysis confirmed the presence of Eef1a2 expression in alpha motor neurons, the same level of confidence was not achieved for Eef1a1 expression levels. This is likely attributable to the sensitivity of the probe used for detecting Eef1a1. Nevertheless, our findings show that Eef1a1 expression persists in alpha motor neurons even beyond the post-natal developmental stage. Future research endeavors will involve unraveling the molecular mechanisms underpinning the neuromuscular phenotype associated with wst/wst mutation. One hypothesis is that the absence of the eEF1A1 to eEF1A2 switch in wst/wst mouse disrupts the efficient delivery of charged tRNAs to ribosomes, potentially resulting in ribosome stalling. This, in turn, may activate the Integrated Stress Response (ISR) and ZAK signaling pathways, ultimately contributing to neuronal death. Therefore, we will characterize the wst/wst mouse model as an informative paradigm for early onset motor neuron disease.

242	Basic and Applied Research in Biological Sciences	Neuroscience	**Cory**	**Diemler**	☑	University of Maine	Microglia Depletion Results in Increased Susceptibility to Ocular Hypertension-Dependent Glaucoma	Microglia depletion increases susceptibility for glaucomatous neurodegeneration in ocular hypertensive mice PURPOSE Microglia responses occur early in the pathogenesis of glaucoma and other neurodegenerative diseases. In recent years, changes in microglial states have been correlated with later glaucoma severity; however, their specific role(s) are not known. We hypothesize that the depletion of microglia with a dietary CSF1R inhibitor would alter glaucomatous optic nerve damage in an aged ocular hypertensive model. METHODS Dietary PLX5622, a CSF1R inhibitor known to decrease populations of microglia in the retina, was introduced to 9.5mo-old DBA/2J mice (a widely used model relevant to ocular hypertension). Microglial depletion was confirmed with retinal tissue RNA-seq analysis (n=4 per diet per sex). Intraocular pressures (IOPs) were measured, and retinal ganglion cell (RGC) function was assessed by measuring pattern electroretinography (PERG) amplitudes and latency at 9, 10.5, and 12mo of age. (n=10 per diet per sex). At 12mo, optic nerves were evaluated for glaucomatous damage using p-phenylenediamine staining (n=12 per diet per sex). Retinas corresponding to the assessed optic nerves were isolated for confocal microscopy (n=6 per diet). RESULTS Pilot studies showed that 75% of retinal microglia are depleted after 3wks exposure to PLX5622. Microglia depletion was further validated by RNA-seq analysis that showed significant downregulation of microglia-specific genes including Tmem119, and P2ry12. 10wks exposure to PLX5622 revealed no significant differences in PERG amplitude and latency, IOP, or RGC soma number between dietary groups. However, analysis of optic nerves showed a significant PLX5622 diet-associated increase in moderate-to-severe optic nerve damage (p= 0.0022). CONCLUSION Our results indicate that reducing the retinal microglial population from 9.5 to 12mo increased susceptibility for glaucomatous neurodegeneration in DBA/2J mice. This suggests a potential beneficial effect of microglia in glaucoma. Experiments are underway to determine whether this overall beneficial effect can be boosted by renewing the microglia pool just prior to IOP onset and optic nerve damage through short term exposure to PLX5622. Future studies will include targeting specific states of microglia through disruption of genes known to control activation including the triggering receptor expressed on myeloid cells (TREM) gene family.
243	Basic and Applied Research in Biological Sciences	Neuroscience	**Brianna**	**Gurdon**	☑	UMaine GSBSE/ JAX	Mapping genetic modifiers of Alzheimer's disease neuropathology endophenotypes in the AD-BXD panel	Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by neuropathology accumulation and cognitive decline. AD is highly heritable with estimated heritability ranging from 60 to 80% yet specific variants, target genes, and cell types that drive AD-related deterioration remain elusive. By incorporating the translationally relevant AD-BXD genetic reference panel, validated genetic mapping strategies, and recent advances in brain-wide cell type and pathology quantification, we are able to identify disease-related genetic modifiers of imaging endophenotypes. Immunohistochemistry was completed to evaluate neurodegeneration (NeuN), gliosis (Iba1 and GFAP), and amyloid pathology (AB1-42) in male and female AD-BXD mice at adult and middle-aged time points. Using the QUINT workflow, hemibrain slices were systematically segmented and registered to the Allen Mouse Brain Atlas to gain a global perspective of cell and pathology coverage. The heritability of regional cell composition was calculated, and quantitative trait loci (QTL) mapping was completed. Within a population of 126 AD-BXD mice, genetic mapping of individual cell composition traits identified a significant QTL associated with cortical neuron load (h = 0.6) at variant rs33120195 on chromosome 17. Lrfn2 was differentially expressed between mice that had the BB vs BD genotype at variant rs33120195. Upon further expansion of the sample size, a sex-specific effect was revealed at both ages studied. AD females with the BB genotype at rs33120195 exhibited decreased cortical neuron load and Lrfn2 expression compared to males. Development of an Lrfn2 overexpression model is underway to validate whether increased expression can rescue cortical neurodegeneration in AD-BXDs.

244	Basic and Applied Research in Biological Sciences	Neuroscience	**Sarah**	**Holbrook**	☑	GSBSE	AAV9-Ighmbp2 gene therapy significantly improves motor performance in severe SMARD1-like mouse model, nmdem3, and CMT2S mouse model, nmdem5	Autosomal recessive mutations in IGHMBP2, a ubiquitously expressed DNA/RNA helicase, have been linked to childhood neuromuscular degenerative diseases (NMDs). C57BL/6J-Ighmbp2em3Cx is a SMARD1-like strain, or Spinal Muscular Atrophy with Respiratory Distress, created via CRISPR-Cas9 targeting of the IGHMBP2 gene and hereafter referred to as EM3. SMARD1 is characterized by muscle weakness starting in the distal extremities and diaphragmatic paralysis leading to respiratory failure. Most patients are diagnosed in early infancy and die in early childhood. The EM3 mouse has more severe muscle atrophy than the historical SMARD1-like model (nmd2J) in the hind limb, diaphragm, and intercostal muscles. The EM3 mouse model also has an average lifespan of ~3 weeks compared to the 2J's ~3 month lifespan. C57BL/6J-Ighmbp2em5Cx is a Charcot-Marie-Tooth disease type 2S model that has not impact on lifespan but does impact motor and sensory function beginning around the 4 week timepoint. Gene therapy has shown promise in another NMD, Spinal Muscular Atrophy (SMA). In collaboration with the Meyer lab at Nationwide Children's Hospital in Columbus, OH, we are testing 2 different AAV9-Ighmbp2 vectors. Each has a different promoter with one having a Chicken β-Actin (CBA) Promoter [higher expression levels than endogenous levels] and the other having a truncated Methyl-CpG binding protein 2 (MECP2 aka P546) promoter [expression levels close to endogenous levels expressed by muscles and neurons]. We performed postnatal day 1 intracerebroventricular injections on EM3 and EM5 mutants and unaffected sibling pups to determine the efficacy of each treatment, respectively, and if there are toxic effects associated with overexpression of IGHMBP2 in wild type mice. Using a variety of assays to determine strength and neuromuscular degeneration, we determined that the P546 promoter is more effective in EM3 mice and that either virus causes the EM5 mice to show no significant difference between mutant mice and wildtype mice.
245	Basic and Applied Research in Biological Sciences	Neuroscience	**Madison**	**Mueth**	☑	University of New England	CUGBP Elav-like family member 4 (CELF4) acts as a negative regulator of nociceptor excitability and hyperalgesia in mice	RNA binding proteins regulate gene function by controlling RNA processing, transport, stability, and translation. Dysfunctional RNA-protein interactions within the nervous system are a contributing factor to neurological dysfunction including neurodegenerative disease and maladaptive pain. Computational analyses of binding sites on pro-nociceptive mRNAs revealed the RNA binding protein, CUGBP Elav-like family member 4 (CELF4), as a candidate regulator of sensory neuron sensitivity. Previously, CELF4 expression and function have been characterized within the central nervous system where Celf4 deficient mice are reported to have abnormal excitatory neurotransmission that causes a complex seizure disorder. Our histological assessments of naïve rodent dorsal root ganglia revealed that CELF4 is highly expressed in capsaicin sensitive (TRPV1+), small to medium diameter sensory neurons, likely nociceptors. Considering these findings, we sought to determine how modulation of CELF4 expression impacts the function of sensory neurons. Genetically modified mice were used to assess the phenotype associated with conditional knockout (KO) of Celf4 from adult sensory neurons. Von Frey and Hargreaves behavioral assays were used to assess sensitivities to mechanical and warm thermal stimuli, respectively. These assays revealed that conditional KO of Celf4 from sensory neurons induces a robust behavioral hypersensitivity to mechanical and thermal stimuli in male and female mice. These reflexive behavioral assays showed no significant sex differences in mechanical or heat sensitivity. In addition, patch-clamp recordings showed acutely dissociated, capsaicin sensitive (TRPV1+) dorsal root ganglia neurons become hyperexcitable following Celf4 KO. Due to the functional changes found in the excitability of these sensory neurons, we used mass spectrometry to measure voltage gated sodium channel protein concentrations in dorsal root ganglia following Celf4 KO. This revealed that KO of Celf4 from sensory neurons increases the concentration of several voltage gated sodium channel proteins . Together these data implicate CELF4 as a tonic suppressor of sensory neuron excitability and therefore a promising target for future therapeutic modulation of sensory neuron sensitivity in neuropathic and inflammatory pain conditions.
246	Basic and Applied Research in Biological Sciences	Neuroscience	**Andrew**	**Ouellette**	☑	The University of Maine	Investigating Dendritic Spine Morphology as a Mediator of Cognitive Outcomes in Aged Diversity Outbred Mice	The intersection of genetic diversity, memory, and synaptic function is a critical component in better understanding age-related cognitive decline. Diversity Outbred mice offer the opportunity to investigate cognitive aging across a genetically diverse population in a controlled lab environment. DO mice exhibit an appreciable amount of variance in Contextual Fear Memory and Acquisition (CFM, CFA), which can be linked to individual differences in genetic background (Ouellette A. et al, 2022, Cell Reports). Here, we investigate the role of dendritic spines as a mediator of individual age-related changes in memory. While there was not a decline in CFM or CFA between 8 and 18mo, we observed expectedly wide range in individual memory outcomes. Thin spine density significantly decreased between 8mo and 18mo, and stubby spine density increased. Spine volume across all spine types increased with age. Apical thin spine density explained 61% of the variance in CFM outcomes, while thin spine volume explained 79% and 43% of variance CFA in apical and basal dendrites respectively. Thin spine density, however, did not associate with CFA outcomes. We have linked dendritic spine density and morphology as a potential mediator of individual outcomes across a genetically diverse population. Our results suggest that there may be an age-related conversion of thin to stubby spine types, and that mice with fewer thin spines are more likely to have better CFM outcomes, coinciding with reports of thin spines as dynamic “learning spines” rather than “memory storage” spines (Hayashi, Y. 2005, Neuron). We also show that the size of these “learning” thin spines may be more important memory acquisition than their total density.

247	Basic and Applied Research in Biological Sciences	Neuroscience	**Megan**	**Tomasch**	☑	University of Maine	The impact of neonatal pain on the development and cellular physiology of the central nucleus of the amygdala	Time spent in the neonatal intensive care unit (NICU) has been shown to increase susceptibility to pain- and anxiety-disorders in later life. Using a rodent model of a typical NICU experience, our lab previously observed altered pain and anxiety responses in adolescent rats that had experienced neonatal trauma and associated this pain-vulnerability with changes to cells expressing corticotropin-releasing factor (CRF) in the central nucleus of the amygdala (CeA). The CeA is comprised of a functionally heterogeneous population of GABAergic neurons that are defined by expression of various biomarkers such as CRF, somatostatin (SOM), dynorphin (DYN), protein kinase C delta (PKC-δ). These cellular populations play distinct roles in anxiety, fear, and pain but have not been explored developmentally. Current research often fails to consider co-expression of multiple markers, including with CRF. We hypothesize that neonatal trauma alters the composition and function of biomarker-identifiable subpopulations within the CeA-CRF system, creating a pain-induced neural plasticity that primes the subjects for altered pain responses and anxiety-like behaviors in later-life. To replicate a NICU experience, neonatal rats receive a small needle prick in the hind paw four times a day, every two hours, for the first week of life. On PD 12, 24, and 48, brain tissue is collected from male and female rats that experienced neonatal pain or were left undisturbed. To assess changes in the cellular composition of the CeA, we performed fluorescent in situ hybridization (fISH) to visualize expression of CRF, SOM, and DYN. We find age-dependent changes in the number, biomarker-phenotype, and patterns of biomarker co-expression. To further understand these changes, acute-slice patch-clamp electrophysiology was conducted at PD 24 to assess CeA-CRF+ cell excitability and the impact of neonatal trauma. Recordings taken from transgenic rats expressing TdTomato in CRF+ neurons have revealed that CeA-CRF+ cells display at least three distinct firing patterns (e.g., regular spiking, late firing, and burst firing) that impact both membrane potential and rheobase. Furthermore, data suggest that neonatal trauma may lead to an altered rheobase and current threshold. Future analysis will examine how these changes differ in biomarker-identified subpopulations.
253	Basic and Applied Research in Biological Sciences	Neuroscience/ Instrumentation/ Systems Development	**Kailey**	**Bell**	☐	The University of Maine	Engineering Neuromuscular Circuitry On-Chip	Human locomotion is the result of precisely coordinated processes between two interdependent tissues: nerve and muscle. Intertwined morphogenic signals that vary in space and time, autonomously driven cellular responses, and electrical/mechanical inputs play a role in the carefully orchestrated dance that takes place during neuromuscular tissue development. A consequence of this complex process is multiple means through which normal development can be disrupted, resulting in neuromuscular disease. Furthermore, these intricacies pose challenges for researchers trying to identify the causes for such diseases or to characterize the processes required for normal development. Traditional methods and tools, such as model organisms and tissue culture, have long been effective in parsing out many of the details, particularly in regard to each tissue's independent developmental processes. These methods continue to be invaluable in expanding our understanding, such as a detailed understanding of the specific morphogens and mechanisms of communication particular to motor neurons and muscles, and form the basis upon which new experimental techniques, such as the one presented here, are derived. Here, we present an innovative, microfabricated 3D co-culture platform which blends many of the dynamic aspects of the in vivo environment with the ease and control of an in vitro model system. This device allows for the independent development of stem cells into two cell types, by providing each with its individual complexity of requisite morphogens, while simplifying the communicative landscape between the cells to interconnecting microchannels. This unique tissue culturing method enables us to probe neuromuscular specific, paracrine cell-cell signaling pathways that contribute to a wider network of signals from other tissues within the developing organism. Ongoing research seeks to characterize and prove the functionality of neuromuscular network formation within the device, prior to investigations into disease modeling, nerve-muscle pairing, and motor neuron subtype specification. The presence of neuromuscular junctions and their functionality is being assessed within the device through a combination of immunofluorescence staining and electrophysiology. Application of high extracellular potassium induces motor neuron release of the neurotransmitter acetylcholine (ACh), which is measured with FM2-10 exocytosis dye. Independently, muscle fibers are stimulated with exogenous ACh application to ensure their capacity for contraction in response to the intended stimulus. Since muscle can also be depolarized by external potassium levels, COMSOL Multiphysics simulations are performed to ensure diffusion of potassium across the microchannels will be insufficient to induce muscle contraction. A positive combined result will strongly support the conclusion that muscle cell contraction seen in co-culture devices upon neuron depolarization is the result of neuromuscular comm

254	Basic and Applied Research in Biological Sciences	Other/Biomedical science	**Logan**	**Douglas**	☑	Maine Health Research Institute/ GSBSE	Effects of Developmental Thyrotoxicosis on Brain Gene Expression and Behavior	Thyroid conditions are not only more common in women, but also often undiagnosed. During pregnancy, they can cause deleterious effects, as the fetus may be exposed to altered levels of thyroid hormone, with important consequences for developmental gene expression and adult outcomes. To understand the effects of maternal hyperthyroidism on development, we are using a mouse model with an inactivated Dio3 gene. Dio3 breaks down thyroid hormone and is highly expressed within the uterus and feto-placenta unit, thereby protecting the developing fetus from maternal levels of thyroid hormone. Inactivation of the Dio3 gene in mice results in an inability to break down thyroid hormone, leading to developmental thyrotoxicosis. Later in adulthood, these mice exhibit multiple neurological abnormalities including hyperactivity, increased aggression, and decreased depression and anxiety-related behaviors, alongside aberrant brain gene expression. Motivated behaviors and relevant neural correlates have yet to be studied within this model, but transiently high developmental expression of Dio3 within reward-related brain areas, such as the bed nucleus of the stria terminalis the nucleus accumbens and the amygdala, suggests that thyroid hormone excess will likely disrupt development of these brain regions, leading to adult abnormalities in the behaviors regulated by these regions. We hypothesize that developmental thyroid hormone excess causes aberrant reward-motivated behaviors in adult life due to abnormal developmental gene expression and programming of specific brain regions related to the reward system. Preliminary gene expression data from Dio3 global knock-out mice model shows altered hypothalamic and striatal expression of Ghsr, Hcrt, Mc3r, and Penk, which are reward and feeding genes relating to ghrelin, orexin, and melanocortin systems. Preliminary results from the self-administration runway and place preference tests suggest sex-specific deficits in motivated behaviors, with male knock-out animals displaying a lack of conditioned place preference for palatable food reward compared to female knock-out mice and wild-type controls of both sexes. These experiments suggest that abnormal thyroid hormone levels during development play a role in the sex-specific programming of the reward system, with important implications for the susceptibility to obesity and addiction.
256	Basic and Applied Research in Biological Sciences	Pharmacology/ Food science	**Carmella St**	**Steven C Sutton**	☐	UNE	Modification of an in vitro intestinal inflammation model that realistically predicts cellular damage from low density nanoplastic.	Carmella St Pierre and Steven C Sutton INTRODUCTION. Ubiquitous plastic has been found in our food and drink as nanoparticles. Toxicology studies with NP (NP) are often conflicting: serious effects are shown in vivo [1] and sometimes in vitro [2] but the more physiologic in vitro intestinal models include a cell monolayer with mucous secreting cells that serve as a barrier to the NP we consume. The rate-limiting step for nanoparticles to reach macrophages and stimulate the release of pro-inflammation cytokines, is the translocation through the cell monolayer. And simulations predict that plastic with a density like the cell culture media (CCM) will take days to reach the cell monolayer. METHODS. A modification of an in vitro intestinal inflammation model was used in these studies. The monolayer consisted of an absorptive cell line (Caco-2) and a goblet cell line (HT-29-MTX) and was grown on plastic insert supports (apical compartment, AP). THP-1 cells differentiated into macrophages were seeded on the bottom of the well (basolateral compartment, BL) [3]. The NP studied were 50 nm polystyrene (PS) nanoparticles (density, d=1.05, 71 µg per insert or 1.68 x 105 particles per insert) and which were digested in vitro [4]. We investigated ways to modify the in vitro model to determine whether the NP was a stressor for inflammation in the gut. RESULTS. While the 96-h incubation resulted in a drastic reduction in the monolayer's barrier integrity (as measured by transepithelial electrical resistance, TEER), translocation of the plastic was still very low. Simulations using the in vitro Sedimentation, Diffusion and Dosimetry model (ISDD) depend most strongly on the particle and CCM densities, the nanoparticle packing into agglomerates, and the distance the particles must diffuse in order to reach the cells [5]. The agglomerates of the NP were measured 2x – 36x larger than the individual particles [4]. The simulations predicted only about 14% of the administered NP would be expected to reach the monolayer in 96 h when the usual 500 µL of CCM was in the AP compartment. However, reducing the AP compartment CCM volume to 100 µL was predicted to result in over 40% of the administered NP reaching the monolayer in just 24-h. Compared to controls (phosphate buffered saline, PBS) a 24-h incubation with a reduced AP volume of NP, resulted in a statistically significant ($p<0.05$) decrease in the TEER and a statistically significant ($p<0.05$) increase in the release of an intracellular enzyme (lactate dehydrogenase, LDH). TEER: 610 ± 11.3 W•cm2 (PBS), 243 ± 28.5 W•cm2 (PS-NH2); LDH: 92.5 ± 12.9 W•cm2 (PBS), 141 ± 13.2 W•cm2 (PS-NH2). CONCLUSION. Reducing the AP compartment volume is an adjustment that overcomes the low-density obstacle of some NP for in vitro intestinal models. We intend to employ this modification in future studies. References supplied upon request.

257	Basic and Applied Research in Biological Sciences	Physiology/Pathophysiology	**Yannic**	**Becker**	☐	Mount Desert Island Biological Labortatory	Heparanase 2 as a regulator of vascular homeostasis in zebrafish	The endothelial glycocalxy (eGCX) is a brush-like glycan layer covering the luminal surface of the endothelium and contributes essential regulatory functions for the circulatory system. Amongst others it is involved in regulation of vascular permeability, sensing of shear forces, and the controlled release and storage of growth factors. Microvascular diseases such as diabetes, sepsis, and ischemia reperfusion injuries coincide with a diminished and altered eGCX. Heparan sulfate (HS) is the central glycan structure of a physiological eGCX and its degradation from the endothelial surface is limited to one endoglycosidase, well known as heparanase. Scarcely known is its homologue heparanase 2 (HPSE2). Intriguingly, HPSE2 possesses no catalytic activity towards HS. In fact, due to its vigorous binding affinity towards HS, HPSE2 inhibits heparanase-mediated HS degradation in a competitive manner. We consider HPSE2 as a potential protective molecule for the vascular system due to prevention of eGCX degradation and ultimately the development of a dysfunctional endothelium. The zebrafish Danio rerio serves as an excellent model organism to address questions about HPSE2 and eGCX. Zebrafish develop rapidly, providing a functional circulatory system within 24 hours post fertilization (hpf), which can be monitored in detail due to larvae transparency. In addition, HPSE2 is highly conserved throughout the entire group of vertebrates. We analyzed the spatiotemporal expression pattern of hpse2 by in situ hybridization in zebrafish larvae, which revealed gene expression in the peripheral nervous system at 24 hpf. Following ontogenesis, we detected expression of hpse2 in hepatic tissue from 72 hpf onwards. Antibody-based strategies localized the protein within blood vessels after induction of hepatic expression. CRISPR Cas9-induced knockout (genomic level) as well as morpholino-induced knockdown (mRNA level) techniques were applied to induce a hpse2 loss of function (LOF) phenotype. We assessed the state of the vascular system in hpse2 LOF fish. As a surrogate, we measured vascular integrity by the capacity to keep fluorescent-labeled protein inside the blood vessels. Both methods inducing hpse2 LOF decreased the amount of labeled protein inside the vasculature. Deeper analysis of the endothelial cells in these fish revealed abnormalities in the vascular architecture as well as ultrastructure endothelial cell morphology. We conclude that HPSE2 is a regulator of the vascular system that maintains vascular homeostasis in zebrafish including vascular integrity and proper vascular development. Mechanistically, we propose that HPSE2 is secreted from the liver into the vascular system where it binds HS on the luminal site of the endothelium. Binding of HS changes the quality and quantity of HS chains, which in turn affects other proteins that interact with HS. We hypothesize that signaling processes essential in maintaining vascular homeostasis are affected by HPSE2.
259	Basic and Applied Research in Biological Sciences		**Bailey**	**Blair**	☑	GSBSE	Uncovering C. albicans Factors that Modulate the Host Immune Response	Candida albicans is the fourth most common bloodstream acquired infection. The first line of defense against these infections, is the innate immune system. Previous work suggests that early immune response is critical in controlling C. albicans infection. However, it has been seen that C. albicans has strategies to evade the host immune system. Evidence suggests that the ability to transition from yeast to hyphal growth may facilitate immune evasion by limiting early phagocyte recruitment and uptake of Candida albicans. Reduced containment of C. albicans can lead to uncontrolled hyphal growth, causing damage that can lead to death. However, the mechanism by which C. albicans limits recruitment or containment is unknown. To uncover factors important in innate immune evasion we utilized the transparent larval zebrafish infection model to screen C. albicans mutants for altered virulence and immune response. Seven mutants with markedly reduced virulence were identified. Many of these mutants also induced an altered immune response. RIM101 and NMD5 were found to play a role in limiting phagocytosis, while CEK1 showed a trend to limit the recruitment of macrophages to the infection site. These results highlight the ability of C. albicans to use multiple strategies that allow it to impair the different steps of the immune response such as recruitment and uptake. This work will provide valuable insight into the mechanisms that C. albicans uses to evade the host immune response to cause disease.

260	Biomedical Engineering & Medical Physics	Diagnostics/ Optical & Non-Optical Image Analysis	**Joshua**	**Hamilton**	☑	University of Maine	Multiscale Computational Analysis of Anisotropic Signatures in the Breast Tissue Tumor Microenvironment	There is a lack of pathologists in every country worldwide and particularly in lower income countries. Indeed, there are only 13 higher-income countries with over 40 pathologists per million people, whereas the average country has 1 to 9 pathologists per million people. Low-income countries also have a lack of preventative radiological imaging such as mammography for breast cancer. This results in patients dying of preventable cancers. For example, a study found 70% of cancer related deaths in India were preventable cancers if caught early but were caused by delays in care. This scarcity of pathologists and clinical imaging highlights the need for innovative approaches to support pathologists and radiologists in the cancer diagnostics pipeline for catching preventable cancers. Computational-based triaging approaches can help streamline the clinical workflow and augment its efficiency. We propose to do this by supporting 1) pathologists via the computational preprocessing of biopsy H&E slides, and 2) radiologists by pre-labeling areas of tumor-prone mammographic tissue. One technique that meets these needs is the 2D Wavelet Transform Modulus Maxima (WTMM) multifractal method and its partner the 2D WTMM anisotropy method. Using longitudinal clinical data, the 2D WTMM multifractal method has found significant differences in the spatial organization of mammographic tissue between benign and malignant mammograms, several years prior to diagnosis. The 2D WTMM anisotropy method has found significant differences in malignant and benign collagen anisotropy through analyzing two-photon imaging of pancreatic cancer biopsy slides. The 2D WTMM anisotropy method was then generalized to breast cancer directly on bright-field images of H&E slides with patient-matched mammograms for 2D WTMM multifractal method analysis. The combination of the two results from both metrics provided the greatest patient diagnostic value. This work also found evidence that H&E images contained similar underlying changes to anisotropy between malignant and benign patients as the more advanced imaging technique and so a whole-slide scan approach of anisotropy analysis was developed. This whole-slide adaptation focuses on removing the need for advanced imaging techniques by utilizing the same scans as pathologists, allowing for the possibility of early adaptation by pathologists due to easier mechanistic understanding of the approach. More specifically for this adaptation a deep learning technique was employed to segment areas on breast cancer biopsy H&E slides into three tissue types: ductal, fat, and other. The 2D WTMM anisotropy method was then used to analyze these slides, revealing significant differences in tissue anisotropy between invasive ductal carcinoma (IDC) and benign patients across tissue categories. Preliminary results from this work also suggest the ability to differentiate sub types of IDC such as whether the tumor was cribriform.
261	Biomedical Engineering & Medical Physics	Materials	**Cameron**	**Andrews**	☐	The University of Maine	Scalable Cross-Linking to Reduce the Plasticity of Rehydrated Cellulose Nanofibril Composites	Medical-grade plastics are used throughout the United States and world every day. The medical industry uses 3,500 tons of plastic each day, which contributed to a global revenue of $50.3 billion in 2022. Of these plastics, 1/3 are used in single-use or limited-use applications. Of these, 91% are non-biodegradable or non-compostable, meaning that over 1,000 tons of plastic are either incinerated or going to landfills every day. This contributes to the microplastic and greenhouse gas crisis that is currently happening globally. Of these plastics, PVC is the most prominent, with other polymers making up the rest of the majority of products used. To combat this, cellulose nanofibrils (CNF) are being explored as a potential alternative to these non-biodegradable plastics. This material is produced at the process development center at the University of Maine, and is the base material for this project. However, CNF readily absorbs water which causes changes in mechanical stiffness. When water is absorbed into the material, hydrogen bonds are removed, the material swells, and there is a significant decrease in mechanical properties. To try to inhibit the absorption of water, a thermal crosslinker, polycup (polyamide-epichlorohydrin), is used. For this project, CNF materials were generated with varying amounts of this cross-linker, and the effect of water content on flexural strength was quantified. The flexural strength, Young's modulus, and Shore D hardness were all tested to examine the properties of this material. The data displayed decreased hardness, Young's modulus, and flexural strength with the addition of polycup. This could be caused by the increase in porosity with the addition of the cross-linker, which may have resulted in higher wettability which would have confounded the results. Overall, polycup did not behave as expected, the total mechanical properties were negatively effected with the addition of the material, and other cross-linking strategies/additives will be explored in the future.
262	Biomedical Engineering & Medical Physics	Materials	**Zach**	**Applebee**	☑	University of Maine	Multi-Component Liquid-Infused Systems: A New Approach to Functional Coatings for Biomaterials	Liquid-infused surfaces (LIS) have found utility across the globe due to their diverse applications across medical fields, including resisting bacterial adhesion and thrombus formation and medical diagnostic equipment enhancement. Recent research has started exploring the broader potential of LIS by incorporating additional components into the liquid matrix. In this work, we present the concept of multi-component liquid-infused systems (MCLIS), in which the coating liquid consists of a primary liquid and a secondary component and review recent examples. At the molecular scale, MCLIS, consisting of silicone oils infused with bacterial quorum sensing inhibitor compounds, have been shown to stop bacterial biofilms from adhering and forming. At the nanoscale, MCLIS made from ferrous magnetic nanoparticles within fluorocarbon-based fluids or silicone oil can change their shape upon exposure to magnetic fields, making them useful for the active removal of adherent fouling organisms. At the microscale, microdroplet arrays using more than one liquid in a defined pattern have been fabricated and used for high-throughput detection of compounds. By introducing an additional element into the liquid matrix of liquid-infused systems, a diverse spectrum of attributes can be imbued into these materials, creating novel opportunities for applications within the biomedical realm and beyond.

263	Biomedical Engineering & Medical Physics	Materials	**Avery**	**England**	☐		Synthesis and characterization of sub-nanometer silver clusters formed by high-speed microfluidic turbine mixing	Noble metal nanoparticles have grown an increasing amount of attention due to their novel chemical and physical properties. These properties depend highly on the number of atoms in the nanoparticle and can be tuned by varying the size. For example, as the size of a nanoparticle approaches the fermi wavelength of an electron, typically below 2nm, the metallic energy band structure begins to transition into discrete energy levels, more accurately characterized by HOMO-LUMO molecular orbitals rather than solid band structures. Accessing these new nanoclusters would greatly enhance properties used in biolabeling and imaging, such as fluorescence, quantized charging, molecular magnetism, and optical chirality. Unfortunately, primary shell Ag13 clusters (0.7 nm) are metastable using current synthesis techniques. Ag13 clusters quickly aggregate (within 1 ms) to more thermodynamically stable Ag nanoparticles (5 nm – 10 nm) making sub-nanometer clusters challenging to synthesize. Using a novel high-speed rotary turbine micromixer, we present a simple synthetic protocol to form stable silver clusters (0.5 nm – 1.2 nm). The turbine, operating at rotational velocities of >20k rpm, enables reagents to be mixed within 50 – 100 µs to form nanoclusters that are stable for at least 60 days.
264	Biomedical Engineering & Medical Physics	Materials	**Adeola**	**Fadahunsi**	☑	University of Maine	Cellulose Nanofibril (CNF) Composite for Osseointegration and Bone regeneration	Bone defects impact the integrity of the skeletal system and have become a global concern as available treatment options are limited, especially in the elderly, where bone tissue renewal cannot compensate for its disintegration, impacting bone porosity and strength, and increasing susceptibility to fractures. Nonetheless, there is an ever-increasing estimate for the prevalence of bone deformities in the human population, and treating bone defects remains a difficulty in medicine. There is a need for the exploration of alternative materials by building scaffolds that can imitate the form, properties, and function of human bone, which favors viability, proliferation, conduction, osteo-integration, and regeneration of bone. Our research work first attempts to fabricate a biomimetic bone scaffold composite material from the naturally ubiquitous cellulose nitro-fibril and hydroxyapatite and evaluate its mechanical properties, in-vitro toxicity, and interaction with MC3T3 pre-osteoblasts; then confirm the ability of the scaffold to accommodate calcification and bone regeneration.
265	Biomedical Engineering & Medical Physics	Materials	**Blake**	**Turner**	☐	University of Maine	Cellulose-Based Hydrogels for use as Nutrient and Drug Depots in Biomedical and Veterinary Applications	Hydrogels are an established method to foster healing in chronic wounds, deep tissue lacerations, and burns. These materials have also been explored in injectable applications for therapeutic injections and vaccines. The objective of this project was to create a vaccine adjuvant specifically for Atlantic salmon aquaculture. Ideally, this hydrogel adjuvant will reduce adverse reactions and number of vaccinations, while maintaining an effective immune response until the salmon is harvested. TEMPO oxidized cellulose nanofibrils (TCNF) were selected for this application because of their bioinert nature. To increase the stability of the TCNF hydrogel, monovalent and divalent salts were added to the gel matrix. This induced ionic bonding between the negatively charged carboxyl groups on the TCNF fiber, creating a stiffer gel with closer fiber aggregation. Hydrogel formulations with various concentrations of sodium chloride and calcium chloride were then evaluated using a cone and plate rheometer. The diffusive properties from the hydrogel formulations were also examined utilizing a dye (Coomassie brilliant blue) analogous to the vaccine payload. All hydrogel formulations were found to have shear-thinning behavior. Sodium chloride hydrogels of all concentrations demonstrated similar dye diffusion rates over 48 hours. Over the same 48 hours, calcium chloride gels of higher concentrations diffused less dye than those of lower concentrations.
266	Biomedical Engineering & Medical Physics	Materials	**Riley**	**Bosco**	☐	Northern Light Health and Bangor High School	Bio-IVP: The Biodegradable Intravenous Packet	One billion intravenous bags are used and discarded in the U.S. per year, prompting the necessity of developing the very first biodegradable intravenous bag. This research study investigated the use of a potato starch bioplastic alternative (Bio-Intravenous Packet or Bio-IVP), which can withstand temperatures of 180 degrees Fahrenheit required for sterilization. In this experiment, leak tests were conducted to compare the integrity of the Bio-IVP to an intravenous bag; this was to determine if the Bio-IVP is secure and functional under load. The prototype was constructed out of a proprietary mixture of potato starch, glycerin, vinegar, and water. A common IV fluid, a saline solution of 0.9% NaCl, was made by boiling water and dissolving table salt in it. Several Bio-IVPs and IV bags were filled with specific volumes and weights of the saline solution, and a variety of stability tests were run by storing the bags at various temperatures for various lengths of time. The bags were visually inspected to examine the saline solution for particulates. Following these various tests, measurements of the liquid were made to determine if there were any changes in volume and mass, denoting either impermeability or leakage. 67% of the Bio-IVPs performed as well as the standard IV bags with no leakage, which means that there is now a viable biodegradable alternative.

268	Health and Social Sciences	Nursing	**Maile**	**Sapp**	☐	University of Maine	Title: Mindfulness-Based Stress Reduction (MBSR) Training Reduces Stress and Enhances Wellbeing in Nursing Students	Authors: Maile Sapp, Ed.M., CMPC; Rebecca Schwartz-Mette, Ph.D.; Kelley Strout, Ph.D., RN; Jade McNamara, Ph.D., RD; Syerra-Marie Carmone, Maizy Weirich, Kayla Parsons, MS, RDN Background: A significant nursing shortage exists, with a projected shortage in Maine of 1,450 RNs by 2025 (NLH, 2022). One contributing factor is burnout, which has been exacerbated by the ongoing COVID-19 pandemic (United States Surgeon General, 2022). The aim of this study was to evaluate the effectiveness of an 8-week Mindfulness-Based Stress Reduction (MBSR) intervention on stress, burnout, and wellbeing in nursing students. Methods: A total of 31 participants participated in the MBSR program, and a control group matched for demographics was identified. All participants (N = 62) completed pre- and post-intervention measures. Measures included the Perceived Stress Scale (Cohen & Mermelstein, 1983), the Satisfaction with Life Scale (Diener et al., 1985), the Oldenburg Burnout Inventory (Demerouti et al., 2002), the Positive and Negative Affect Schedule (Watson et al., 1988), and the Five-Factor Mindfulness Questionnaire (Baer et al., 2006). Analysis and Results: A series of analysis of (co)variance (ANCOVA) models tested whether the MBSR group differed from the control group post-intervention. Pre-intervention levels of each variable were controlled in each model. MBSR participants reported significantly lower levels of perceived stress ($F = 63.64$, $p < .001$), higher levels of satisfaction with life ($F = 7.35$, $p < .01$), and greater gains in mindfulness skills ($F = 4.55$, $p < .05$) than did control participants. MBSR participants also reported marginally significantly higher levels of positive affect ($F = 3.49$, $p = .068$). No significant group differences were observed for negative affect or burnout. Conclusion: Findings suggest a generally positive impact of mindfulness training and broad-based wellness interventions on stress and wellbeing in nursing students. There were significant gains in participant mindfulness skills and satisfaction with life, and significant reductions in perceived stress. Notably, changes in mindfulness skills can be challenging to demonstrate (Ghawadra et al., 2019). Implementation of these interventions within nursing education programs has the potential to offer more support to nursing students, increase skill development towards enhanced well-being, and improve stress management. Keywords: Mindfulness, nursing, burnout Disclosure: The current study is part of a larger, ongoing project (WellNurse) which is supported by the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS) as part of an award totaling $1.5 million with zero percentage financed with non-governmental sources. The contents are those of the author (s) and do not necessarily represent the official views of, nor an endorsement by HRSA, HHS or the U.S. Government.
271	Health and Social Sciences	Other/Genomics, Pathology, and Oncology	**Michael**	**Babcock**	☑	University of Maine GSBSE	Improving Cancer Patient Access to Precision Medicines: Maximizing Tissue for Molecular Profiling	Purpose. Pre-analytical prioritization of tissue specimens is critical to provide 1.) a pathology diagnosis of cancer, 2.) tumor molecular profiling, and 3.) tissue archiving for clinical trials and translational research studies. Molecular profiling performed by next generation sequencing (NGS) occurs after patient tumor material is processed for a cancer diagnosis, thus the most common preanalytical factor resulting in molecular profiling failure is due to insufficient formalin-fixed, paraffin-embedded (FFPE) tumor tissue material analysis. For instance, approximately 30% of tumor biopsies from advanced lung cancer patients are insufficient for molecular subtyping due to limited tissue. Adequate tumor cellularity (minimum 30% content in approximately 25 mm2) and sufficient nucleic acid (DNA and/or RNA) are the two most common factors associated with the ability to produce quality next generation sequencing (NGS) results. There is therefore a critical need in maximizing tissue utilization to provide for a cancer diagnosis and provide molecular biomarker results for patients to gain access to precision medicines and clinical trial studies. To address this need, unused FFPE tissue from histopathology sectioning used in pathology diagnosis were used as specimen sources for molecular profiling. Methods. Retrospective review of pathology and molecular profiling results (n=1643) from deidentified cancer patients from the Northern Light, Eastern Maine Medical Center system between 2020 and Mar 1, 2023 when unused, level 2-3 histopathology tissue sections from tissue processing were used as a specimen source. Data review included tumor %, tumor areas (mm2), clinical mutational findings, and extended clinical molecular profiling reports. Results. FFPE tumor biopsy tissue (n=1643) represents 60.3% of solid tumors tested locally by NGS (n=992/1643). 80.9% were lung cancers (n=803/992), 4% were biopsies cases (n=31/803) with <30% tumor content. 50% of tumors (n=812/1643) had at least one clinically actionable biomarker based on NCCN recommended guidelines. 58.6% of patient tumors (n=34/58) with <30% tumor tissue had at least one actionable finding, 20 were lung biopsies. Real-time PCR analysis send out results of RNA based biomarkers (n=218) for MET exon 14 skipping (n=2) and NTRK RNA fusion analysis (n=38) yielded 19.4% (n=40/218) quantity not sufficient (QNS) reports due to insufficient tumor present in tissue blocks for molecular analysis. In-house MET exon 14 skipping analysis using unused tissue levels yielded results on both cases, one case had MET exon 14 skipping. Conclusions. This pre-analytical improvement in clinical molecular pathology resulted in 7.1% of locally tested cancer patient cases (n=58/812) being eligible for a precision medicine treatment that without tissue stewardship would otherwise not have had genomic profiling performed due to tumor material not meeting clinical testing requirements.

272	Computational Biology & Medicine	Modeling	**Rajat**	**Rai**	☑	Graduate Studies in Biomedical Engineering and Sciences	Retinal Venous Vulnerability in Primary Open Angle Glaucoma: Effects of Intraocular Pressure and Blood Pressure	Purpose: Primary open angle glaucoma (POAG) is a major cause of irreversible blindness with risk factors including elevated intraocular pressure (IOP), and both high and low blood pressure (BP). This study investigates the joint influence of IOP and BP on retinal hemodynamics, emphasizing venous circulation. Methods: A synthetic dataset of 2,500 eyes with varied IOP [5–45] mmHg, systolic BP (SBP) [90–200] mmHg and diastolic BP (DBP) [40-120] mmHg was created. Mean pressure (P), mean flow (Q), and mean resistance (R) were estimated using a validated mathematical model. The values of these markers were then analyzed in relation to different values of IOP and mean arterial pressure (MAP; MAP = 1/3 SBP + 2/3 DBP). Clinical data from a population-based Greek study were similarly analyzed. Differences in the simulated and clinical markers between healthy and POAG eyes were then measured. Results: Synthetic dataset analysis showed P and R vary significantly depending on different IOP-MAP combinations. Notably, eyes with low MAP and high IOP demonstrated a drastic increase in R in the venules accompanied with a dramatic decrease in P in the central retinal vein (CRV). Clinical data showed that venules in POAG eyes had significantly higher R than healthy eyes ($p < 0.01$), accompanied by decreased P values in the CRV of POAG eyes compared to healthy eyes ($p = 0.01$). Conclusion: The study highlights the increased susceptibility to venous collapse in POAG eyes and the importance of considering the venous side of retinal circulation in POAG when determining the combined impact of risk factors.
273	Basic and Applied Research in Biological Sciences	Molecular Biology	Samuel	Broadbent	☐	BS in molecular and cellular biology	Tissue-Clearing and Molecular Labeling of Early Axolotl Developmental Stages	Tissue clearing has emerged as a powerful tool for whole system interrogation. Most tissue-clearing methods focus on tissue able to withstand the usual harsh chemical treatment. However, fragile tissues like embryos show deformation and damage after such approaches. Further, embryos from different animals vary greatly in their cellular composition during development. Amphibian embryos have a high-yolk cellular content which degrades during development. This is in stark contrast to most mammalian embryos where the yolk is outside a body with a high-water content. Modern tissue-clearing approaches either maintain morphology or enable isotropic sample expansion or shrinkage of water rich embryos. However, there are so far no tissue-clearing approaches for yolk-rich embryos. Here, I show a tissue clearing approach that maintains system morphology in initial developmental stages of axolotl embryos. Further, I demonstrate the compatibility of this method with different molecular labeling approaches. Finally, I show whole embryo imaging from initial cleavage stages up to early organogenesis using light-sheet microscopy.
274	Basic and Applied Research in Biological Sciences	Physiology/Pathophysiology	Mohamed	Zaid	☑	PhD, Biomedical Engineering	Sex-based differences in ophthalmic and central retinal artery velocity: From the heart to the eye analysis	The study aims to investigate sex-based differences in ocular and systemic vascular biomarkers in early-stage open-angle glaucoma (OAG) patients from the Indianapolis Glaucoma Progression Study (IGPS). Additionally, it seeks to compare clinical and theoretical values of blood pressure (BP) and the peak-systolic velocity (PSV) in the ophthalmic artery (OA) and central retinal artery (CRA) as predicted by the novel EYE2HEART model. The EYE2HEART mathematical model of systemic and retinal circulation is used to characterize the hemodynamic impact of known structural and functional differences in men and women. On average, women are considered to have higher heart rate (HR; by 5%), left ventricular (LV) systolic elastance(ELS; by 15%), LV diastolic elastance (ELD; by 30%), right ventricular (RV) systolic elastance (ERS; by 15%) and retinal capillary density (by 5%). However, women also have smaller arterial diameter and length (by 10%), and smaller OA and CRA (by 5%). These differences are used as model inputs to study sex-related differences in the circulation. Markers of systemic and retinal circulation - systolic BP (BP SYS), mean arterial BP (MAP), PSV in the OA, and CRA - simulated by the model are compared to those measured clinically within IGPS and within the literature. Early-stage OAG eyes are selected when the visual field Humphrey's mean deviation (MD) is >-6 dB (n=804). Differences in markers are analyzed using a Kruskal-Wallis test. Results show that markers simulated by the model align consistently with the mean clinically obtained values. Notably, women exhibit a statistically significant lower BP SYS (127.0 vs 121.4 mmHg) and MAP (90.6 vs 85.9 mmHg). This reduction results in a significantly lower OA PSV (26.2 vs 24.1 cm/s). Smaller CRA diameter compensates for the lower velocity in the OA, where there is no significant difference in the CRA PSV (9.4 vs 9.6 cm/s). In conclusion, model findings emphasize the importance of accounting for sex-specific differences in cardiovascular function when assessing ocular hemodynamics. Results highlight the potential of the EYE2HEART model for predicting and understanding these relationships in the context of diseases.

275	Basic and Applied Research in Biological Sciences	Biology	Shawn	David	☑	Pre-doctoral associate	Role of T cells in Clonal hematopoiesis of indeterminate potential (CHIR)	Myelodysplastic syndromes (MDS) are malignant bone marrow disorders characterized by ineffective hematopoiesis and high risk to transformation into acute myeloid leukemia. One of the defining features of MDS is higher T cell exhaustion and loss of cytotoxicity on T cells, similar to many cancers wherein immune function declines with progression of cancer. To better understand progression of such bone marrow disorders to leukemia and devise therapeutic strategies to prevent leukemic states, it is important to study pre-MDS states. One of the most frequently reported pre-MDS states is clonal hematopoiesis of indeterminate potential (CHIP) where expansion of blood cells occurs from a single hematopoietic stem cell (HSC) harboring a pre-leukemic mutation. Although CHIP is an asymptomatic condition found in elderly population, it is known to be associated with increased risk of cardiovascular disease, chronic obstructive pulmonary disease, all-cause mortality and leukemic transformation. The most common mutation in CHIP is known to inactivate DNA methyltransferase 3A or Dnmt3a. Dnmt3a-mutated HSCs are known to have a self-renewal or proliferative advantage over non-mutated HSCs which is enhanced by the inflammatory environment associated with CHIP. However, we have very limited understanding of how the inflammatory environment can cause T cell dysregulation to drive progression of CHIP to malignant transformations. Therefore, the goal of this study is to understand the functional significance of T cells in Dnmt3a-mutated CHIP. Based on preliminary results from differential gene expression analysis on human CHIP samples, we hypothesized that increased antigen presentation by highly proliferating Dnmt3a-mutated stem and progenitor cells (HSPCs) can cause chronic overstimulation of T cells leading to changes in T cell signatures and subsets that eventually lead to ineffective immune surveillance and an environment permissive to further clonal expansion of mutant HSPCs in a feed-forward loop. By employing our Dnmt3a-mutated mouse model of CHIP, mice expressing ovalbumin and OT-I mice here, we explore the state of T cells in CHIP and study the interaction between Dnmt3a-mutant HSPCs and T cells. Although we observe no signatures of progenitors of exhausted T cells, we find changes in the T cell subsets that could potentially drive exhaustion of T cells. Lack of T cell activation and proliferation upon interaction with mutant antigen presenting cells (APCs) suggests T cell dysfunction possibly occurs in more inflammatory environments or aged environments. This suggests that future experiments such as transplanting Dnmt3a-mutant HSPCs into WT old mice, adoptive transfer of OT-I T cells into Dnmt3a-mutant mice and looking at changes in cytokine expression profiles in mutant mice will reveal molecular changes in CHIP that gives rise to T cell dysfunction observed in MDS or leukemia. The new insights gained will guide future strategies to abrogate clonal expansion and prolong immune health in pre-MDS states which could potentially decrease the severity and progression of pre-MDS states to malignant transformations.
276	Basic and Applied Research in Biological Sciences	Biology	Chenhao	Yang	☑		Notch signaling regulates PVAT phenotype and function	Background. Obesity is an established risk factor for cardiovascular diseases (CVD) and paracrine signaling between adipose tissue and blood vessels is likely to influence disease progression. As a component of the vasculature, perivascular adipose tissue (PVAT) is a critical regulator of vascular function due to its secretion of vasoactive substances to blood vessels. Notch signaling which has a broad role in embryonic development, also plays a crucial role in regulating metabolic homeostasis Hypothesis. Notch signaling overactivation leads to reduced mitochondrial respiration in PVAT and increased contraction of PVAT-adjacent blood vessels. Methods. C57BL/6 mice were fed a high fat diet (60 kcal% Fat%) for 12 weeks to induce obesity. Gene expression was analyzed in PVAT to identify targets involved in PVAT whitening. We also generated a transgenic conditional model of Notch1 constitutive activation in mature adipocytes using an Adipoq-Cre driver and examined PVAT's physiology and function of PVAT-adjacent vessels in Ad/N1ICD mice compared to control non-Cre mice. Results. Expression of Notch 1 and downstream Hes1 genes were found to be upregulated in PVAT of wild type mice after 12-week high fat diet treatment. Here, we further studied Notch's regulation on PVAT phenotypes and function. In vitro, Ad/N1ICD differentiated PVAT exhibited a significant decrease in mitochondrial respiration and ATP production rates compared to control cells. We also found that there were significant changes in expression of signaling components of mitochondrial fission and fusion biogenesis, and PINK1 mitophagy pathway in Ad/N1ICD PVAT compared with control groups as demonstrated by proteomics and immunoblot data. Moreover, vessel wire myography data revealed that PVAT-adjacent aorta from Ad/N1ICD mice showed significant increased vasocontraction compared to the aorta from control mice. Conclusion. Overactivation of Notch signaling could lead to impaired mitochondrial function in PVAT and altered vasoactivity of PVAT-adjacent blood vessels. This study advances our knowledge on how PVAT metabolism could regulate vasculature health.

277	Basic and Applied Research in Biological Sciences	Molecular Biology Food science Microbiology/Virology	Marissa	Kinney	☐		Using Steamed Broccoli Sprouts to Better Understand Bacterial Glucosinolate Metabolism	Inflammatory bowel diseases (IBD) lead to dysfunction of the gastrointestinal (GI) tract, resulting in disruption to overall health. These diseases can affect people of all ages and are present on a global scale. Research has demonstrated that diets high in cruciferous vegetables, such as broccoli, are associated with decreases in GI inflammation. Broccoli contains glucoraphanin, which through metabolism by gut bacteria, can become an anti-inflammatory compound, sulforaphane. Recent research has validated the use of steamed broccoli sprouts in the diet of mice to reduce inflammation and resolve symptoms of IBD. Isolated microbiota samples obtained from various locations in the GI of these mice are being investigated for the presence of glucoraphanin-metabolizing genes from a common gut bacteria, Bacteroides thetaiotaomicron (B. theta). Similar analyses being conducted on human fecal samples from individuals who consumed steamed broccoli sprouts for 28 days have demonstrated decreases in the presence of B. theta. This result was not anticipated and has strengthened beliefs that B. theta is not the primary species performing glucoraphanin metabolism, thus prompting further analyses of the fecal samples from mice and humans for glucoraphanin-metabolizing genes of other common GI bacteria. Genomes of isolates from the gut of mice which have high quantities of glucoraphanin-metabolizing genes will be sequenced for identification. This information will help to identify potential bacterial candidates for future research on probiotic development.
Faculty Posters								
Poster number	**Session**	**Topic area**	**First name**	**Last name**	**GSBSE student?**	**Organization /Affiliation**	**Abstract Title**	**Poster abstract Text (not to exceed 500 words). Abstract must state the purpose, significant results and main conclusion of work.**
100	Basic and Applied Research in Biological Sciences	Biology/Neuroscience	**Peter**	**Caradonna**	☐	University of New England	UNE COBRE Histology & Imaging Core Services	The mission of the Histology and Imaging Core at the University of New England is to provide COBRE investigators, the greater UNE research community, and external academic and commercial partners with access to expertise, training and specialized instrumentation related to tissue embedding, sectioning, staining, immunohistochemistry, microscopy, and image analysis. The Histology and Imaging Core also offers consultation services to guide investigators in choosing the best methods for their research. In addition to providing services related to routine histological processing, the Core also specializes in methyl methacrylate embedding of non-decalcified tissue, static & dynamic bone histomorphometry analysis, and optical tissue clearing.

111	Health and Social Sciences	Other/ Oncology	**Lory**	**Gaitor**	☐	The Jackson Laboratory	Enhancing Oncology Research Engagement: A Comparative Analysis of Remote and Traditional Study Conduct in a Multi-site, Multi-cohort Observational Study	Purpose: The Maine Cancer Genomics Initiative (MCGI) conducted a multi-site, multi-cohort observational study spanning 19 oncology practices across the state. This study aimed to engage adult cancer patients and oncologists across diverse regions in Maine, enhancing participation in oncology research. Here we explored the effectiveness of remote study conduct and compared patient choices for traditional paper and electronic survey completion. Additionally, we assessed clinician survey completion rates. Further consideration will be given to patient demographics and research site metrics. Methods: Between July 2017 and October 2020, nine research sites successfully enrolled over 1,600 adult cancer patients and 79 physicians. The MCGI Central Office research site (Site 1) adopted a remote approach, focusing on rural regions in northern and coastal Maine. Other study sites (Sites 2-9) executed the study traditionally, engaging patients and clinicians during clinic visits. Sites are separated into small- or large sites depending on staffing and enrollment numbers. Patient participants were surveyed at four time points during the one-year study period, with the option to complete surveys online or on paper. Clinician participants completed one optional survey for each of their study patients. Key Findings: • Patients across all sites clearly chose paper surveys over online surveys (around 90%). This choice remained consistent at all survey time points. • Patient survey completion rates varied across all sites; smaller-scale sites (Sites 1-6) generally outperformed larger-scale sites (Sites 7-9). The smallest scale study site consistently had the highest completion rate across all survey time points. Across all sites, a consistent trend was observed showing a gradual decline in survey completion rates throughout the study period (from ~90% to ~70%). • Clinician survey completion rates averaged 70% overall but varied across sites ranging from 44%-100% completion. • Patients demonstrated varied choices based on gender and age. Females favored online surveys over paper survey completion. Moreover, patients across a wide age range engaged in the study, and patients who chose to complete online surveys tended to be younger than those who chose to complete paper surveys. Conclusion: The MCGI study provides valuable insights on how to successfully conduct multi-site, multi-cohort observational research using diverse engagement methods. Our analysis shows a prevailing choice for paper surveys across all sites, regardless of size, suggesting an inclination for traditional approaches. This study underscores the need for flexible study designs to accommodate diverse participant choices in multi-site research. While remote study activities are on the rise and more accepted, traditional methods remain appealing to certain demographics. Recognizing participant choices is vital for improving engagement and data quality in healthcare research.
105	Health and Social Sciences	Nursing	**Colleen**	**Marzilli**	☐	University of Maine	Be A JEDI Pro	Medical racism, or a lack of culturally competent care, negatively impacts health outcomes and create health disparities. Medical racism is a function of implicit and explicit bias that is present in healthcare settings and within healthcare professionals, including nurses. There are strategies that can be leveraged to address and reduce medical racism. Both individuals and organizations have a responsibility to understand and address medical racism in practice. Building healthier communities requires health professionals committed to providing care that eliminates medical racism. It is essential that the medical community understand the issues at the center of medical racism and health disparities, and create a workable framework to systematically eliminate medical racism. There are many concepts and theories that address medical racism or similar concepts. These concepts include cultural competence, cultural bias, cultural sensitivity, cultural humility, cultural and linguistic appropriate services, and many others. However, it is important to have a workable framework for healthcare professionals in any setting to provide care that is free from medical racism. The purpose, or aim of this study is to identify core concepts that are essential for eliminating medical racism and its synonym terms. Using the meta-aggregation method, quantitative and qualitative data sources were reviewed and themes identified. In total, a critical appraisal of existing research, including a review of 89 articles identified seven key themes and eight strategies. The significant results of this study identified themes including “Be A JEDI Pro”, or belonging, allyship, justice, equity, diversity, inclusion, and professionalism. Nurses play a key role in addressing the needs of the most vulnerable, and as we strive to move our society towards a more just, equitable, diverse, and inclusive society, with a professional culture of belonging and allyship, all nurses have a shared responsibility to lead as we move towards a collective goal of supporting “Be a JEDI Pro” initiatives. There are innovative techniques that nurses, natural leaders in the healthcare setting and beyond, can use to support these critical initiatives including the CARE acronym, or compassion/communication, awareness/asking, resourcefulness/reliability, and encouragement/evidence-based practice. In conclusion, “Be A JEDI Pro” initiatives is a framework and the CARE technique are key strategies to address medical racism. Traditional paradigms must be challenged to move from the current model of practice to a practice model focused on creating a culture of belonging to support health equity and eliminate disparities.

106	Basic and Applied Research in Biological Sciences	Biochemistry/ Other/ Toxicology	**Jennifer**	**Newell**	☐	University of Maine	Antioxidant Effects of Partridgeberry Leaf Extract	Mitchella repens, or partridgeberry, is a plant that is native to eastern North America and has an extensive history within the medicinal practices of Indigenous tribes. Historical records indicate Indigenous Americans treated pain associated with childbirth, and rheumatism with tea created from their leaves. The leaves of many plants contain polyphenols, which are known plant compounds that can act as antioxidants. Previous research has indicated that the berries of partridgeberry are rich in polyphenols, but there is little research examining the polyphenolic content in the leaves of partridgeberry. Antioxidants provide a range of health benefits as they inhibit free radicals created from oxidative stress. Too much oxidative stress induced from the heavy metal manganese (Mn) plays a critical role in the development of age-related neurodegenerative diseases. Our hypothesis is that partridgeberry leaf extract (PLE) may act as an antioxidant against Mn-induced oxidative stress. Three separate partridgeberry leaf extracts (PLE) were created and the total phenolic content of the PLE was determined to be 1432 +/- 280 mg gallic acid equalivalents/g dry leaves. A dose-response survival curve was created in nematode worms, Caenorhabditis elegans, pre-treated with increasing concentrations of PLE and the resultant LD50 was calculated to be 26.05%. Total reactive oxygen species (ROS) was measured in the presence of PLE and manganese (II) chloride and a two-fold decrease in the total ROS was observed. Similarly, PLE alone increased glutathione levels and prevented loss of glutathione in Mn-treated worms. As oxidative stress is implicated in mitochondrial damage, an ATP assay was performed to evaluate the impact of PLE on C. elegans mitochondrial function. Data analysis suggest a protective effect with up to a two-fold increase in ATP production in response to Mn. This is the first study to show polyphenolic compounds in PLE act in vivo as antioxidants in Mn-treated worms.
114	Basic and Applied Research in Biological Sciences	Physiology/Pathophysiology	**Anastasia**	**Paulmann**	☐	Mount Desert Island Biological Laboratory	The African Turquoise Killifish (Nothobranchius furzeri): A Novel Model for Investigating Vascular Aging	Aging stands as a paramount risk factor for a myriad of diseases, making the study of the underlying mechanisms a vital endeavor. The "Vascular Theory of Aging" posits that microvascular rarefaction, characterized by the diminishing density of capillary vessels in organs, is a driving force behind the aging process. Given the shifting demographics towards an aging population, comprehending vascular aging is becoming increasingly critical, particularly in the context of preventing cardiovascular diseases. Current aging research predominantly relies on model organisms such as C. elegans or Drosophila, which have provided invaluable insights into various molecular pathways. However, these models lack essential mammalian features, including a detailed representation of vascular systems, bone structures, and adaptive immune systems. Addressing these limitations requires a novel vertebrate model capable of encompassing the complexity of mammalian aging. The African Turquoise Killifish (Nothobranchius furzeri) emerges as a compelling candidate for studying aging processes. This species exhibits a remarkably short lifespan, a consequence of its adaptation to the challenging environmental conditions of West Africa. It further distinguishes itself with rapid sexual maturation and a unique embryonic diapause mechanism. These characteristics make the African Turquoise Killifish an excellent candidate for investigating aging, particularly in the context of vascular aging. This abstract presents our hypothesis and preliminary work in establishing the African Turquoise Killifish as a robust model organism for studying vascular aging. We envision that this model will facilitate a deeper exploration of the intricate processes underlying aging, with a specific focus on microvascular rarefaction. Our research seeks to unravel the complex interplay between age-related vascular changes and their implications for overall health.

113	Biomedical Engineering & Medical Physics	Other/Medical Device Development and Testing	**Suzanne**	**Wendelken**	☐	The Roux Institute, Northeastern University	A Novel, Non-invasive Airway Device for Bag-mask Ventilation and Rescue Breathing	Background: Manual ventilation by bag-masking is often challenging or ineffective due to the poor seal of the mask around the patient's face, which can rapidly lead to life-threatening hypoxia and hypercarbia. Both inside and outside of the hospital setting, it can be difficult or impossible to establish a mask seal for patients with beards, dentures, or a large face or neck diameter. In the field, a poor mask fit can occur due to liquid, dirt, mud, protective equipment interference, and broken or missing teeth. This necessitates the use of two hands to establish the mask seal, and an additional person to deliver breaths. Here, we present a design and proof-of-concept testing of a 3D-printed prototype that addresses the poor-mask-seal problem. Methods: Interviews were conducted with potential users, including airway expert clinicians and first responders to guide the design requirements, performance metrics, and identify appropriate safety standards. The device was designed addresses the poor-mask-seal problem by utilizing a seal inside the mouth, differing from the external seal provided by the traditional masks. The intraoral seal allows the user to immediately establish a good seal and jaw position with one hand. A modular, nested accessory piece was designed to stent open soft tissue and provide the same benefit as an oral pharyngeal airway (OPA), which is often used in conjunction with the traditional mask. The connector portions conform to standard ventilation tubing size, and will be able to be used with suction, laryngeal mask airways (as a tube stabilizer and bite block), and standard masks. The prototype was designed using CAD software and 3D-printed using a flexible, food-grade resin. Results: The prototype was successfully demonstrated by an airway expert (anesthesiologist) bag-masking a healthy volunteer. The user found the device sufficient for rapidly establishing and maintaining a mask seal, and delivering breaths using a standard bag-mask device. Compared to the traditional mask, the user remarked that it required less effort to position and hold the device in place, and, easier to deliver breaths due to the improved seal. The volunteer found the device to be comfortable, and less intrusive than the traditional mask. Experiments are underway to quantify the performance and usability of the device, in addition to guiding product design iterations. New testing results will be presented and discussed. Conclusion: A functional prototype of a non-invasive airway device was designed and constructed using 3-D printed. The device addresses the mask leak problem primarily by utilizing an intraoral seal. Initial user testing showed promising results that the device is as effective and easier to use than the traditional mask. Further testing is needed to quantify the results in anticipation of medical device approval applications. U.S. Provisional Patent Application No. 63/506,646, "Noninvasive Ventilation Device"

2024 MERGE Posters

120	**"Bridging the Gap: accessing mental healthcare in Lincoln County"**	
	Authors: Robert Krulee OMS-II 1 , James W Jarvis, MD, FAAFP 2	
121	**"Improving Dermatological Care in Kennebec County: Reducing The Disparity of Rural Healthcare"**	
	Authors: Tyler Nussinow OMS II, Thomas Gearan MD	
122	**"Expanding Perinatal Support for Individuals with Opioid Use Disorder in Washington County"**	
	Author: Abigail DeSchiffart	
123	**"Rural Mental Health: A Look at Available Local Data and Community Perspectives"**	
	Authors: Ryan Hibbs, OMS-II, Annie Derthick, PhD	
124	**"Developing a Rural Learning Platform in Maine"**	
	Authors: Madeleine McCormick, David McLellan MBA, Kneka Smith EdD, MPH, Kalli Varaklis MD, MSEd	