MIDWEST SDB

IN CINCINNATI

2025 REGIONAL MEETING

Cincinnati Children's Hospital

September 19-21

midwestsdbincincy.org


DEV BIO
SOCIETY FOR
DEVELOPMENTAL BIOLOGY


Nikon


Cincinnati
Children's®
Division of Developmental Biology


Xenbase


IWAKI AQUATIC
SYSTEMS AND SERVICES


DSHB
ANTIBODIES FOR RESEARCH


PARSE
BIOSCIENCES


Leica


Roche


SCIDAP
scientific
data analysis
platform


XENOPUS 1


GENE TOOLS, LLC
www.gene-tools.com
Morpholinos


Miltenyi Biotec


BD


Developmental
Dynamics
development, evolution, disease, stem cells, repair, regeneration
www.wiley.com/developmentaldynamics

Midwest SDB 2025 Abstract Book

SHORT TALKS

Short talk 1

Nirpesh Adhikari[1], Paul P.R. Iyyanar[1], Chuanqi Qin[1], Zhaoming Wu[1], Hee Woong Lim[2,3], Yu Lan[1,3,4], Rulang Jiang[1,3,4]

The ALX family transcription factors determine frontonasal identity of the neural crest-derived craniofacial mesenchyme

[1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [2]Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [3]Departments of Pediatrics and Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [4]Division of Plastic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

Biallelic disruptions of any of the three *Aristaless-like homeobox* (*ALX*) genes, *ALX1*, *ALX3*, and *ALX4*, cause frontonasal dysplasia, a class of congenital craniofacial disorders characterized by hypertelorism and median facial clefting. Studies in mice showed that *Alx1*, *Alx3,* and *Alx4* exhibit partially overlapping patterns of expression during craniofacial development. Whereas *Alx3* mutant mice do not have obvious craniofacial defects and *Alx4* mutant mice exhibit parietal foramina, *Alx1* mutant mice show frontonasal defects including short snout, flat nasal bridge, upper lip notching, and hypoplastic premaxilla, and die soon after birth. In this study, we show that *Alx1/Alx3* compound mutant mice have severe nasal clefting with absence of upper and lower incisors. Through RNA-seq analyses followed by in situ hybridization-based visualization, we found that *Alx1/Alx3* mutants exhibit disruption of frontonasal mesenchyme identity with ectopic expression of jaw mesenchyme markers *Dlx1*, *Dlx2*, *Lhx6,* and *Lhx8* during embryonic development. Furthermore, transgenic overexpression of *Alx1* in the post migratory cranial neural crest cells (CNCC) resulted in duplication of the premaxilla accompanied by ectopic expression of *Alx4* and *Pax7* genes and reduction in expression of *Dlx1, Dlx2*, *Dlx3,* and *Dlx5* genes in developing maxillary/mandibular mesenchyme. Together these data indicate that *ALX* family transcription factors are necessary and sufficient to determine the frontonasal identity of the CNCC-derived craniofacial

mesenchyme. This work was supported by the National Institutes of Health National Institute of Dental and Craniofacial Research (NIH/NIDCR) grant DE029417.

Short talk 2

Benjamin Akande[1], Sylvie Earlewine[1], Grace Leonard[1], John Postlethwait[2], Saulius Sumanas[3]

The *pendulum* swing: a novel zebrafish mutant with defects in cardiopharyngeal development.

[1]Miami University, USA; [2]University of Oregon, USA; [3]University of South Florida, USA

The development of the cardiac outflow tract (OFT) is driven by the interaction of late-differentiating second heart field (SHF) and neural crest cells (NCC). Perturbation of this interaction has been implicated in OFT defects, which account for ~1/3 of congenital heart defect (CHD) cases annually. However, the cellular and molecular etiology of OFT defects remains elusive. Here we report a novel zebrafish mutant called *pendulum* (*pen*) with extensive cardiac deformities. At 72 hours post fertilization (hpf), the *pen* mutants display pericardial edema, retrograde blood flow, defective bulboventricular valve formation, reduced OFT smooth muscle, and increased endocardial cell numbers. Additionally, we observed craniofacial dysmorphology in *pen* mutants at 96 hpf. Altogether, these results suggest compromised SHF and NCC interaction. Transcriptomic profiling of *pen* mutants, at 72 hpf, revealed hyperactivation of TGF-β signaling, a hallmark of Loeys-Dietz Syndrome–a disorder characterized by mutations in TGF-β receptors, OFT abnormalities, and craniofacial dysmorphology. While the *pen* causative gene remains unidentified, these findings suggest that pen may model aspects of LDS and uncover a novel role for TGF-β signaling in OFT morphogenesis. This project is funded by Miami University Committee for Faculty Research.

Short talk 3

Elena Albizzati, Xuyao Chang, Inah Yang, Sarah Potter, Paloma Merchan-Sala, Artem Barski, Kenneth Campbell, Jason Tchieu

The *Nuclear Factor I* gene family dictates the temporal progression of cortical development by establishing radial glia cell identity

Cincinnati Children's Hospital Medical Center, USA

Cortical development depends on precise temporal control of progenitor competence to ensure the orderly generation of neuronal and glial lineages. Radial glial cells (RGCs), which are the primary neural stem cells of the embryonic forebrain, proliferate early in embryogenesis, undergo a sequential series of fate switches that produce the canonical layered architecture of the neocortex. This temporal progression is driven by intrinsic transcriptional programs in coordination with extrinsic signals, yet the molecular mechanisms that regulate these intrinsic timers remain poorly understood. Here, we explore the role of *Nuclear Factor I* (*NFI*) genes, a family of transcription factors essential for brain development implicated in distinct neurodevelopmental disorders. Using conditional mouse models, we determined the independent and combinatorial functions in RGCs and their effect on cortical development. While single and double knockout (KO) mice (*Nfia, Nfib, Nfix*) lead to mild delays in corticogenesis, triple KO (cTKO-ABX) causes severe cortical defects, marked by a significant accumulation of progenitors that fail

to exit the proliferative state and are unable to commit to cell fates beyond early neurogenesis. To extend these findings to humans, we derived human pluripotent stem cell (hPSCs) lines endogenously FLAG-tagged for each *NFI* gene. Using ChIP-seq and epigenomic analyses, we found extensive, overlapping, and stage-dependent binding in critical regulatory regions, including numerous enhancer sites within opening chromatin regions. These results underscore the role of *NFIs* as core regulators of RGCs maturation in chromatin remodeling and transcriptional regulation. Together, this work suggests that *NFIs* drive RGCs maturation through developmental states and highlights how redundant, but specialized transcription factors coordinate intrinsic timing mechanisms crucial for the developing neocortex.

Short talk 4

Farina Aziz, Jaehwan Kim, Jason Knott, Alyssa Virola-Iarussi

The Power of Paralogues: *Sox15* and *Sox21* in Early Embryonic Development in Mice

Michigan State University, USA

Preimplantation development in mammals, marked by a series of precisely orchestrated molecular events from fertilization to implantation, provides an excellent model for studying the initiation of pluripotency. In mice, SOX2 is the earliest marker of pluripotency, labeling pluripotent inside cells as early as the 16-cell stage, when other pluripotency factors such as OCT4 and NANOG are expressed in both pluripotent and non-pluripotent cell types. However, *Sox2* is dispensable for formation of the pluripotent epiblast cells in the blastocyst, suggesting that closely related *Sox* paralogues may compensate for the loss of *Sox2* during blastocyst formation. We discovered that the closely related *Sox* paralogues, *Sox15* and *Sox21* are reproducibly detected at both the mRNA and protein level alongside *Sox2* during preimplantation development. Moreover, *Sox15* and *Sox21* mRNA and protein persist in *Sox2* null embryos indicating potential compensatory roles. Using knock-out mouse models, we showed that losing any three alleles of *Sox2* and *Sox21* impairs blastocoel formation, suggesting a dose-dependent, combinatorial role of these paralogues in cavitation. To test whether *Sox15* can also compensate for the loss of *Sox2* and/or *Sox21*, we are currently analyzing embryos with combined heterozygous or null alleles. Furthermore, we found *Sox21* expression is independent of NANOG and OCT4, but, like *Sox2*, is regulated by TFAP2C, highlighting shared regulatory inputs. These data suggest that distinct yet overlapping regulatory pathways involving *Sox* paralogues converge to regulate early embryonic development. These studies will advance our understanding of how *Sox* paralogues cooperatively regulate gene networks that establish early cell identity during embryonic development and may further our knowledge on the pathways involved in early embryo viability.

Short talk 5

Nicole Edwards[1], Scott Rankin[1], Adhish Kashyap[1], Alissa Warren[1], Zachary Agricola[1], Alan Kenny[1], Matthew Kofron[1], Yufeng Shen[2], Wendy Chung[3], Aaron Zorn[1]

Disrupted endosomal trafficking of polarity proteins causes congenital trachea-esophageal separation defects

[1]Cincinnati Children's Hospital, USA; [2]Columbia University Medical Center, USA; [3]Boston Children's Hospital, Harvard Medical School, USA

Trachea-esophageal birth defects are life-threatening congenital anomalies that arise when the trachea and esophagus do not correctly separate from the common anterior foregut tube. The genetic causes of trachea-esophageal defects are not well known, and how the trachea and esophagus normally separate during embryonic development is poorly understood. In a collaborative network called the CLEAR Consortium, we are using animal models and patient genome sequencing to discover new trachea-esophageal defect-related genetic variants and determine their disease mechanisms. Using the complementary advantages of mouse and *Xenopus* embryos, we recently defined a novel endosome-mediated epithelial remodeling process that is critical for the separation of the foregut into tracheal and esophageal tubes. We aimed to determine the cellular mechanisms regulated by endosomal trafficking by mutating the key endosomal pathway proteins Dynamin, Rab5a, and Rab11a in *Xenopus*. We found that disrupting endosome trafficking leads to trachea-esophageal fistulas, occluded trachea-esophageal lumens, and epithelial cell disorganization in the transient septum connecting the trachea and esophagus. We found that endosome-dependent asymmetric internalization of E-Cadherin is required for trachea-esophageal separation. We also found significantly altered localization of the polarity protein Vangl2 in endosomal mutant embryos, suggesting that Vangl2 may be trafficked by Rab11a+ recycling endosomes to maintain epithelial apical-basal cell polarity cues in adherent epithelial cells. We observed similarly disrupted polarity in *Xenopus*mutants of novel patient variants in genes predicted to function in endocytosis, suggesting that disrupted endosomal-mediated epithelial remodeling may be a common disease mechanism underlying human trachea-esophageal anomalies. This work is funded by NICHD P01 HD093363.

Short talk 6

Joyce Fernandes, Nelchi Prashali, Jackson Riffe

Glial remodelling during reorganization of peripheral abdominal nerves in Drosophila

Miami University, United States

During metamorphosis in Drosophila, five pairs of posterior abdominal nerves fuse to form a terminal nerve trunk (TNT), coincident with a reduction in size of the abdominal ganglion. The 4 day-period of metamorphosis results in the transformation of a crawling larva into an adult that is capable of vastly new behaviors such as flight and reproduction. The study of such transformations is relevant for understanding neural plasticity that underlies fine tuning of neural circuits in humans during key periods of early childhood as well as adolescence. We have previously shown that four layers that ensheath larval peripheral nerves have distinct temporal and spatial patterns of reorganization (Subramanian et al. 2017). The perineurial glial (PG) cell layer is the outermost cellular layer that surrounds each nerve. The

number of cells in this layer increases 3-fold during the first day of metamorphosis, as the overlying acellular layer, the neural lamella begins a process of degradation. Using markers for proliferation has revealed that this increase is due to in-situ proliferation of glia. This phenomenon is of interest because previously differentiated glial cells are able to re-enter the cell-cycle. Our working hypothesis is that breakdown of the overlying ECM layer, which is coincident with the increase in PG cells, releases proliferative signals. Preventing ECM breakdown by targeted manipulation of matrix metalloproteinases has revealed that glial cell proliferation is reduced by 15-20% ($p<0.05$), and there is a 30% reduction in eclosion ($p<0.01$). This work has been funded by Miami University.

Short talk 7

Ignacio Garcia Gomez[1], Jemma L Webber[1,2], Berta Soria-Izquierdo[1], John C Clancy[1,3], Anne Duggan[1], Yingjie Zhou[1], Charles P Murphey[1], Trevor D.M Harriman[1], Jianghong Wang[1], Mary Ann Cheatham[1], Jaime García-Añoveros[1]

Targeted Cell Interconversions Reveal Inner Hair Cell Induction of Supporting Cell Identity and Distribution in the Organ of Corti.

[1]Northwestern University, USA; [2]Creighton University, United States; [3]UC Santa Cruz, United States

The organ of Corti is a highly organized sensory epithelium within the cochlea, essential for auditory transduction. Structurally, it comprises hair cells and supporting cells, partitioned into inner and outer compartments by the tunnel of Corti. The inner compartment contains inner hair cells (IHCs-the primary sensory receptors for hearing) associated with inner border (IBCs) and inner phalangeal cells (IPhCs). The outer compartment features outer hair cells (OHCs-amplify and fine-tune auditory signals) and Deiters' cells (DCs), while the tunnel of Corti itself is formed by inner and outer pillar cells (IPCs and OPCs). To elucidate if and how IHCs and OHCs influence the differentiation and spatial organization of supporting cells during development, we genetically manipulated hair cell identity at specific developmental stages. By targeting genes critical for hair cell differentiation (Insm1;Tbx2) we induced transdifferentiation between IHCs and OHCs. In Insm1 cKO mice, embryonic conversion of some OHCs to IHCs (oc-IHCs) yielded a mixed outer compartment, while various Tbx2 cKO models enabled controlled conversion of OHCs to IHCs (ic-OHCs) embryonically or postnatally. To discern the role of IHC-derived FGF8 in supporting cell patterning, we analyzed supporting cell phenotypes in Fgf8 cKO mice. Our developmental analysis reveals that the fundamental inner versus outer identity of supporting cells is not dictated by the hair cell type they surround. Nevertheless, IHCs play pivotal roles in supporting cell development by inducing OPC formation at the expense of DCs via embryonic FGF8 signals, modulating IPC abundance, and specifying IPhCs while promoting their unique wrapping behavior. These results demonstrate that while initial supporting cell fate is independent of hair cell subtype, IHCs actively orchestrate supporting cell identity and distribution during cochlear organogenesis, thus establishing the intricate architecture required for hearing. R01DC015903

Short talk 8

Simon Han[1], Vinit Adani[2], Edward Farrow[1], Bhavalben Parmar[2], Ching-Fang Chang[1], Kimberly Cochran[1], Paige Ramkissoon[1], Ezekiel Esteban[1], Kelsey Elliott[1], Brian Gebelein[1], Martín García-Castro[2], Samantha Brugmann[1]

A dual role for Gli3 signaling in cranial neural crest development

[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]University of California, Riverside, Riverside, CA, USA

Cranial neural crest cells (CNCC) are a multipotent cell population that undergoes several cellular processes including specification, epithelial-to-mesenchymal transition, migration, and differentiation into a plethora of cell types essential for the form and function of the craniofacial complex. A wealth of studies across various model systems have established dogma as to the molecular mechanisms and signaling cascades that contribute to CNCC development. Wnt, FGF, and BMP signaling have repeatedly been reported as essential to CNCC specification, migration, differentiation; however, the Sonic Hedgehog signaling pathway has received limited attention for any specific role in this process. Herein, we propose two distinct temporal roles for the transcription factor Gli3 in CNCC development. *Gli3* is co-expressed with several CNCC induction and specification markers in chick, mouse, and human embryonic stem cell (ES)-derived NCCs. Loss of Gli3 prior to CNCC specification led to reduced expression of key markers of CNCC specification. Conditional loss of *Gli3* post specification, specifically impaired ectomesenchymal differentiation. These results demonstrate dual novel roles for Gli3 in early CNCC specification and later in CNCC differentiation. Research was supported by the National Institute of Dental and Craniofacial Research (F31 DE033565 to S.J.Y.H., R35 DE07557 to S.A.B.).

Short talk 9

Karen Hicks

Seasonal reproductive development in the moss *Physcomitrium patens*

Kenyon College, United States

Many organisms make use of environmental cues, such as daylength and temperature, to synchronize reproductive development with favorable climatic conditions, thereby increasing their reproductive success. The genetic mechanisms that regulate reproductive development in response to seasonal cues are largely conserved across flowering plants, however it was not known if this conservation extended beyond angiosperms to other land plant lineages, including mosses. Using transcriptomic approaches, we found that *P. patens* homologs of conserved angiosperm flowering time genes are differentially expressed within days after a shift in temperature and/or daylength, suggesting that they might play a conserved role in seasonal reproduction. We constructed higher order mutants in several *P. patens* homologs and found that they initiated reproductive development similar to wild type. Our results suggest that, while photoreceptors and components of the circadian clock may be functionally conserved across the land plant lineage, a distinct and novel downstream pathway regulates reproductive development in response to seasonal cues in *P. patens*, and perhaps in other bryophytes. We are using several genetic approaches to identify this novel downstream pathway in order to build a

molecular genetic framework for the mechanism of seasonal reproductive development. Funding has been provided by NSF and Kenyon College.

Short talk 10

Teemu Hakkinen[1], Lucas Pineiro[2], Irene Jin[2], Ophir Klein[1], Zev Gartner[1], Tyler Huycke[1,2]

Growing together: coordinated cell and tissue mechanics drive intestinal villus elongation

[1]University of California, San Francisco, USA; [2]University of Michigan, USA

Tissue folds are structural motifs critical to organ function. In the developing intestine, an initially flat epithelial layer bends into a periodic pattern of folds, giving rise to millions of finger-like villi essential for nutrient absorption. We recently discovered an active physical mechanism, driven by the underlying mesenchyme, that simultaneously patterns and sculpts the intestinal epithelium to initiate villus formation. However, the mechanisms underlying the subsequent elongation of villi into their mature, functional forms remain unresolved. By combining live imaging of whole intestinal tissues from mice, mathematical modeling, and in vivo functional studies, we first demonstrate that villus extension results from a space-filling tissue growth process. Mechanistically, this process involves spatiotemporally coordinated epithelial remodeling, differential growth, and trans-epithelial fluid flux. We then show that villus expansion is constrained laterally by neighboring villi to promote anisotropic tissue extension and proper villus architecture. Together, our findings reveal that intestinal villus elongation requires a coordinated interplay between local epithelial-stromal cell mechanics and global geometric packing constraints that direct growth at the tissue scale. This work is supported by R01DK126376 to ZG and OD and University of Michigan lab startup funds to TH.

Short talk 11

Kelli Johnson[1], Xiangning Dong[1], Yu-Hwai Tsai[1], Angeline Wu[1], Sydney Clark[2], Sha Huang[1], Rachel Zwick[3,4], Ian Glass[5], Katherine Walton[1], Ophir Klein[4,6], Jason Spence[1,7,8]

Mapping mesenchymal diversity in the developing human intestine and organoids

[1]Department of Internal Medicine, Division of Gastroenterolgy, University of Michigan Medical School, USA; [2]Department of Cellular and Molecular Biology, University of Michigan Medical School, USA; [3]Department of Cell Biology, NYU Grossman School of Medicine, USA; [4]Program in Craniofacial Biology and Department of Orofacial Sciences, University of California, San Francisco, USA; [5]Department of Pediatrics, Genetic Medicine, University of Washington, USA; [6]Department of Pediatrics, Cedars-Sinai Guerin Children's, USA; [7]Department of Cell and Developmental Biology, University of Michigan Medical School, USA; [8]Department of Biomedical Engineering, University of Michigan and University of Michigan College of Engineering, USA

The organization of diverse mesenchymal populations during human intestinal development is critical for tissue architecture and function yet remains poorly defined. To construct a comprehensive, tissue-scale map of the developing human small intestine, we leveraged single-cell RNA-sequencing data to build a custom Xenium spatial transcriptomics gene panel covering the diversity of cell types in the human intestine. Analysis was focused on the developing mesenchyme populations (also referred to as

fibroblasts or stroma) given the lack of spatiotemporal information about these cell populations. We defined 5 broad mesenchymal populations occupying discrete locations within the lamina propria and submucosa – the subepithelial cells (SEC), lamina propria fibroblasts (LPF), submucosal fibroblasts (SMF), smooth muscle cells (SMC), and *CXCL13*+ fibroblasts. Our data reveal dynamic spatial remodeling of fibroblast communities during development and establish molecular markers to distinguish these populations. We leverage this high-resolution atlas to benchmark pluripotent stem cell-derived human intestinal organoids and to demonstrate how this foundational resource can be used to dissect intestinal stromal signaling in a spatial manner, with broad implications for modeling development, regeneration, and disease. Funding: This work was supported in part by: 2019-002440 (Seed Network) and 2021-237566 (Pediatric Network) from the Chan Zuckerberg Initiative DAF to J.R.S., the Intestinal Stem Cell Consortium (NIDDK and NIAID, U01DK103141 to J.R.S), the NIDDK (R01DK137806 to J.R.S; RC2DK140862 to J.R.S and O.K; R01DK121166 to K.D.W; F32DK138694 to K.J), and the University of Michigan Center for Gastrointestinal Research (NIDDK 5P30DK034933). I.G. and the University of Washington Laboratory of Developmental Biology was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD- 5R24HD000836).

Short talk 12

Dominic Kolonay

MED12 is a Regulator of Calcium Gene Expression in the Developing Heart

The Ohio State University, USA

The Mediator complex serves as a bridge linking basic machinery and transcription factors (TFs) to control transcription, but detailed molecular mechanisms of gene regulation by Mediator in cardiac development are not well-understood. Mediator is comprised of head, middle, tail, and transiently associated CDK8 submodules. The CDK8 kinase submodule has been shown to regulate Mediator-RNA Polymerase II interaction. MED12 is an essential Mediator component within the kinase submodule and is required for CDK8 kinase activity. Human MED12 mutations cause developmental delays and cardiac abnormalities, indicating the need for mechanistic understanding of MED12's role in the heart. Med12 null mice are embryonic lethal, and mice harboring cardiomyocyte specific deletion of Med12 exhibit heart failure with reduced ejection fraction in early postnatal development. RNA-seq of P1 mouse knockout ventricles show significant dysregulation of calcium handling genes, while calcium tracings revealed impaired contractility. To determine how MED12 regulates cardiac gene expression we performed immunoprecipitation and mass spectrometry. We identified key developmental TFs MEF2, CREB, and SRF that interact with MED12 and we demonstrated that MED12 regulates calcium handling genes through MEF2. CUT and RUN experiments show altered recruitment of TFs to DNA with dysregulated MED12. Current studies are focused on determining domains of MED12 required for TF interactions, and the molecular mechanisms disabled when those interactions do not occur. Collectively, our data demonstrates that MED12 acts as a regulator of cardiac transcription, and altered levels lead to progressive heart failure in part by dysregulation of gene expression in a developing heart. Therefore, determining how MED12 interacts with TF partners will allow us to better understand how the regulation

of gene expression progresses in cardiac development. This work is funded by R01HL166520 and T32HL134616.

Short talk 13

Gopal Kushawah

Cth1 is a spatio-temporal maternal RNA decay factor essential for zebrafish development.

Stowers Institute for Medical Research, USA

The maternal-to-zygotic transition shifts regulatory control from maternal to zygotic messenger RNAs (mRNAs) primarily through the degradation of maternal RNA. While the temporal dynamics of the maternal mRNA decay are well-characterized, spatial mechanisms remain underexplored. To resolve the spatio-temporal dynamics of maternal RNAs decay, we integrated SLAM-seq with single-cell RNA-seq data to map the spatial dynamics of maternal RNAs during early zebrafish development. Among the maternally deposited transcripts, *cth1* emerged as one of the most abundantly deposited and rapidly degraded transcript, suggesting a tightly regulated role during early embryogenesis. To dissect its function, we generated *cth1* loss-of-function mutants using CRISPR-Cas9. However, attempts to generate maternal-zygotic mutants failed due to early embryonic lethality resulted from impaired gametogenesis in *cth1* mutants. To circumvent this limitation, we employed CRISPR-Cas13d knockdown strategy. Embryos with *cth1 knockdown* exhibited delayed development and severe anterior–posterior axis defects. Knockdown phenotype rescue assays and RNA in situ revealed that the *cth1* 3′ untranslated region (3′UTR) is indispensable for its function, localization, and transcript stability. Using a 3′UTR reporter library, we identified discrete, evolutionarily conserved regions within the *cth1* 3′UTR that confer transcript instability, suggesting that these cis-elements are functionally important across fish species. Transcriptome profiling revealed that *cth1* selectively targets transcripts with highly polyadenylated tails and AU-rich 3′UTRs, highlighting its role as a selective post-transcriptional regulator. Altogether, our findings identify *cth1* as the first maternal RNA decay factor in zebrafish that governs maternal transcript clearance with precise spatial and temporal control—an essential mechanism for orchestrating embryogenesis. Funding: Stowers Institute.

Short talk 14

Elaine Kushkowski

Zebrafish neural crest progenitor domain revealed by ventral gastrula fate map

University of Chicago, USA

Over the past century, fate maps have provided key insights into the early development in a variety of vertebrate and invertebrate species. Fate maps characterize relationships between early cell positions and later cell fates, revealing the spatial organization of cell fate progenitor domains during embryogenesis. In zebrafish, previous gastrula-stage fate maps identified the origin of germ layers and mapped many cell fates that arise from dorsal and marginal gastrula domains. However, these fate maps did not fully resolve the ectoderm and mesoderm progenitor domains in the ventral half of the embryo. Further, the progenitor domain that gives rise to the neural crest, a multipotent cell type that is central

to vertebrate development and evolution, was not identified in previous fate maps. This study examines how ventral regions of the zebrafish gastrula contribute to the pharyngula body plan. We generated a fate map by labeling small regions of progenitor cells in the 6 hours post fertilization (hpf) gastrula and characterizing the cell fates and anteroposterior (AP) positions of labeled progeny in the 30 hpf pharyngula. We identify progenitor domains that give rise to the full extent of the neural crest, cranial placodes, lateral line system, and epidermis, as well as parts of the central nervous system and somitic mesoderm. In addition, we find that the neural crest progenitor domain may be divided into sub-domains based on overlap with other cell fates and later position along the AP axis. Overall, this study reveals the progenitor domains of several key developmental cell types within the ventral gastrula, broadening our understanding of early embryonic organization in zebrafish. Our findings highlight that fate maps continue to provide foundational context for investigating complex morphological and molecular processes during early development.

Short talk 15

Hyunwook Lee[1,2,3,4,5], Abigail Jaquish[6,7], Sharlene Fernandes[1,2], Barbara Zhao[1,2,3,4,5], Amber Elitz[1,2,3,4,5], Kathleen Cook[1,2], Sarah Trovillion[1,2], Natalia Bottasso-Arias[1,2], Simon Han[3,4,5], Samantha Goodwin[1,2], Nicholas Russell[1,2], Amanda Zacharias[1,2,3], Samantha Brugmann[3,8,9], Jeffrey Whitsett[1,2,3], Debora Sinner[1,2,9], Xin Sun[6,7], Daniel Swarr[1,2,9], William Zacharias[1,2,3,9,10,11]

The BAF complex is required for respiratory development and alveolar type 1 cell fate acquisition

[1]Perinatal Institute, Cincinnati Children's Hospital Medical Center, USA; [2]Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, USA; [3]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA; [4]Medical Scientist Training Program, University of Cincinnati College of Medicine, USA; [5]Development, Stem Cells & Regenerative Medicine Graduate Program, University of Cincinnati College of Medicine, USA; [6]Department of Cell and Development Biology, University of California San Diego, USA; [7]Department of Pediatrics, University of California San Diego, USA; [8]Division of Plastic Surgery, Department of Surgery, Cincinnati Children's Hospital Medical Center, USA; [9]Department of Pediatrics, University of Cincinnati College of Medicine, USA; [10]Division of Pulmonary and Critical Care Medicine, University of Cincinnati College of Medicine, USA; [11]Department of Internal Medicine, University of Cincinnati College of Medicine, USA

Mammalian embryonic lung morphogenesis requires the formation of a definitive lung bud from the foregut endoderm, branching morphogenesis, specification of a proximal to distal axis, and differentiation of airway and alveolar epithelial lineages. While the transcriptional and signaling regulators required for respiratory development have been increasingly well characterized, the epigenetic factors that regulate lineage-specifying transcription factors and cell-specific control of gene expression remain unknown. Here, we identify a key role of the canonical BAF complex during lung epithelial development and particularly AT1 cell fate acquisition. Loss of canonical BAF complex activity in the foregut endoderm leads to complete failure of lung formation, while selective deletion of a single key subunit, ARID1A, leads to failure of proximal-distal axis specification and specification of AT1 cells in the distal saccular epithelium. In place of AT1 cells, we identified a high proliferative epithelial cell state defined by expression of Sox9, a distal epithelial progenitor marker, joint activation of the YAP/TAZ and

Wnt signaling pathways, and loss of BMP4 signaling response. Conditional loss of ARID1A in AT1 cells postnatally during alveologenesis, the final stage of lung development, caused no discernable changes in alveolar epithelial cell populations while conditional loss of ARID1A in AT2 cells impaired AT2-AT1 cell differentiation. Using embryonic lung organoids, we demonstrate that exogenous BMP4 is sufficient to rescue AT1 cell differentiation in ARID1A-deficient epithelium, while YAP/TAZ and Wnt signaling require functional BAF complex. Together, these data demonstrate a requirement for the BAF complex for lung bud formation, proximal-distal patterning, and AT1 cell fate acquisition. HL was supported by the L.B. Research Foundation and GM063483 (NIH), DS by HL156860 and HL144774 (NIH), and WJZ by HL156860, HL166245, and AI150748 (NIH).

Short talk 16

Lauren Todorov[1], Kouhei Oonuma[2], Takehiro Kusakabe[2], Mike Levine[1], Laurence Lemaire[1,3]

Neural crest lineage in the protovertebrate model Ciona

[1]Princeton University, USA; [2]Konan University, Japan; [3]Saint Louis University, USA

Neural crest cells serve as multipotent progenitors arising from the neural plate border and populating the whole embryo. They give rise to key characteristics of vertebrates, including the "new head". These cells are unique to vertebrates. We investigated their evolutionary origins using Ciona, which belongs to tunicates, the closest living relative to vertebrates. Previous studies have identified Ciona sensory pigment cells as potential rudimentary neural crest cells. However, these cells would represent the differentiated derivative, not the progenitor. Here, we explore their developmental path, taking advantage of the invariant lineage-based development of Ciona. Our findings reveal that the pigment cell lineage also produces neural progenitor cells, which develop into part of the juvenile nervous system after metamorphosis (Todorov et al., Nature 2024). In vertebrates, neural progenitors are one of the main cell types derived from neural crests. These data suggest that a population of multipotent progenitor cells was located at the neural plate border of the last common ancestor of tunicates and vertebrates. Consequently, it seems that multipotentiality, one of the core properties of neural crest cells, existed before the emergence of vertebrates. This work was supported by a grant (NS076542) from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health (NIH), the National Institute of General Medical Sciences of the NIH (grant number T32GM007388). It was also funded by the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI, 19H03213, 23H02492, 23K27185, 19K16150, and 23K05786), the Hirao Taro Foundation of KONAN GAKUEN for Academic Research as well as the Office of Vice President for Research and the Provost Office of Saint Louis University (RI award 01978 and grant 001654).

Short talk 17

Geremy Lerma[1], Kamorah Ryhlick[2], Joseph Beljan[1], Olivia Carraher[1], Alyssa Pennel[1], Gavin Muniz[2], Sharon Amacher[1,3,4]

Validation of Duchenne muscular dystrophy candidate modifiers using a CRISPR-based approach in zebrafish

[1]The Ohio State University, Department of Molecular Genetics, USA; [2]The Ohio State University, Department of Biology, USA; [3]The Ohio State University, Department of Biological Chemistry and Pharmacology, USA; [4]The Ohio State University, Center for RNA Biology, USA

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease for which there is no cure. There is a critical need for additional therapeutics. Human genome-wide association studies (GWAS) have identified candidate DMD genetic modifiers that could serve as therapeutic targets. Because many GWAS-identified single nucleotide polymorphisms (SNPs) lie in noncoding, putative regulatory regions, it can be challenging to identify which gene(s) are regulated by these SNPs and how gene expression is altered to modify disease severity even with extensive *in silico* modeling. We analyzed expression of zebrafish orthologs of putative DMD modifiers and showed almost all are comparably expressed in wild-type and *dmd* mutant zebrafish at three different stages of disease. To model decreased expression of candidate modifiers we pursued a zebrafish CRISPR-based screening approach and used birefringence to assess muscle organization. We validated our strategy by testing zebrafish orthologs of two extensively studied DMD modifiers, *LTBP4* and *THBS1* and showed that they ameliorate DMD in zebrafish. We then tested novel candidates from a more recent GWAS and provide evidence that supports that *galntl6, man1a1, etaa1a, etaa1b,* and *adamts17* are *bona fide* DMD modifiers. Our findings demonstrate the utility of zebrafish for DMD genetic modifier screening and characterizing modifier function. This work is supported by NIH grant R01GM11764, an Ohio State University President's research Excellence Accelerator Award, a sub-contract of NIH grant R01NS085238, a T32 training grant T32GM086252, an Ohio State Herta Camerer Gross Fellowship, and Parent Project Muscular Dystrophy award AWD-117298.

Short talk 18

Muzi Li, Abubacarr Jalloh, Ezra Amiri, Koray Kasan, Urs Schmidt-Ott

Activition Versus Repression: Divergent Initiation of Axis Specification in Drosophila and the Midge Chironomus

U Chicago, USA

Embryos of the fruit fly Drosophila and the midge Chironomus specify the anterior-posterior body axis using unrelated, structurally distinct transcription factors. In Drosophila, this process is governed by the homeodomain protein Bicoid, which functions as anterior determinant in the syncytial early embryo and activates numerous target genes by promoting chromatin accessibility. In Chironomus, which lacks the *bicoid* gene, the C-clamp transcription factor Panish fulfills the same role but how it functions remains unknown. By combining genetic perturbations with genomic approaches, we found that Panish functions as a transcriptional repressor that breaks symmetry along the anterior-posterior body axis by directly repressing a zygotically expressed posterior determinant, encoded by the Chironomus ortholog of *tailless* (*Cri-tll*). In turn, Cri-Tll represses anterior cell fates by directly inhibiting the expression of genes required

for anterior patterning, including homologs of *optix*, *orthodenticle*, *sloppy-paired*, and *giant*. Preliminary data suggest that these genes suppress ectopic abdomen specification in the anterior embryo by repressing *caudal*. Therefore, despite their analogous role in symmetry breaking, Bicoid and Panish differ not only in their targets but also in their mechanisms of gene regulation. A developmental gene network may thus be subject to rapid evolution through co-option of transcription factors with completely different actions. This work has been supported by NIGMS under Award Number R01 GM127366.

Short talk 19

Zong-Yuan Liu

Endogenous FGFs drive ERK-dependent cell fate patterning in 2D human gastruloids

University of Michigan, USA

During gastrulation, the three germ layers are established, and the body plan is laid out. Secreted cell signaling molecules, known as morphogens, play a crucial role in this process. Studies in mouse have identified four different families of morphogens—BMP, Wnt, Nodal, and FGF. While the functions of the first three are relatively well understood, the role of FGF signaling, particularly in human development, remains elusive. Here, we explore the role of FGF/ERK signaling in micropatterned human pluripotent stem cells, also known as 2D human gastruloids. We find that a ring-like pattern of phosphorylated ERK forms in an FGF-dependent manner and expands in parallel with the emergence of primitive streak (PS)-like cells but further inward. We demonstrate that this pattern of ERK activity is due to FGF receptor (FGFR) activation with FGFR1 localized on the basal side. Transwell and scratch assay further reveal that the ERK pattern is driven by an endogenous FGF ligand gradient. Using single-cell RNA sequencing, we identified FGF4, FGF8, and FGF17 as the primary ligands expressed in the PS region, and confirmed their presence using RNA fluorescent in situ hybridization (FISH). Intriguingly, these FGF ligands display unique expression profiles spatially: FGF4 is restricted within, FGF8 at the inner boundary, and FGF17 mostly in the protrusions of the PS region. Functional studies revealed that knockdown of FGF4 severely diminishes ERK activity and PS differentiation, whereas knockdown of FGF8 leads to the expansion of PS. FGF17 knockdown has a small reduction on ERK activity and PS differentiation, but it significantly reduces mesoderm differentiation. In summary, we have greatly advanced our understanding of FGF/ERK signaling in human gastrulation and provided evidence that different FGF ligands may play distinct roles during this process. This work is supported by NSF RECODE 2033654 and NIGMS312 R35GM138346.

Short talk 20

John Klem[1], Tae-Hwi Schwantes-An[2], Michael Suttie[3], Rae`den Gray[1], Hieu Vo[1], Leah Wetherill[2], CIFASD CIFASD[1,2,3], Charles Lovely[1]

Mutations in the Bmp signaling components sensitize zebrafish and humans to ethanol-induced jaw malformation

[1]University of Louisville School of Medicine, USA; [2]Indiana University School of Medicine, USA; [3]Oxford, United Kingdom

Fetal Alcohol Spectrum Disorders (FASD) describe a continuum of ethanol-induced developmental defects, including facial malformations. While ethanol-sensitive genetic mutations contribute to facial malformations, the impacted cellular mechanisms remain unknown. Formation of the facial skeleton requires complex signaling interactions between the neural crest (NC), that form the facial skeleton, and the endoderm. Proper endoderm morphogenesis is central to these interactions. Bone Morphogenetic Protein (Bmp) signaling is a key regulator of endoderm morphogenesis providing a possible ethanol-sensitive mechanism. Here, we establish an *in vivo* FASD model, where mutations in the Bmp pathway sensitize embryos to ethanol-induced facial defects by disrupting endoderm morphogenesis. Ethanol-treated Bmp mutants display significant changes in endoderm morphology, disrupting the tissue interactions driving facial development. Using quantitative morphometric readouts, we show that changes in endoderm shape mirror shape changes to the facial skeleton. We go on to show that our zebrafish data are predictive with variants in human *BMPR1B* associating with ethanol-related differences in jaw volume.Strikingly, ethanol does not impact Bmp signaling directly but increases apoptosis in migratory NC in Bmp mutants. This suggests that Bmp-dependent signals from the endoderm are ethanol sensitive leading to NC defects. Overall, our results show that the zebrafish model remains a powerful, efficient model to simultaneously generate a deeper mechanistic understanding of gene-ethanol interactions on the complex tissue interactions and provide a conceptual framework for ethanol-induced structural birth defects in human. Ultimately, this work will connect ethanol exposure with concrete cellular events that could be sensitive beyond the facial skeleton. This research was supported by R00AA023560 and R01AA031043 to CBL, U24AA030169 to LW, and U01AA014809 to MS.

Short talk 21

Anna Lubertozzi[1], Angad Dhillon[2], Jessica Martin[2], Timothy Plageman[2]

Afadin Orchestrates Tricellular Adherens Junctions and Lens Tissue Architecture

[1]Department of Molecular Genetics, Ohio State University, USA; [2]College of Optometry, Ohio State University, USA

The vertebrate lens develops from a simple epithelium into a highly ordered tissue composed of elongated lens fiber cells arranged in a precise lattice-like architecture essential for transparency and refractive function. In mice, we observed that this pattern begins to emerge around postnatal day 1 and is nearly complete after postnatal day 7. This relatively unique tissue organization generates an array of anisotropic cells with regularly spaced tricellular junctions. Tricellular junctions have been linked to the organization and integrity of various epithelial tissues and are locations where biomechanical forces are concentrated, but their role in the vertebrate lens remains unclear. To explore how resident proteins

might contribute to lens fiber cell patterning, we screened a panel of known adherens junctional proteins using immunofluorescent staining of lens tissue and identified four—afadin, delta-catenin, p120-catenin, and ZO-1—as enriched at tricellular adherens junctions (tAJs) in the lens. We focused on afadin function due to its early and robust localization to tAJs during the time when lattice organization first forms and its previous association with tAJs. Using a lens-specific conditional knockout mouse model, we found that loss of afadin results in microphthalmia, disrupted lens fiber cell elongation, and severe disorganization of the lens fiber cell lattice. These defects correlate temporally with afadin's initial enrichment at tAJs and are accompanied by failure to recruit other tricellular junction proteins. Together, our findings suggest that afadin is a critical regulator of tAJ assembly and plays an essential role in coordinating lens fiber cell architecture during lens development. This work is supported by NEI R21EY034213.

Short talk 22

Esther Ushuhuda[1], Jenniluyn Nguyen[2], Natalie Pfaltzgraff[1], Matthew Kofron[1,3], Maria Mikedis[1,3]

MEIOC prevents continued mitotic cycling and promotes meiotic entry during mouse oogenesis

[1]Cincinnati Children's Hospital Medical Center, USA; [2]Whitehead Institute, USA; [3]University of Cincinnati College of Medicine, USA

In multicellular organisms, germ cells' transformation into haploid gametes requires that they transition from mitosis to meiosis, whereby they stop mitotic cycling and enter the meiotic cell cycle. In mammals, transcriptional activator STRA8-MEIOSIN mediates the decision to enter the meiotic cell cycle by triggering the G1-to-meiotic S phase transition. However, the molecular basis by which mammalian germ cells prevent continued mitotic cycling before entering the meiotic cell cycle remains unclear. Here, we investigate MEIOC's role in the mitosis-to-meiosis transition during mouse oogenesis by analyzing proliferation, cell cycle transcriptomics, and cell cycle-associated protein expression. MEIOC was previously shown to destabilize mRNA and repress the mitotic program after meiotic entry. Here, we demonstrate that MEIOC prevents continued mitotic cycling prior to meiotic entry in oogenic cells. We find that the mitosis-to-meiosis transition involves the repression of G1/S cyclin CCNA2 at the transcript and protein levels, and that MEIOC downregulates CCNA2 protein expression. In addition, MEIOC promotes entry into meiotic S phase by increasing *Meiosin* transcript abundance and consequently activating the STRA8-MEIOSIN transcription factor. Given that STRA8-MEIOSIN upregulates *Meioc* expression, MEIOC and STRA8-MEIOSIN form a positive feedback loop to reinforce timely meiotic initiation. We also demonstrate that BMP signaling halts mitotic cycling and promotes meiotic entry by upregulating MEIOC. We conclude that, in mouse oogenic cells, the transition from mitosis to meiosis occurs as two molecularly regulated steps– (i) halt of mitotic cycling and (ii) entry into the meiotic cell cycle – and that MEIOC modifies the cell cycle program to facilitate both steps in this transition. Funded by NICHD award R00HD097285.

Short talk 23

Sejuti Naurin, Oliver Voecking, Jakub Famulski

Chemokine signaling during optic fissure fusion in zebrafish

University of Kentucky, USA

A critical stage of vertebrate eye morphogenesis is the formation and closure of the optic fissure (OF), a transient ventral gap in the retina that enables hyaloid vasculature entry. Timely and precise OF fusion is essential for normal retinal development; failure leads to coloboma, a congenital blinding disorder. Although transcriptional regulators like *pax2a* (zebrafish homolog of *pax2*) are known to be key for OF fusion, the molecular mechanisms driving OF fusion remain unclear. In this study, I used Tg[*rx3:GFP*] zebrafish to generate a high-resolution single-cell transcriptome of OF fusion with temporal and spatial precision. Focusing on *pax2a*-expressing cells, I identified several novel genes enriched in the OF; *cldn19*, *ackr3b*, *cxcl12a*, and *spry4* whose expression is markedly reduced in *pax2a*^{noi} coloboma mutants, suggesting a regulatory link. Interestingly, *cxcr4a*, a receptor in the *cxcl12* pathway, was absent in scRNA-seq but present in the OF by WISH. *Cxcr4a* expression in the OF was eliminated upon DMH4 treatment, an angiogenesis inhibitor, suggesting *cxcr4a* is expressed by migrating hyaloid vasculature. Prior work from our lab showed that OF-associated hyaloid vasculature is essential for delivering the basement membrane (BM) remodeler *mmp2*, which is required for initiation of OF fusion. To test whether chemokine signaling plays role in OF fusion and hyaloid vasculature migration, I have generated *cxcl12a*, *ackr3b*, and *cxcr4a* mutant lies. Preliminary data indicates delayed OF fusion in these mutants. I am currently generating double mutants for the ligands (*cxcl12a*, *cxcl12b*) and receptors (*ackr3b*, *cxcr4a*) to further investigate the coloboma phenotype. To our knowledge, this is the first study to explore the role of chemokine signaling in OF fusion, offering novel insight into the molecular mechanisms of this critical developmental process. Funding source: CHARGE syndrome foundation 2025

Short talk 24

Jose Raúl Perez-Estrada[1], Jared A Tangeman[1,2,3], Stacy Bendezu-Sayas[1,3], Carlos M Charris-Dominguez[1,3], Hiruni Lokuyaddehige[1,3], Caroline Kennelly[1], Nimra Safdar[1,3], Erika Grajales-Esquivel[1,2], Chun Liang[1,2,3], Katia Del Rio-Tsonis[1,2,3]

Modulating RPE Plasticity to Bypass Neurocompetence Barriers

[1]Department of Biology, Miami University, Oxford, OH, USA, US; [2]Center for Visual Sciences, Miami University, Oxford, OH, US; [3]Cell, Molecular, and Structural Biology Program, Miami University, Oxford, OH, US

The retinal pigment epithelium (RPE) is a monolayer of cells essential for retina health and physiology. RPE is a plastic tissue that can regenerate neural retina in some embryonic amniotes via cell reprogramming. In chicken embryos, RPE reprograms into neural retina after retinectomy and FGF2 stimulation at embryonic day 4 (E4) but not at day 5 (E5) or later. We hypothesized that signaling pathways and intrinsic cell fate control during eye development are coupled mechanisms restricting RPE neurocompetence. To identify transcription factors and signaling pathways that could promote RPE reprogramming in the late embryonic state, we used single-nucleus multimodal profiling to identify the differences in molecular signatures of chicken RPE cells spanning stages E3 through E7. This analysis

identified nine up-regulated and nine down-regulated transcription factor-encoding genes and accompanying changes in motif accessibility that coincided with RPE neurocompetence restriction. Our findings suggest that enhanced activity of the Hippo-YAP pathway could restrict RPE neurocompetence. Inhibition of the Hippo-YAP pathway significantly increased cell proliferation in E4 and E5 RPE explants in the absence of FGF2 but did not induce retina formation. However, it affected the expression of several genes, including EMT regulators and cell cycle-related genes, while suppressing RPE identity genes. Interestingly, transient inhibition of the Hippo-YAP pathway in E4 RPE explants extended the neurocompetence window to E5, promoting RPE reprogramming in response to FGF2. These findings indicate that cell proliferation regulated by the Hippo-YAP pathway may control RPE reprogramming and restrict neurocompetence at later stages. Funding: NEI R01 EY034980 KDRT.

Short talk 25

Rebekah Rushforth

Functional tubulin autoregulation is required for proper neurogenesis

Nationwide Children's Hospital, USA

Tubulin autoregulation is the process by which cells maintain the appropriate concentration of soluble tubulin in the cell. The TETRATRICOPEPTIDE REPEAT DOMAIN CONTAINING PROTEIN 5 (TTC5) protein is crucial for mediating this process. Three recently published patient cohorts identified variants in *TTC5* that cause severe developmental brain malformations. We aim to demonstrate that dysfunction in tubulin autoregulation is responsible for cortical malformations. We hypothesize that loss of tubulin autoregulation alters tubulin structures and causes defective corticogenesis. To test this, we generated a novel *Ttc5* null mouse model. RT-qPCR of *Ttc5*-null mouse embryonic fibroblasts and mixed cortical cultures have shown that loss of *Ttc5* notablyincreased total tubulin mRNA concentrations. Therefore, the *Ttc5*-null mouse demonstrates defective tubulin autoregulation. Furthermore, *Ttc5*-null mice faithfully recapitulate human brain malformation phenotypes including hypoplasia of the corpus callosum, ventriculomegaly, and microcephaly. Mechanistic studies into neurogenesis show changes in cell cycle dynamics with reduced proliferation at embryonic day (E)12.5, but increased proliferation at E14.5 in *Ttc5*-null mice. Examination of key tubulin structures revealed both a decrease in the length of the primary cilia and mitotic spindle defects resulting in severe chromosomal alignment and segregation errors. Both the mitotic spindle and primary cilia play essential roles in cell cycle progression and timing. Surprisingly, we found the alterations in the mitotic spindle are likely driven by increased gamma tubulin foci. We confirmed an increase of gamma tubulin mRNA via RT-qPCR. Gamma tubulin has not been previously associated with tubulin autoregulation. This finding thereby expands the paradigm of tubulin autoregulation and demonstrates the mechanism for tubulin autoregulation brain malformations. This work is funded by The Wexner Research Institute recruitment funds (R.W.S.).

Short talk 26

Bitan Saha, Krishna S. Krothapalli, Joshua S. Waxman

Nr2f1a-Notch signaling interactions balance chamber-specific endocardial identity necessary for cardiac development

Cincinnati Children's Hospital Medical Center, USA

Hypoplastic Left Heart Syndrome (HLHS) is a congenital heart defect characterised by underdeveloped ventricular chambers due to impaired endocardial development and dysregulation of cardiac jelly, an extracellular matrix (ECM) present between the myocardium and endocardium. While NR2F2, a transcription factor predominantly associated with atrial cardiomyocyte differentiation, has surprisingly been associated with HLHS, how loss of NR2F factors underlie HLHS is not understood. Using a knock-in transgenic reporter for endogenous expression of zebrafish *nr2f1a*, the functional homologue of mammalian NR2F2, we find that *nr2f1a* is uniquely expressed in the atrial endocardium starting at 48 hours post fertilization. We find that pharmacological inhibition of Notch signaling, which is active in the ventricular endocardium, led to an expansion of *nr2f1a* expression into the ventricular endocardium. Conversely, *nr2f1a* mutants had expanded Notch activity in the atrial endocardium. Single cell RNA-seq data revealed that ECM remodelling factors are excluded from the atrial endocardium during chamber development and suggested *hyal2b*, a hyaluronidase, as a candidate effector of chamber-specific cardiac jelly degradation. We observed increased deposition of cardiac jelly associated with ectopic *nr2f1a* expression and *hyal2b* loss in the ventricular endocardium, while cardiac jelly was virtually absent in *nr2f1a* mutant hearts, which have ectopic *hyal2b* expression expanding into the atrial endocardium. CRISPR-mediated knockdown of *hyal2b* in *nr2f1a* mutants was able to restore proper cardiac jelly deposition. Altogether, our findings highlight a novel mutually-antagonistic interaction between Notch signaling and Nr2f transcription factors that dictates ventricular and atrial endocardial identity necessary for chamber-specific morphology via regulation of ECM deposition, which may provide insights into the etiology of HLHS. This work has been funded by the American Heart Association.

Short talk 27

Elizabeth Schock[1,2], Joseph Nguyen [2], Carole LaBonne[2]

The C-terminal domain of SoxB1 and SoxE factors mediates differential protein function during neural crest cell formation

[1]University of Kentucky, USA; [2]Northwestern University, USA

Transcription factors play critical roles during embryonic development, which includes directing cells towards specific cellular identities. Interestingly, there are many instances where transcription factors from the same family direct cells towards different fates despite having highly similar DNA-binding domains. One example of this is Sox factors during formation of neural crest cells. While SoxE factors (Sox8/9/10) promote neural crest formation, SoxB1 factors (Sox1/2/3) block neural crest cell fate adoption and instead hold cells in a neural plate border progenitor state. To understand how such opposing roles can arise in transcription factors from the same family, we generated Sox3-Sox9 chimeras where either the DNA-binding domains or the C-terminal regulatory domains (CRD), which contain transactivation domains, of the proteins are swapped. We assessed the ability of these constructs to

promote or inhibit neural crest formation using Xenopus embryos. Our findings indicate that functional differences in Sox3 and Sox9 factor activity during neural crest formation can be attributed to differences in the CRDs, and not the DNA-binding domains, of these protein subfamilies. We next assessed how differences in the CRDs of Sox3 and Sox9 impact global genomic occupancy using ChIP-seq. Interestingly, we find that Sox factor CRDs are critical determinants of genomic binding specificity. Through motif analysis, we identify differences in the core Sox consensus sequences and find unique putative binding partners for Sox3 and Sox9. These data suggest that the Sox family CRDs modulate DNA specificity both through partner selection and differential affinity towards different consensus sequences. Funding Sources: F32DE029113 (BS), K99DE031825 (BS), 597491-RWC (CL)

Short talk 28

Courtney Stockman[1], Thomas Taylor[1,2], Jenna Green[1], Kimberly Wagner[1], Anne-Karina Perl[1,2,3]

Matrix Fibroblast-Derived CXCL12 Regulates Capillary Maturation and Secondary Septation During Alveologenesis

[1]Division of Neonatology and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, USA; [2]Molecular and Developmental Biology Graduate Program, Cincinnati Children's Hospital Medical Center, USA; [3]Department of Pediatrics, University of Cincinnati College of Medicine, USA

Alveologenesis, the final stage of lung development, depends on coordinated interactions among fibroblasts, endothelial cells, and epithelial cells to establish a functional alveolar-capillary interface. When communication between these cell types is disrupted, secondary septation fails, resulting in enlarged airspaces and reduced surface area for gas exchange. Recent studies have identified distinct fibroblast subsets that actively instruct alveolar development. Matrix fibroblasts, once thought to be passive structural cells, have emerged as key regulators of septation through both extracellular matrix production and paracrine signaling. One such signaling axis is CXCL12–CXCR4. We investigated the role of this pathway by conditionally deleting CXCL12 from Meox2? matrix fibroblasts during postnatal alveolar development using a tamoxifen-inducible Meox2-CreERT2 driver. Loss of CXCL12 led to alveolar simplification and reduced secondary septa. Immunostaining revealed a marked loss of differentiated CAP2 endothelial cells alongside persistence of CAP1 cells, consistent with a failure in capillary maturation. These findings suggest that fibroblast-derived CXCL12 promotes the endothelial cell transition from CAP1 to CAP2, a step necessary for proper vascular development during alveologenesis. Our data highlights a previously unrecognized role for matrix fibroblasts as instructive regulators of vascular maturation and suggest that disruption of CXCL12 signaling may contribute to impaired microvascular development in neonatal lung disease. This study was supported by NIH/NHLBI grants R01 HL167030 and R01 HL164418. We thank the CCHMC Transgenic Animal and Genome Editing Core for the design and generation of the Meox2CreERT2 mouse.

Short talk 29

Kimberly J. Bussey[1], Kolla Kristjansdottir[2], Ying He[2], Garilyn Jentarra[1], Rosa Ventrella[2]

Utilizing the genetics and genomics competencies developed by the Association of Professors of Human and Medical Genetics (APHMG) to monitor student progress and identify Precision Medicine Program strengths and areas of improvement

[1]Precision Medicine Program, College of Graduate Studies, Midwestern University, Glendale, AZ, 85308, USA; [2]Precision Medicine Program, College of Graduate Studies, Midwestern University, Downers Grove, IL, 60515, USA

Precision medicine is an emerging field that necessitates a comprehensive understanding of genetic, genomic, and clinical data to tailor medical care to individual patients. To align educational efforts with the needs of this evolving discipline, our Precision Medicine Program curriculum at Midwestern University was mapped to the Association of Professors of Human and Medical Genetics (APHMG) core competencies, which are divided into three areas: medical knowledge, patient care, and communication. To optimize our educational approach, we compared the courses in our program to the APHMG core competencies. We demonstrated that our curriculum builds throughout the program, beginning with foundational genetic concepts and ending with patient-focused skills. To determine how well the program was meeting these core objectives, we administered an evaluation to students entering the program and after completion of their first year of core courses to identify their confidence using a Likert scale and knowledge using multiple choice questions that were mapped to each core competency. Results demonstrated that core competency confidence increased from 2.6 to 3.8 on a four-point scale (paired t-test; $p<0.0001$), and quiz scores increased from 29% to 61% (paired t-test; $p<0.001$). However, gaps in confidence were identified in the specific competencies of population genetics and molecular genetics. These findings suggest a need for curricular adjustments to enhance student competency in understanding how population genetics can be used to predict disease risk and deciding which genetic test should be used in specific clinical situations. Targeted interventions to address these weaknesses throughout the program will ensure that graduates will feel confident in applying these essential concepts in practice. Continuing to monitor student progress will allow for curriculum development in response to the evolving landscape of medical genetics education.

Short talk 30

Gabriella Voit, Elizabeth Falat, Jennifer Gutzman

Understanding Laminin-111's Role in Mediating Mechanical Stress During Zebrafish Brain Morphogenesis

University of Wisconsin Milwaukee, USA

Brain morphogenesis is a complex process that requires the integration of biochemical signaling and mechanical forces to shape the developing tissue. While molecular pathways guiding brain development are well studied, the biomechanical mechanisms regulating tissue architecture remain less understood. To address this gap, we investigated the role of basement membrane mechanics in neuroepithelial tissue folding using the zebrafish midbrain-hindbrain boundary (MHB) as a model. The basement membrane protein laminin-111 is highly expressed during MHB morphogenesis and is essential for structural

integrity. Previous work in our lab has shown that mutations in these genes prevent MHB folding leading to structural defects. However, the role of laminin-111 in regulating cell adhesion and mechanical stress distribution remains unclear. To understand overall tissue stress we utilized a biocompatible oil microdroplet-sensor technique. Our findings indicate that laminin-111 mutants exhibit increased tissue stress at the MHB compared to wild-type embryos, suggesting a loss of mechanical integrity. We also identified loss of epithelial integrity in the neuroepithelium observed by disrupted Cdh2 immunostaining and by leakage of 500 kDa dextran from the ventricles following injection. Together, our study reveals that laminin-111 is required to maintain tissue tension, adhesion, and epithelial integrity, providing new insights into the biomechanical regulation of brain morphogenesis. By uncovering how laminin-111 regulates neuroepithelial mechanics, this study contributes to the broader understanding of epithelial tissue engineering and organ morphogenesis. This work has been funded by the UWM Discovery and Innovation Grant.

Short talk 31

Andrew Vontell[1,2], Jack Kucinski[1,2], Alexi Tallan[1,2], Katherine Silvius[2], Cenny Taslim[2], Benjamin Stanton[1,2,3,4], Genevieve Kendall[1,2,3]

Developmental consequences of DNA binding by a rhabdomyosarcoma fusion oncoprotein *in vivo*.

[1]Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, OH, USA, USA; [2]Center for Childhood Cancer Research, The Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA, USA; [3]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA, USA; [4]Department of Biological Chemistry and Pharmacology, The Ohio State University College of Medicine, Columbus, OH, USA, USA

Rhabdomyosarcoma is a pediatric soft-tissue sarcoma with features of arrested myogenesis. The most aggressive subtype is driven by the fusion of transcription factors PAX3 and FOXO1. The resulting PAX3::FOXO1 oncoprotein retains the DNA binding domains of PAX3, consisting of a paired domain and a homeodomain, coupled with the transactivation domain of FOXO1. This chimeric protein acts as an aberrant transcription factor, and we have demonstrated that it functions as a pioneer factor across cellular contexts. We developed an embryonic zebrafish PAX3::FOXO1 mRNA injection model to understand PAX3::FOXO1 epigenetic and transcriptional activity in a developmental context. We found that *in vivo* PAX3::FOXO1 binds inaccessible chromatin through partial homeobox motif recognition and activates targets through composite paired box/homeobox motif recognition. Interestingly, a conserved activity of PAX3::FOXO1 in zebrafish and in patient tumors is the activation of neural pathways. We hypothesize that the pioneering of these neural programs is critical for tumorigenesis. Here, we probed the structure-function relationship of PAX3::FOXO1's paired and homeobox domains in the pioneering of this neural signature. We observed that deletion of either domain reduces the extent to which PAX3::FOXO1 can activate neural pathways through different mechanisms. Both the paired and homeodomain deletions result in differential embryonic survival outcomes and less severe developmental phenotypes compared to the full-length fusion protein, demonstrating *in vivo* consequences for the alteration of the neural signature. Overall, our approach supports functionally evaluating the requirements for pioneering function of PAX3::FOXO1 to better understand its role in

tumorigenesis. This work was supported by NIH/NCI R01 CA272872, Alex's Lemonade Stand A Award, V Foundation for Cancer Research V Scholar Award and Nationwide Children's Hospital startup funds.

Short talk 32

Chung-Ju Yeh[1], Zipora Yablonka-Reuveni[2], Christoph Lepper[1]

FGFR1 and FGFR4 are essential regulators of muscle stem cell quiescence

[1]The Ohio State University, USA; [2]University of Washington, USA

Quiescence – the reversible growth-arrested G_0 state – is critical for the long-term maintenance of many adult stem cells and hence the tissues' life-long regenerative capacity.Failure to maintain stem cell quiescence during aging leads to stem cell loss and reduced ability to regenerate after injury. However, our knowledge about the complex molecular signaling networks required for maintaining stem cells in quiescenceis incomplete. Here, we discovered an essential role for FGFR1 and FGFR4 in regulating skeletal muscle stem cell, i.e. satellite cell (SC), quiescence via inhibiting their precocious differentiation. Moreover, SC-specific *Fgfr1/4*-deficiency results in impaired muscle regeneration and decreased SC self-renewal. Our data reveal that the activated *Fgfr1/4*-deficient SC-lineage prematurely exits the cell cycle and undergoes terminal differentiation, which is regulated via the ERK signaling axis. This study advances our foundational knowledge on the molecular mechanisms governing SC quiescence, and reveals FGFR1/4 as promising therapeutic targets with the potential to enhance regenerative outcomes in skeletal muscle repair. This research is funded by NIH grant R01AR078231, NIA grant AG021566, and NIA grant AG035377.

Short talk 33

Coral Zhou

Mechanisms of genome scaling during embryogenesis and evolution in Xenopus

University of Kansas, USA

Across the tree of life, genome size varies by six orders of magnitude. Polyploidy is an extreme form of genome size expansion in which entire copies of the genome are duplicated. During evolution, polyploidy correlates with bursts of speciation and genetic innovation. Within a species, including in humans, polyploid cells have specialized functions in many tissues including the liver, eye and reproductive organs. Across this huge range, the ratio between genome size, nuclear size, and cell size is largely conserved for a given species and cell type. Deviations in nuclear-to-cytoplasmic (N/C) ratio are strongly correlated with pathologies such as aging and cancer through unknown mechanisms. In the Zhou Lab, we use naturally polyploid African clawed frogs Xenopus to discover the basic molecular mechanisms that sense and adapt to changes in genome size during embryogenesis and evolution. Gaining this knowledge is critical for understanding how genomes maintain homeostasis while creating new functions during health and disease. This work is supported in part by the NIH Center of Biomedical Research Excellence (COBRE) Center for Molecular Analysis of Disease Pathways at the University of Kansas.

POSTERS

Poster Session 1

Poster 1

Mayowa Akinwale[1], Ansley Conchola[1], Renee Hein[1], Zhiwei Zhao[1], Xiangning Dong[1], Peggy Hsu[1], Tristian Frum[1], Ian Glass[2], Jason Spence[1]

STAT3 Driven Regulation of Lower Airway Progenitor Cells in the Developing Lung

[1]University of Michigan, USA; [2]University of Washington, USA

Recent progress in single cell genomic technologies has begun to uncover the heterogeneity of cell types and cell states within developing and adult human tissues at homeostasis and in diseased states. We have identified a unique SCGB3A2^{+}SFTPB^{+}CFTR^{+} progenitor secretory cell population, termed lower airway progenitor (LAP) cells in the developing lung using an 8 – 21 weeks post conception single-cell RNA sequencing (scRNA-seq) dataset. LAP cells are most abundant in the non-cartilaginous small airways and lineage tracing has shown LAP cells can give rise to pulmonary neuroendocrine cells (PNECs) and a subset of multiciliated cells more abundant in the distal airways. Despite recent advances in our understanding of LAP cell differentiation capacity, how LAP cells are regulated and the specific drivers behind the differentiation process remains unclear. Here, using an available multiomic (scRNA-seq + scATAC-seq) dataset from isolated distal regions of human fetal lungs enriched for LAP cells, the single-cell multiomic inference of enhancer and gene regulatory networks (SCENIC+) analysis pipeline was used to identify LAP cell-enriched transcription factors and predicted gene targets. Among prospective identified transcription factors, STAT3 was a top candidate based on its expression in vivo and functional experiments in organoids using pharmacologic inhibitors to test its role during differentiation. Additionally, we leveraged a spatial transcriptomics dataset to identify mesenchymal cells adjacent to pSTAT3^{+} LAP cells. By integrating these spatial data with multiome data, we employed CellChat to infer putative ligand-receptor interactions mediating communication between the neighboring mesenchyme and LAP cells. This analysis revealed candidate signaling pathways that may modulate LAP cell

differentiation and self-renewal, providing new insights into the cellular crosstalk governing LAP cell fate decisions. Funding: NIH R01

Poster 2

Skylie Antle[1], Kendall Martin[2], Hee-Woong Lim[2], Joshua Waxman[2], Jennifer Schumacher [1]

Analyzing expression patterns and function of cardiac genes during zebrafish embryogenesis

[1]Miami University, USA; [2]Cincinnati Children's, USA

Congenital heart disease (CHD) is highly prevalent in live births and requires concise diagnoses and treatment plans. Although there is a vast amount of cardiovascular genetics research in the field of CHD, there are still gaps in knowledge regarding the gene networks that drive heart development and function. *Danio rerio* (zebrafish) make excellent model organisms because they lay up to hundreds of large transparent embryos that are externally fertilized, allowing for easy collection and analysis. Single-cell RNA sequencing was performed on isolated hearts to identify new genes involved in cardiac function. Sequencing analysis categorized genes based on their co-expression with *myh7* in the ventricle at 96 hours post fertilization (hpf). Five genes with little to no previous expression data in the heart were selected for further molecular analysis. These genes were also predicted to be related to human cardiomyopathy, however, there have been limited prior studies conducted on them. Using molecular cloning techniques, we created RNA probes for each gene to conduct in situ hybridization (ISH). We conducted ISH at multiple stages throughout embryonic development and confirmed each gene's expression in the ventricle at 96 hpf. Across these five genes, expression was also found in the atrium, somites, pectoral fin, craniofacial muscles, adaxial cells, liver and gut at different developmental stages. We can conclude from ISH analysis that these genes have unique temporal and spatial characteristics. Moving forward, we plan to use CRISPR gene editing to help us understand the developmental function of each of these genes. Creating novel models to determine the functional significance of these genes can help further our understanding of human cardiomyopathy. This research was funded by Miami University's Undergraduate Summer Scholar Program.

Poster 3

Clare Austin, Thomas Gallagher, Monica Blatnik, Kathryn Thompson, Danielle Pvirre

Investigating the role of P-bodies in Pnrc2-mediated oscillatory gene transcript decay

The Ohio State University, USA

The segmentation clock is an oscillatory gene network that regulates somitogenesis, the formation of embryonic segments that later develop into vertebrae and musculature. Pnrc2, an mRNA decay adaptor, is necessary for rapid decay of oscillatory gene transcripts in the zebrafish presomitic mesoderm (PSM). When Pnrc2 function is lost, over 1700 transcripts, including known oscillatory gene transcripts *her1* and *dlc*, are overexpressed. Despite overexpression of many transcripts, *pnrc2* mutant embryo development is overtly normal, with some developmental delay and reduced post-embryonic viability. We show that lack of an overt embryonic mutant phenotype is likely because accumulated transcripts are poorly translated. The mechanism by which Pnrc2 regulates rapid decay and translational

repression of oscillatory gene transcripts is unknown. Among the genes we looked at *ddx6* and *ddx61,* DEAD-box helicases that are associated with P-body assembly and are upregulated at both the transcript and protein level in *pnrc2* mutant embryos. Pnrc2 is known to interact with P-body-associated proteins in human cultured cells, consistent with our hypothesis that P-bodies contribute to oscillatory gene transcript regulation. To characterize Pnrc2 localization and identify Pnrc2 interactors, we are using CRISPR-mediated knock-in to tag endogenous Pnrc2 with an ALFA epitope sequence, which will allow us to detect the endogenous Pnrc2 protein using the ALFA nanobody. Using an ALFA nanobody-GFP fusion, we will observe Pnrc2 localization during somitogenesis in wild-type and *pnrc2*mutant embryos and co-localization with Ddx6 and Ddx61. Using an ALFA nanobody-Halo tag fusion, we will use co-immunoprecipitation and mass spectrometry to identify Pnrc2 interactors. I predict Pnrc2 interacts with P-body proteins in the zebrafish PSM and will colocalize with Ddx6 and Ddx61. This study will identify the role of P-bodies in Pnrc2-mediated oscillatory gene transcript decay during somitogenesis.

Poster 4

Nairi Azazian, Farina Aziz, Fei Fang, Liangliang Sun, Amy Ralston

Proteomics of Modified Pre-Implantation Mouse Embryos

Michigan State University, USA

During embryonic development, the inner cell mass (ICM) of a developing embryo differentiates into epiblast (EPI) and primitive endoderm (PE). Many proteins are involved with this process, however, the involvement of specific proteins in forming EPI and PE cell types have not been fully explored. The goal of this project is to characterize the protein content of wild type and reprogrammed embryos. Using mass spectrometry-based proteomics, I aim to identify and characterize differentially expressed proteins that modulate differentiation. Moreover, using RNA sequencing I will perform a comparative analysis of mRNA and protein expression of reprogrammed versus wild type embryos. My projects are funded by National Institute of Health award R35 GM131759 to AR.

Poster 5

Chidiogo Azuka, Jian Liu, Xiangshu Jin

Structural Basis of Fsc1-Mediated Autophagosome-Vacuole Fusion in Fission Yeast Autophagy

Michigan State University, USA

To support growth and development, cells maintain a stable internal environment through the process of homeostasis, ensuring optimal conditions for cell function and metabolism. Integral to maintaining homeostasis, eukaryotic cells engage in the critical degradation process of autophagy. During autophagy, intracellular materials, including cell organelles and macromolecules, are sequestered in specialized double-membraned vesicles and transported to the lysosomes, where hydrolytic enzymes degrade them. Autophagy proceeds in three main steps: initiation, elongation, and fusion, and is conserved across eukaryotic species. Fission yeast, *Schizosaccharomyces pombe*, shares several features with humans and serves as a model organism for autophagy studies. Discovered in a genome-wide screen,

fsc1 protein is a novel autophagy factor in *S. pombe* and is implicated in the less-understood autophagosome-vacuole fusion step during autophagy. Deletion of the *fsc1* gene blocks this crucial autophagic step, thereby disrupting the entire autophagy process. To investigate the molecular basis of this critical autophagy step, we solved crystal structures of *S. pombe* fsc1 protein in two space group symmetries and analyzed its structural interactions to understand possible mechanistic routes underpinning fsc1-mediated autophagosome-vacuole fusion. Our results show that Fsc1 exists as a dimer, with its amino acid sequence organized into five tandem fasciclin-like fold domains, without any different intervening structural motif. The dimeric association state of Fsc1 is supported by data from size-exclusion chromatography (SEC), indicating that this is the biologically relevant state required for fusion during autophagy. Furthermore, we have identified a significant binding groove in the fourth domain of Fsc1, which could serve as a potential binding site for binding partner proteins that act just upstream and downstream of Fsc1 during the autophagy workflow.

Poster 6

Sushant Bangru[1,2], Lucas Garcia[2], Kazunori Ando[1], Rebecca Myatt[1], Pierre Gillotay[1], Stefano Di Talia[2], Kenneth Poss[1,3]

Interrogating signaling and growth dynamics in regenerating scales in zebrafish

[1]Morgridge Institute for Research, USA; [2]Department of Cell Biology, Duke University, Durham, NC, USA; [3]Department of Cell & Regenerative Biology, University of Wisconsin, Madison, WI, USA

Vertebrate appendage regeneration remains a major challenge with mammals only able to repair fractures—and occasionally digit tips—and lack the ability to regenerate complex structures like limbs. In contrast, zebrafish robustly regenerate complex skeletal structures, including fins, jaws, and dermal scales. Zebrafish scales—small, flattened dermal bones—rapidly regenerate within 7–10 days post-injury, accurately restoring their original size, shape, and position. Their optical transparency, and regenerative efficiency make scales uniquely suited for dissecting vertebrate regenerative mechanisms. Tissue regeneration involves precise spatial and temporal coordination of extracellular signals and intracellular gene regulation across substantial distances. Recent work uncovered traveling waves of ERK activity propagating through regenerating zebrafish scales. These waves appear crucial for organizing osteoblast proliferation, and differentiation, but the detailed relationship between ERK wave dynamics and osteoblast behavior remains unclear. To address this gap, we employed live quantitative imaging of ERK activity in mutants exhibiting altered scale morphology. Our analysis revealed correlations between wave parameters and scale size, and geometry. Further, we have developed genetic tools for altering cell numbers and optogenetic modulation of ERK signaling to elucidate mechanisms regulating osteoblast hypertrophy and scale morphogenesis. Transcriptomic analyses of regenerating osteoblasts uncovered genome-wide regulatory changes accompanying their transition from proliferative to hypertrophic states. Collectively, these findings highlight signaling waves as essential spatiotemporal regulators of osteoblast behavior during regeneration. Our integrative approach advances understanding of vertebrate regenerative biology, revealing fundamental cellular mechanisms that orchestrate complex tissue regeneration. Funding NIH R01-AR076342.

Poster 7

Joydip Barua, Aidan Kraan, Jake Gallagher, J. Andrew Jones, Mark Charlton-Perkins

Müller Glia Potassium Ion Buffering Prevents Retinal Inflammation and Protects Retinal Ganglion Cells

Miami University, USA

Retinal inflammation, driven by autoimmune disorders, infections, neuromyelitis optica, and optic nerve dystrophies, affects hundreds of millions worldwide. The development of the retina depends on Müller glia (MG). These radial-like glial cells arise early in retinal morphogenesis and persist into adulthood as essential support for other retinal neurons, including the retinal ganglion cells (RGCs) that make up the optic nerve. A central question is how these developmentally established glial cells modulate inflammation and preserve neural integrity under stress. We and others have shown that *Kcnj10a*, encoding an inwardly rectifying potassium channel, is highly expressed in MG from the earliest stages of retinal development, suggesting a critical role in establishing ionic homeostasis and neuron-glia interactions. Using a zebrafish model, with its transparent embryos and rapid retinal development, we employed a CRISPR-Cas9 strategy to knock down *Kcnj10a* in MG selectively. Knockdown impaired optomotor responses and altered electroretinogram b-waves, consistent with disrupted early visual circuit function. Histological analysis revealed reactive gliosis in MG, increased microglial invasion, and a significant loss of RGCs, while sparing other retinal neurons. Strikingly, administration of psilocybin during retinal development significantly reduced inflammation in *Kcnj10a*-deficient retinas, underscoring its potential as a modulator of neuroinflammatory processes. These findings highlight MG's developmental role in shaping both ionic buffering and inflammatory responses. By targeting glial support mechanisms established during development, we propose new therapeutic strategies for promoting RGC survival and mitigating neuroinflammatory disease. Funding Sources: Miami University, Advanced Research Team

Poster 8

Stacy Bendezu-Sayas[1,2], Carlos Charris-Dominguez[1,2], Erika Grajales-Esquivel[1,2], Jared A. Tangeman[1,2], Katia Del Rio-Tsonis[1,2]

Intercellular Signaling Orchestrates Gene Regulatory Networks in Transitional States of RPE Reprogramming

[1]Department of Biology, Miami University, Oxford, OH, USA; [2]Center for Visual Sciences at Miami University, Oxford, OH, USA

The retinal pigment epithelium (RPE) of the embryonic chicken exhibits remarkable plasticity and can reprogram into neural retina following injury and treatment with fibroblast growth factor 2 (FGF2). In this study, we performed an in-silico characterization of gene regulatory networks (GRNs) and chromatin dynamics driving RPE reprogramming. We analyzed retinectomized chicken (Gallus gallus) eyes at embryonic day 4 (E4; regenerative) and E5 (non-regenerative) at 6 and 24 hours post-retinectomy across both stages and treatment conditions. We built a chicken-specific single-cell multi-omics pipeline using Nextflow and Docker to ensure a reproducible workflow. We uncovered regulatory networks orchestrating these transitions, including transcription factors (TFs) from the YBX family, which have been associated with stress tolerance. FGF2 is known to activate ERK and AKT signaling in RPE. Notably,

in cancer cells YBX1 can inhibit downstream PI3K/AKT and MEK/ERK signaling, suggesting context-dependent regulation. Accordingly, YBX1 emerged as a key regulator, although its role in dedifferentiation remains unclear. Importantly, signals from SLIT, UNC5, and non-canonical WNT (ncWNT) pathways were enriched during early stages of FGF2 treatment, appearing to redirect cellular communication toward a neurogenic trajectory rather than maintenance of a non-reprogramming state. We observed a marked increase in these interactions at E4 within the first 24 hours post-treatment, suggesting a fine-tuned mechanism in which intercellular signaling converges on nuclear GRNs to engage chromatin modifiers and epigenetic machinery, reconfiguring the transcriptional landscape while preserving cell viability and plasticity. Funding: NEI R01 EY026816 and EY034980, the John W. Steube Endowed Professorship to KDRT, and NINDS F99 NS129167 to JAT.

Poster 9

Bayla Bessemer[1,2], Rolf Stottmann[1,3]

Hydroxysteroid 17-Beta Dehydrogenase 7 (*Hsd17b7*) is Required for Proper Mammalian Development

[1]Nationwide Children's Hospital, Steve and Cindy Rasmussen Institute for Genomic Medicine, USA; [2]The Ohio State University, Department of Veterinary Biosciences, USA; [3]The Ohio State University, Department of Pediatrics, USA

Disorders of cholesterol biosynthesis are genetic diseases that can have life-threatening impacts on early development. Clinical features can include intellectual disability and craniofacial developmental anomalies. Current therapeutic strategies have little impact on clinical progression. Hydroxysteroid 17-Beta Dehydrogenase 7 (*Hsd17b7*) is a gene which encodes for a post-squalene cholesterol biosynthesis enzyme. “Rudolph,” the hypomorphic allele of *Hsd17b7*, reflected striking defects to the central nervous system and skeletal development. The objective of this study is to investigate the consequences of *Hsd17b7* complete deletion on the central nervous system. Loss of *Hsd17b7* throughout the embryo with a $Sox2^{cre/wt}$ is lethal by embryonic day 9.5. To overcome this, we have utilized mouse Cre-LoxP systems to ablate *Hsd17b7* from the neural crest using *Wnt1cre2* and the forebrain with *Emx1cre*. Phenotypes in mutant mice are characterized through gross examination, histology, skeletal preps, and immunohistochemistry at different points in embryonic development and post-natal development. The $Wnt1^{cre/wt}$; $Hsd17b7^{flox/wt}$ mice reveals a striking cerebellar dysgenesis linked to changes in the midbrain-hindbrain boundary, hypoplasia of the midbrain, and severe hydrocephaly linked to malformation of the subcomissural organ.The $Emx1^{cre/wt}$; $Hsd17b7^{flox/flox}$ mice have a severely hypoplastic forebrain. Based on the noted phenotypes, we suspect that loss of *Hsd17b7* in the *Wnt1cre2* mice leads to early apoptosis of the *Wnt1* lineage, directly compromising development of the cerebellum. Loss of *Hsd17b7* in the *Emx1cre* mice is anticipated to hinder smoothened function through decreasing access of cholesterol, thereby inhibiting proper hedgehog signaling. Funding for this work is from the Abigail Wexner Research Institute at Nationwide Children’s Hospital and R01NS141189(R.W.S.). B.B. was funded by TOSU Graduate School Dean’s Distinguished University Fellowship.

Poster 10

Yoshitha Bhargav, Jonathan Hildebrand, Nelchi Prashali, Joyce Fernandes

Formation of the terminal nerve trunk in Drosophila: reorganization of axons and glia

Miami University , United States of America

During metamorphosis in Drosophila, five pairs of posterior abdominal nerves fuse to form a single terminal nerve trunk which is accompanied by reorganization of four ensheathing layers within each nerve (Subramanian et al 2017). This reorganization of peripheral nerves is an example of significant restructuring of the nervous system that occurs during the four-day transition period, and is associated with the shift in locomotor control. The TNT is first evident at 36 hours (approximately 1.5 days into metamorphosis). Electron micrographs of cross sections of unfused nerves (larva, 24 hours) and the TNT (48 hours and Adult) were examined to follow the organization of axons and the four ensheathing layers-wrapping glia, sub perineurial glia, perineurial glia and the neural lamella. At 24 & 48 hours the neural lamella is absent, wrapping glia have retracted from axon bundles, while the perineurial and subperineurial layers are mostly intact. In the adult stage, all glial layers and the neural lamella are present. Counts of axon profiles show that their number of axons decreases from larval into the 24-hour stage (Larva: 83.64?2.73, 24h: 44.2?2.25), a difference that is statistically significant. The reduction is consistent with death of neurons that has been reported in the literature (reviewed in Truman and Riddiford, 2024). There is no significant difference in the number of axon profiles at 48 hours ((39.78?3.54), and by the adult stage there is a significant increase (60.78?2.75), reflecting the presence of adult specific neurons. These studies have set the stage for further analysis of axon-glia interactions that shape neural plasticity during metamorphosis using peripheral nerves as a model.

Poster 11

Md Eleus Hussain Bhuiya, Jeremy Lynch

Molecular Remodeling of the Oosome During Pole Cell Formation in *Nasonia vitripennis*

Department of Biological Sciences, University of Illinois Chicago, USA

Germ cell specification in the parasitoid wasp *Nasonia vitripennis* relies on a uniquely large, RNA protein-rich germ plasm known as the oosome. Contrasting the polar granules of *Drosophila melanogaster*, the oosome is composed of a mesh-like RNA network, a distinctive Nv-Tudor (Nv-Tud) protein shell, and almost complete exclusion of mitochondria. Despite its unusual architecture, the molecular changes accompanying oosome fragmentation and pole cell formation remain poorly defined. We used multiplexed HCR RNA-FISH combined with super-resolution SRRF imaging to analyze oosome organization in both control and RNAi-treated embryos. Knockdown of Nv-*oskar* (Nv-*osk*), Nv-*vasa* (Nv-*vas*), or Nv-*aubergine* (Nv-*aub*) transformed the normal mesh-like oosome into small punctate granules, indicating that each of these factors is essential for maintaining oosome integrity. Nv-*tud* knockdown reduced oosome size and significantly impaired pole cell formation, suggesting that Nv-Tud contributes both to oosome stability and germ cell development. Comparative analyses of before (0–3 h) and after (3–6 h) pole cell formation revealed distinct spatiotemporal localization patterns of different transcripts and proteins. In pole cells, Nv-Osk decreased remarkably, Nv-Tud dispersed from its shell-like arrangement, Nv-Vas shifted to a shell-like distribution, and Nv-Aub formed larger granules with higher

intensity. Moreover, Nv-*osk* and Nv-*coronin* transcripts decreased remarkably, and Nv-*rrm* moved outward after the formation of pole cells. In contrast, Nv-*bark* RNA maintained a stable mesh structure across both stages. These results provide the first high-resolution evidence of coordinated transcript–protein remodeling during oosome-to-pole-cell transition in *N. vitripennis*.

Poster 12

Jeyoon Bok[1], Yung Su Kim[1], Fangyi Cheng[1], Chongjian Gao[1], Zhuowei Zhou[1], Norio Kobayashi[1], Aoife Tang[2], Xufeng Xue[1,3], Pulin Li[4], Jianping Fu[1]

A controllable human spinal cord model with full dorsoventral patterning

[1]University of Michigan, USA; [2]Greenhills School, USA; [3]Cincinnati Children's Hospital Medical Center, USA; [4]Whitehead Institute for Biomedical Research, USA

Morphogen gradients orchestrate the dorsoventral (DV) patterning of the spinal cord (SC), giving rise to specified neural progenitor domains, the dorsal roof plate (RP), the ventral floor plate (FP), and delaminating neural crest cells (NCCs). While foundational insights have emerged from animal models, our comprehension of processes such as retinoic acid (RA) signaling, the dynamics of NCC migration, and human-specific developmental characteristics is still incomplete due to the constraints of existing systems. To address this, we developed a human pluripotent stem cell (hPSC)-based model of the SC, referred to as microfluidic SC-like structures (μSCLSs). We generate these structures by applying engineered, antiparallel morphogen gradients across micropatterned hPSC colonies using a microfluidic platform. Our μSCLS model achieves a robust recapitulation of complete DV organization, including all 11 progenitor domains, the RP, and the FP, and its transcriptional signatures align with those of the embryonic human SC. This platform enabled us to uncover a novel RA-BMP signaling crosstalk that clarifies the role of RA during SC DV patterning, which had remained ambiguous. Furthermore, we demonstrate that NCCs undergo lineage-specific ventral migration in response to chemoattractants, which allows for direct visualization and mechanistic dissection of their sensory versus sympathetic fate trajectories. As a controllable, reproducible, and human- relevant system, this model provides a powerful tool for probing human SC development, neural crest biology, and disease modeling with unprecedented detail.

Poster 13

Benjamin Callaway[1], Caiden Duskey[1], Diego Pareja[1], Isabella Klapwijk[1], Aiden Krann[1], Kwangmin Choi[2], Lisa Martin[2], Katherine Chaney[2], Carlos Prada[3], Nancy Ratner[2], Mark Charlton-Perkins[1]

CRISPR-based Zebrafish Model that Provides Novel Insights into Putative Modifier Genes of Neurofibromatosis Type-1

[1]Miami University of Ohio, USA; [2]Cincinnati Children's Hospital, United States; [3]Lurie Children's Hospital, United States

Neurofibromatosis type 1 (NF1) is a common pediatric genetic disorders, causing tumors of the peripheral (PNS) and central (CNS) nervous system. Optic pathway gliomas (OPGs) are particularly concerning because they emerge early in childhood and can impair vision. Mouse models have clarified

aspects of peripheral tumors like plexiform neurofibromas (PNFs), but they fall short in modelling the CNS features of NF1. Importantly, twin studies suggest that the diversity of disease severity is regulated in part by modifier genes. The rapid development, transparency, and genetic accessibility of zebrafish provide an ideal system to study tumor initiation and how modifiers influence disease expression. We generated a novel zebrafish NF1 model to explore the developmental origins of PNFs and OPGs and to test how genetic modifiers influence tumor onset and severity. NF1 knockdown animals displayed hallmark symptoms, including reduced body size, eye proptosis, and skin hyperpigmentation. Abnormalities were evident in both systems: Schwann cell hyperplasia and disrupted barbel growth marked PNS involvement, while thickened optic nerves and OPGs were observed in the CNS in up to 20% of animals. This system also enables modifier screening. Introducing a heterozygous mutation in APC, a candidate modifier identified from exome sequencing of 109 NF1 patients, drastically increased the OPG penetrance. While only ~20% of NF1 animals developed OPGs, over 90% did so in the NF1; APC double-mutant background. Moreover, some of these tumors showed features consistent with higher-grade gliomas, including elevated proliferative indices. Our zebrafish model provides a tractable platform for studying pediatric NF1 tumor emergence, the interplay of developmental programs and genetic background, and for identifying strategies to prevent or delay pediatric tumor progression. Project funding sourced from Miami University of Ohio and NIH NINVS 1R01NS133200-01A1.

Poster 14

Colby Cassidy[1,2], Tushar H. Ganjawala[2], Prativa Amom[2], Breana D. Anderson[1,2], Radmehr Molaei[1,2], Jorin T. Hanson[1,2], Amanda L. Zacharias[2,3]

Roles for fate specifying transcription factors in collective cell migrations and fate transformations in *C. elegans* embryogenesis

[1]University of Cincinnati, USA; [2]Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center , USA; [3]University of Cincinnati College of Medicine, USA

In vertebrates, cell fate specification is directly linked with collective cell migrations. In *C. elegans*, the role for lineage determining factors is established in gastrulation, but the role for fate specifying transcription factors in gastrulation and other collective cell movements including ventral cleft closure remained unknown until recently. We previously evaluated the roles of the transcription factors (TFs) that specify neuronal, muscle, skin, and pharyngeal fates to determine their roles in collective cell movements. We found that the skin TFs *elt-1* and *nhr-25* are required to promote mesoderm gastrulation, pharynx organization, and ventral cleft closure, while both muscle fate TFs *hlh-1* and *unc-120* also promote ventral cleft closure. To determine whether fate transformations played a role in disrupting these collective cell movements, we examined the expression of various cell fate markers in mutant embryos. In particular, we wanted to test the hypothesis that cells would express the fate markers of "sister" lineages, since their shared history suggests that they might have the factors necessary to activate these genes. With the exception of the well studied intestinal lineage transformation, we observed no wide-scale expansions of fate, as indicated by transcription factor expression, suggesting that additional mechanisms besides mutual antagonism (as has been shown in the C lineage) exist to prevent cells from adopting inappropriate fates. Such mechanisms may involve

chromatin reorganization, as other reports indicate increases in closed chromatin regions at roughly the same time cell fate transcription factors begin to be expressed. Our findings are also consistent with the hypothesis that the cell fate transcriptions are regulating cellular migration. Funding provided by the UC Clermont Cronin Career Scholar Fund to CC and NIH HD117015 and a Cincinnati Children's Trustee Award to ALZ.

Poster 15

Xuyao Chang[1], Wenqi Li[2], Satoshi Matsui[3], Cindy Huynh[1], Craig Erickson[1], Feng Guo[4], Gustav Cederquist[5], Lorenz Studer[5], Makiko Iwafuchi[1], Amelle Shillington[1], Constantinos Chronis[6], Jason Tchieu[1]

ZMYND11 Functions in Bimodal Regulation of Latent Genes and Brain-like Splicing to Safeguard Corticogenesis

[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Texas Southwestern Medical Center, USA; [3]Osaka University, Japan; [4]Indiana University Bloomington, USA; [5]Memorial Sloan Kettering Cancer Center, USA; [6]University of Illinois College of Medicine, USA

Despite the litany of pathogenic variants linked to neurodevelopmental disorders (NDD) including autism (ASD) and intellectual disability, our understanding of the underlying mechanisms caused by risk genes remain unclear. Here, we leveraged a human pluripotent stem cell model to uncover the neurodevelopmental consequences of mutations in ZMYND11, a newly implicated risk gene. ZMYND11, known for its tumor suppressor function, encodes a histone-reader that recognizes sites of transcriptional elongation and acts as a co-repressor. Our findings reveal that ZMYND11-deficient cortical neural stem cells showed upregulation of latent developmental pathways, impairing progenitor and neuron production. In addition to its role on histones, ZMYND11 controls a brain-specific isoform switch involving the splicing regulator RBFOX2. Extending our findings to other chromatin-related ASD risk factors revealed similar developmental pathway activation and splicing dysregulation, partially rescuable through ZMYND11's regulatory functions. Funding resources: Cincinnati Children's Research Foundation (CCRF) [Tchieu], U.S. Department of Defense (United States Department of Defense) - W81XWH-22-1-0533 [Tchieu], Starr Foundation - Tri-SCI-2023-013 [Studer], Memorial Sloan-Kettering Cancer Center (MSKCC) - P30CA008748 [Studer], U.S. Department of Health & Human Services | National Institutes of Health (NIH) - R01GM143161 [Iwafuchi]

Poster 16

Electra Coffman, Timothy Plageman Jr.

Arvcf stabilizes adherens junctions and Ankyrin-B protein within the lens fiber cell membrane

The Ohio State University, USA

The ocular lens is an avascular transparent tissue responsible for focusing light onto the retina to produce a clear image. Uniquely, it grows throughout an organism’s entire lifespan without cell turnover. Structural disruptions in these long-lived lens cells can lead to cataracts, a disease marked by lens opacification. Although age is the highest global risk factor for cataract development, how cell architectural changes contribute to its progression remains poorly characterized. We previously

established that mice lacking *Arvcf*, a gene encoding a cell adhesion protein, develop disrupted adherens junctions (AJs) as soon as 1 month with premature cortical cataracts by 5 months of age. To identify important functional components of lens cell AJ's, protein lysates from control and mutant lenses were analyzed by fractionation, immunoprecipitation, western blot, and/ or mass spectroscopy. Loss of *Arvcf* induces a dramatic decrease in N-Cadherin as well as its catenin binding partners. It was also observed that N-cadherin associates well with both Arvcf and Ankyrin-B (AnkB) and that Arvcf strongly associates with the AnkB binding partner NrCAM. *In vivo* immunofluorescence further demonstrates that Arvcf facilitates the recruitment of AnkB to the short side lens fiber cell junctions by at least one month. As AnkB knock-out mutants ($AnkB^{-/-}$) also possess cell adhesive disruptions, we hypothesized that Arvcf and AnkB function in a similar pathway to maintain lens fiber cell N-Cadherin and lens transparency over age. It was observed that AnkB is required for maintaining Arvcf in the broad side lens fiber cell junctions as soon as E17.5. These data suggest that Arvcf may function in part to facilitate the association of Ankyrin-B to the cadherin-catenin complex and vice versa. These findings further suggest a shared role for Arvcf and AnkB in stabilizing lens fiber AJs which may provide molecular clues into cataract development.
Funded by NIH R01EY033815.

Poster 17

Parna Dutta[1,2], Kyle McCracken[1,2]

Investigating the mechanisms of pharyngeal foregut pattern based on in vivo developmental trajectories

[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati, USA

The pharyngeal endoderm forms in the anterior foregut and contributes to multiple organs including thyroid, thymus, parathyroid gland, and ultimobranchial body. Despite the clinical importance of these structures, comparatively little progress has been made in the directed differentiation of pharyngeal derivatives, which likely reflects our limited understanding of patterning events in the anterior-most region of the endoderm. To address these knowledge gaps, we first sought to define the molecular compartmentalization within the Sox2-expressing foregut. Analyses using scRNA-seq and wholemount IF staining revealed a complementary expression pattern of Otx2 and Gbx2 that segregated anterior and posterior domains of the E8.5 mouse pharyngeal foregut, respectively. Otx2 was expressed in the anterior tip of the foregut pocket that likely represented the region forming the first pharyngeal pouches and the thyroid. Gbx2 was expressed more posteriorly in the region of the second through fourth pouches. Interestingly, this pattern mirrored the known forebrain-hindbrain domains of these factors that is established in the neuroectoderm during gastrulation. Using multiple established protocols, we found that endoderm differentiated in vitro invariably expressed high levels of OTX2 in nearly all (>95%) cells. Conversely, neither nascent endoderm nor subsequent foregut stages contained any cells expressing GBX2. These data indicated that hPSC-derived endoderm is biased to adopt an anterior identity. Ongoing studies include genetic lineage tracing to delineate the fate of the early Gbx2-positive endoderm, as well as genetic perturbation experiments to investigate the functional roles of Otx2 and Gbx2 in endoderm patterning. Ultimately, we anticipate these novel insights into

endoderm and foregut patterning will inform directed differentiation strategies for pharyngeal foregut derivatives. CCHMC

Poster 18

Sylvie Earlewine[1], Grace Leonard [1], Benjamin Akande[1], John Postlewait[2], Saulius Sumanas[3], Jennifer Schumacher[1]

Insights into cardiopharyngeal development from a novel zebrafish mutant.

[1]Miami University , USA; [2]University of Oregon, USA; [3]University of South Florida , USA

Vertebrate heart formation depends on coordinated signaling among the first heart field, the second heart field (SHF), and neural crest cells (NCC). The cardiac outflow tract (OFT) and the adjacent bulboventricular (BV) valve are formed by both SHF and NCC lineages, and malformations in these structures account for nearly 30% of congenital heart defects (CHD). The cellular and molecular regulators of OFT development remain poorly understood. Here, we report a novel zebrafish mutant, *pendulum (pen)*, that provides a new model to study OFT development. In *pen* mutants, we observe an increased endocardial cell population and reduced smooth muscle cells in the OFT, as well as defective gene expression in the BV valve. These abnormalities were accompanied by extensive pericardial edema and reduced heart rate, consistent with impaired cardiovascular function. Additionally, the *pen* mutants display retrognathia at larval stages. To further characterize this phenotype, we performed two-color acid-free cartilage and bone staining, which revealed extensive craniofacial cartilage abnormalities that are consistent with SHF-NCC defects. Taken together, the *pen* phenotype suggests a defect in SHF-NCC proliferation and differentiation. To mechanistically understand how cell interactions may be affected in *pen* mutants, we performed transcriptomic profiling via bulk RNA-seq on 72 hpf pen embryos. This revealed hyperactivation of TGF-β signaling, a hallmark of Loey-Dietz Syndrome–a disorder characterized by OFT abnormalities and craniofacial dysmorphology. Our results highlight *pen* as a valuable model for dissecting the cellular and molecular basis of CHD. Ongoing studies aim to discover precisely how SHF-NCC interactions may be affected in *pen* mutants. This work is supported by Miami University Committee for Faculty Research.

Poster 19

Anna Evans, Noah Pison, Nathaniel Wells

A new tool in the box: Establishment of Xenopus embryonic stem cell

University of Wisconsin-Milwaukee, USA

Xenopus laevis is an efficient model system used for decades to answer fundamental questions in cell and developmental biology in both physiological and pathological contexts. While a powerful in vivo model, it offers a limited in vitro toolbox. We aim to establish a Xenopus laevis embryonic stem cell line and expand the versatility of this model. Animal caps (AC) are fated to become the ectoderm during normal development. However, ACs are competent to respond to inducing molecules and form derivatives from all three germ layers. In this regard, AC can be compared to mammalian ESCs. However, AC cannot be maintained in a pluripotent state over time. We therefore propose to develop a culture

medium that would allow the maintenance of pluripotency factors as well as increase cell self-renewal to obtain true ESCs. AC cells were isolated and cultured in mTesR or N2B27 base medium complemented with combinations of factors (CHIR/XRV, FGF2, and activin). After 5 days of culture, we performed qPCR and RNA-seq followed by GO term analysis. Preliminary results concluded that animal cap cells cultured in CHIR/XRV gave no lineage-specific terms and showed maintenance/upregulation compared to controls of pluripotent genes klf4, c-myc, lin28, sox2 and vent2 (homolog of human Nanog). In addition, the cells can be kept in culture longer than the controls. While the results are promising, the media composition needed fine-tuning. We therefore started to test a combination of Leukemia inhibitory factor (LIF) with BMP4. Our preliminary results showed that not only the previous pluripotent genes are maintained but the pluripotency factors Pou5f3 (homolog of human Oct4) are overexpressed compared to CHIR/XRV condition. All together our results showed that our culture media allowed a better survival of AC cells and the maintenance of pluripotent genes expression over time. Funding: NIH, R21OD033669 FOA PAR-21-167: Development of Animal Models and Related Biological Materials for Research.

Poster 20

Edward Farrow[1,2], Smitha Rao[3], Simon J. Y. Han[1,2], Xuyao Chang[1], Cindy Hyunh[3], Samantha A. Brugmann[3,4,5,6], Hee-Woong Lim[4,7], Jason Tchieu[3,4,5], Kenneth Campbell[3,4,5], Brian Gebelein[3,4,5]

Establishment of a doxycycline-inducible system to study the regulatory functions of the homeodomain transcription factor GSX2 in human lateral ganglionic eminence (LGE)-like progenitors *in vitro*

[1]Cincinnati Children's Hospital Research Foundation, Graduate Program in Development, Stem Cells and Regenerative Medicine, Cincinnati, OH, United States; [2]University of Cincinnati College of Medicine, Medical-Scientist Training Program, Cincinnati, OH, United States; [3]Cincinnati Children's Hospital Medical Center, Division of Developmental Biology, Cincinnati, OH, United States; [4]University of Cincinnati College of Medicine, Department of Pediatrics, Cincinnati, OH, United States; [5]Cincinnati Children's Hospital Medical Center, Center for Stem Cell & Organoid Medicine, Cincinnati, OH, United States; [6]Cincinnati Children's Hospital Medical Center, Division of Plastic Surgery, Cincinnati, OH, United States; [7]Cincinnati Children's Hospital Medical Center, Division of Biomedical Informatics, Cincinnati, OH, United States

Transgenic mouse models have demonstrated the critical role of the homeodomain transcription factor Genetic-Screened Homeobox 2 (Gsx2) in the developing basal ganglia. However, the technical limitations of the existing *in vivo* approaches, such as progenitor heterogeneity and lack of temporal resolution, have impeded the investigation of direct regulatory targets. This study engineered a Dox-inducible human embryonic stem cell (hESC) line to investigate the function of GSX2 protein in directed differentiation cultures that model developing lateral ganglionic eminence-like (LGE-like) progenitors. Transcriptomic, chromatin accessibility, and genomic binding studies revealed that GSX2: (1) binds to both high- and low-accessibility chromatin using varying binding site preferences; (2) alters chromatin accessibility largely through indirect mechanisms; (3) functions primarily as a transcriptional repressor; and (4) regulates key conserved target genes of both progenitor maturation and regional specification. These results provide insight into the key regulatory roles and targets of GSX2, thereby establishing a new tractable experimental system to investigate basal ganglia development. This work was supported

by the National Institute of Neurological Disorders and Stroke (R01NS124660 to K.C. and B.G.) and a Center for Stem Cell and Organoid Medicine (CuSTOM) grant from CCHMC to K.C. and B.G.

Poster 21

Andrew Fernandes

Early retinoic acid signaling is necessary to produce a population of Wnt signaling cardiomyocytes that drive atrioventricular valve development.

Cincinnati Children's Medical Center, USA

Perturbations of retinoic acid (RA) signaling have been associated with congenital valve defects (CVDs). Although the earliest role of RA signaling is to restrict cardiomyocyte specification, the requirements for early RA signaling in valve development are poorly understood. Here, we investigated a requirement of early RA signaling on the development of the zebrafish atrioventricular valve (AVV), the mammalian mitral valve equivalent. Inhibition of RA production beginning at 3 hours post-fertilization (hpf) and zebrafish *aldh1a2* mutants indicated that RA-deficient embryos lack AVVs at 72 hpf, despite having enlarged hearts with intact endocardial lining. Examination of overlapping atrioventricular canal (AVC) cardiomyocyte markers; and *bmp4* and *tbx2b*, showed that the myocardial AVC was expanded in RA-deficient embryos. Despite this expansion, endocardial valve markers, including *klf2a* and *notch1b*, were lost in RA-deficient embryos, implying that early RA signaling loss affects a factor(s) that signals from the AVC myocardium to the endocardium to promote AVV induction. Canonical Wnt signaling within AVC cardiomyocytes promotes proper AVV development. In contrast to other AVC cardiomyocyte markers, we found that RA-deficient zebrafish embryos lack myocardial *wnt2bb* expression. Consistent with the loss of *wnt2bb* expression, we found that RA-deficient embryos lack Wnt reporter expression within AVC cardiomyocytes by 36 hpf. Furthermore, inhibition of canonical Wnt signaling treatment from 32-40 hpf was sufficient to reduce valve size and endocardial cushion cell number. CRISPR-mediated knockdown of *wnt2bb* recapitulated the loss of Wnt reporter expression in AVC cardiomyocytes and the reduction of cushion cells by 72 hpf. Altogether, our results suggest that early RA signaling is necessary to establish a population of *wnt2bb*+ AVC cardiomyocytes that promote appropriate AVV development, which may provide insights into mechanisms underlying vertebrate CVDs. F31HL172346-02

Poster 22

Carlos Ferran-Heredia

Identifying and Modeling the Origins of the Metanephric Mesenchyme

Cincinnati Children's / University of Cincinnati, USA

The mammalian kidney derives from the metanephric mesenchyme (MM) which arises from the posterior-most region of the intermediate mesoderm (IM) and contributes to the stromal and nephron progenitors of the kidney. The MM is also characterized by its abilities to self-renew and drive complex morphogenetic events through interactions with the ureteric bud. Although putative MM-like cells have been differentiated from human pluripotent stem cells (hPSCs) to generate nephron-containing kidney organoids, they do not exhibit key properties of the MM. We hypothesize that these in vitro protocols do

not recapitulate the early steps of MM formation in vivo, which are currently undefined. To address this critical knowledge gap, we sought to precisely delineate the differentiation pathway that generates the MM in the embryo. We used in vivo genetic lineage tracing models and wholemount IF staining to trace gastrulation-stage axial progenitors through embryonic development and to characterize MM formation. Our model demonstrates that the nephron and stromal progenitor pools of the MM derive from neuro-mesodermal competent axial progenitors in the caudal lateral epiblast. These axial progenitors commit to a mesodermal fate by ~E8.5 to form the early tailbud mesoderm and subsequently the MM. In contrast to the conventional view of early mesoderm formation in the gastrula, the labelled axial progenitors exhibited potential to form neural but not endodermal derivatives. Likewise, testing of published nephron progenitor cell protocols suggests that these putative MM-like cells do not differentiate through a neuro-mesodermal competent state. Collectively, these data support that published protocols do not mimic the steps of MM formation in vivo and highlight the need to redefine how MM-like tissue is formed in vitro. This work is funded by CCHMC.

Poster 23

Bassit Fijabi[1], Seth Teague[1], Emily Freeburne[1,2], Hina Khan[1], Craig Johnson[1], David Brückner[3], Idse Heemskerk[1]

Combinatorial Cell Signals Encode Cell Fate and Positional Information in 2D Human Gastruloids

[1]University of Michigan, United States; [2]Bio-Techne, United States; [3]University of Basel, Switzerland

Understanding how cell signaling orchestrates precise cell fate decisions and tissue patterning represents a fundamental challenge in developmental biology that has been compounded by the inability to effectively address combinatorial signaling effects. Here, we present a framework integrating iterative immunofluorescence, information theory, and machine learning to relate combinatorial cell signaling to cell fate in human 2D gastruloids. By simultaneously measuring spatially-resolved activities of six cell signaling pathways and expression of seventeen fate marker genes at the single-cell level, we construct a comprehensive "signal-to-fate" map that accurately predicts fate marker gene expression patterns - including after changes in exogenous BMP4 concentration. We additionally predict phenotypical changes in response to pharmacological perturbation of FGF/ERK and Hippo/YAP signaling. Our analysis reveals that cell fate is encoded by the collective state of multiple signaling pathways. We show that spatial patterning precision is limited primarily by intrinsic signaling noise, and that the distribution of positional information among pathways is dynamically reallocated to maintain robust patterning under diverse conditions. Finally, we leverage the knowledge that only certain combinatorial signaling patterns are biologically permissible to improve quantitative predictions of fate marker gene expression. These findings establish a generalizable quantitative framework for relating cell signaling to fate and positional information in developmental systems. This work was funded by the National Institute of General Medical Sciences (NIGMS R35GM138346) and National Science Foundation RECODE (2033654). BF was supported by the University of Michigan Postbaccalaureate Research Education Program (NIH R25 GM086262) EF was partially supported by the National Institutes of Health Cellular Biotechnology Training Program (T32GM145304).

Poster 24

Nicole Franks[1], Hannah Schrader Dear[1], Ella Markley[1], Alexander Holtz[2], Jane Song[3], Craig Johnson[1], Paola Medina-Cabrera[1], Daniela Hernandez[1], Praise Joel[1], Marina Pasca di Magliano[1,4,5], Deneen Wellick[6], Benjamin Allen[1]

***Gli^{FHV}* mice: a novel tool to investigate GLI processing and localization.**

[1]Department of Cell & Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA; [2]Center for Regenerative Medicine, Boston Medical Center, Boston, MA 02118, USA; [3]Genentech, South San Francisco, CA 94080, USA; [4]Department of Surgery, University of Michigan, Ann Arbor, MI 48109, USA; [5]Rogel Cancer Center, University of Michigan, Ann Arbor, MI 48109, USA; [6]Department of Cell and Regenerative Biology, University of Wisconsin, Madison, WI 53705, USA

GLI proteins (GLI1-3) are the transcriptional effectors of mammalian Hedgehog (HH) signaling. However, studies of GLI function have been hampered by the lack of robust GLI antibodies. To address this, we utilized CRISPR-based gene editing to generate endogenous epitope-tagged *Gli* alleles for each *Gli* gene (*Gli1^{FLAG}, Gli2^{HA}, Gli3^{V5}*). Through breeding, we established a novel mouse model, *Gli1$^{FLAG/FLAG}$;Gli2$^{HA/HA}$;Gli3$^{V5/V5}$*, referred to as *Gli^{FHV}*. Importantly, *Gli^{FHV}* animals are viable and fertile with no overt phenotypes. Sanger and long-read sequencing confirmed proper editing of each *Gli* allele, while qPCR and western blot analysis confirmed similar gene expression and protein levels, respectively, between wildtype and *Gli^{FHV}* animals. We utilized these mice to assess GLI localization in the developing limb, finding that all three GLIs localize to primary cilia with distinct distributions. Further, we generated immortalized *Gli^{FHV}* mouse embryonic fibroblasts (MEFs), demonstrating that these cells are HH-responsive and that GLIs localize to primary cilia and nuclei in a HH-dependent fashion. Using these MEFs, CUT&RUN qPCR analysis confirmed GLI2 enrichment at known *Gli1* and *Ptch1* enhancer regions. These animals and cell lines provide a valuable resource for analyses of GLI processing, localization and function throughout embryogenesis, postnatal development, and in adults.

Poster 25

Jacob Gafranek

Investigation of the response to cryoinjury in the zebrafish atrium reveals abbreviated regenerative dynamics

Cincinnati Children's Hospital Medical Center, USA

Myocardial infarction (MI), which occurs following cessation of blood flow to the heart muscle and is most often caused by advanced coronary artery disease, remains the leading cause of death in the United States. Adult mammals cannot recover necrotic tissue resultant from MI, and instead form a dense fibrotic scar. By contrast, zebrafish and neonatal mammals have shown remarkable regenerative capabilities following cardiac injury. However, any potential chamber-specific regenerative mechanisms remain to be explored. Here, we endeavor to elucidate the regeneration dynamics exhibited within the atrial chamber and compare them to what has been shown in the ventricle. Initial histological and RNA-sequencing analyses both showed that atrial cardiomyocytes undergo dedifferentiation similarly to the ventricle, evidenced by the upregulation of cardiac progenitor markers, and proliferative markers around the insult, in addition to deposition of collagen and fibrin within the injury by 3 days post injury(dpi).

However, we find that following cryoinjury, the atria exhibit remarkably rapid recovery with almost complete scar resolution by ~14dpi, in contrast to the ventricles which take ~60 days and this faster scar resolution was mirrored by accelerated collagen cycling within the atrium. Furthermore, transplant and *ex vivo* culture experiments suggest that a TGF-b signaling-mediated increased ability of atrial epicardial-derived cells to migrate into the wound correlates with and may contribute to the accelerated atrial regeneration. Altogether, our data demonstrate that, while zebrafish atria employ aspects of the canonical ventricular regenerative roadmap, autonomous characteristics support a comparatively accelerated ability to regenerate. We do not yet understand the comprehensive mechanism by which the atrium achieves abbreviated recovery, but our findings implicate robust epicardial activation in driving this remarkable feat. F31HL147399, MCB Divisional Training Grant

Poster 26

Prashant Gaikwad[1], Thomas Rülicke[2], Simon Hippenmeyer[3], Matthew Kofron[1,4], Cristina Cebrian[1,4]

A streamlined method for optical clearing of non-perfused mouse tissues for light sheet microscopy imaging.

[1]Cincinnati Children's Hospital Medical Center, United States of America; [2]University of Veterinary Medicine Vienna, Austria; [3]Institute of Science and Technology Austria, Austria; [4]University of Cincinnati College of Medicine, United States of America

Tissue clearing is essential for high-resolution, three-dimensional imaging of intact biological structures. However, many existing protocols require whole body perfusion, extended processing times and the use of harsh or toxic chemicals that compromise tissue integrity and/or endogenous fluorescence. Here, we present a simplified clearing method that uses non-toxic reagents to achieve efficient optical transparency across a range of neonatal, young adult and older mouse organs, including kidney, adipose tissue, heart, lung, liver and brain, within 4 to 7 days. We have used the MADM (Mosaic Analysis with Double Markers) mouse model with recombination driven by Hprt-cre to generate discreet groups of cells clonally labeled with eGFP, TdTomato or both across different organs. We can successfully clear a panel of tissues from non-perfused mice as well as kidneys that have been stored at 4C post-fixation. This protocol preserves endogenous fluorescence as shown by light sheet microscopy of whole cleared kidneys. It is also compatible with intravascular delivery of fluorochrome-conjugated tracers such as Lycopersicon Esculentum (Tomato) lectin, enabling visualization of organ vasculature, or 10,000 MW dextran to visualize filtration and excretion by the nephrons. This streamlined approach offers a rapid, safe and effective alternative for 3D tissue imaging applications. This work is supported by NIDDK Award Number R01DK129238 to CC.

Poster 27

Thomas Gallagher, Monica Blatnik, Clare Austin, Kathryn Thompson, Danielle Pvirre, Angelina Morgan, Michael Kearse, Sharon Amacher

Pnrc2 promotes rapid mRNA decay and coordinately supports embryonic development with P-body factors Ddx6 and Ddx61

The Ohio State University, USA

Somitogenesis, the sequential segmentation of vertebrate embryonic mesoderm, is controlled by the segmentation clock, a molecular oscillator that controls periodic gene expression and is regulated by a negative feedback loop driven by Hes/Her transcriptional repressors. In zebrafish, Pnrc2 is required for decay of *her1* mRNA and additional oscillatory gene transcripts. Using RNA-Seq, we have identified >1700 overexpressed transcripts in *pnrc2* mutants during segmentation. Despite accumulation of numerous mRNAs including those encoding key developmental regulators, *pnrc2* mutants appear overtly normal, with some developmental delay. Our previous work suggested that accumulated mRNAs are not over-translated in *pnrc2* mutants, though the underlying mechanism(s) was unknown. We now show that accumulated mRNA of several oscillatory genes have shortened poly(A) tails, suggesting they are inefficiently translated. Polysome profiling shows that several oscillatory gene transcripts have normal levels of ribosome engagement in *pnrc2* mutants but accumulated mRNA is disengaged from ribosomes. Inhibiting deadenylation leads to somite defects in *pnrc2* mutants, which we hypothesize is due to over-translation of Pnrc2-regulated transcripts. Among overexpressed transcripts examined, transcripts encoding the P-body protein, Ddx61, are both overexpressed and engaged with ribosomes, leading to increased Ddx61 protein. Co-depletion of Ddx61 and its ohnolog Ddx6 enhances *her1* mRNA accumulation and later leads to strong morphological defects in *pnrc2* mutants. Ddx6/Ddx61 depletion in wild-type embryos has no effect on *her1* expression, though low rates of similar morphological defects are observed later. Together, our results show that Pnrc2 promotes decay of tail-shortened, ribosome-disengaged mRNAs and co-regulates *her1* mRNA decay with Ddx6 and Ddx61, two P-body factors that sustain normal development in decay-deficient *pnrc2* mutants. Funding: R01GM117964, R35GM146924, T32GM141955

Poster 28

Tushar Ganjawala[1], Julia Jeffrin[1], Amanda Zacharias[1,2]

Investigating effect of maternal hyperglycemia on embryo development by recording real-time metabolic response in *C. elegans* embryos using genetically encoded fluorescent biosensors identifies key roles for glycolysis in promoting specific cell migrations

[1]Divisions of Neonatology and Pulmonary Biology, Children's Hospital Medical Center, USA; [2]Department of Pediatrics, University of Cincinnati Medical School, USA

Infants born to mothers with pre-existing diabetes are at 2-4 fold greater risk of having congenital anomalies. Despite this, how maternal hyperglycemia changes cellular metabolism to impact embryonic development is not fully understood. The *C. elegans* embryo is a simple, accessible model system that enables us to characterize the metabolism of individual cells throughout embryonic development and determine how hyperglycemia impacts cell fate specification and morphogenesis. We generated

transgenic worms carrying a genetically-encoded ratiometric fluorescent sensor, HYlight, that measures glycolysis pathway activity. We used this reporter to identify embryonic cells with changes in glycolysis from their mother and/or sister cells during development. We also found that glycolysis increases non-uniformly in embryos of mothers exposed to high glucose levels. In parallel, we analyzed embryos homozygous for a loss of function mutation in the *C. elegans* 6-phosphofructokinase homolog, pfk-1.1, which renders cells unable to perform glycolysis. We found that cells divide more slowly, and specific cell migration events are disrupted, including the migration of the ABpl cell, which establishes the left-right axis, and ventral cleft closure. In contrast, gastrulation of cells fated to form the intestine, which happens between ABpl migration and ventral cleft closure, occurs normally. This indicates that different developmental events are programmed to use (or not use) glycolysis over other forms of energy generation, raising the question of the identity of these programming factors. *C. elegans* is a promising model system to uncover both how metabolism influences developmental events and how developmental factors control cellular metabolism. Funded by NIH R21HD117015 and a Trustee award from CCHMC to ALZ.

Poster 29

Karla Akari Garcia[1], Kelly Tseng[1], Irene Vorontsova[2]

Conserved Mechanism in Vision Development: Lens Nucleus Centralization in Xenopus Laevis

[1]University of Las Vegas, Nevada, United States; [2]University of Auckland, New Zealand

Developing eye optics, determined by the lens and cornea, must coordinate with the axial length of growing eyes to focus light onto the retina to form an image. It was found that zebrafish (Danio rerio) lens nuclei are initially anteriorly localized in optical axes in larvae, then centralize at older stages, resembling non-aquatic species. An anteriorly placed lens nucleus increases optical power, enabling a functional optical system in larvae, where eye axial length is short. To assess if alike mechanisms occur in other aquatic animals, we studied the clawed frog, Xenopus laevis, a fully aquatic species similarly relying on vision for survival at stages where eyes are small and lens-retina distance is short. We found the X. laevis tadpole lens nucleus also moved from an initial anterior location (st. 44) to centralization during the prometamorphosis period. Similarly, in eyes regenerated after st. 27 ablation, young tadpole lens nuclei are anteriorly localized then centralize before metamorphosis, recapitulating the same pattern as control developing eyes. Moreover, lens nuclei localization in optical axes in developing and regenerated X. laevis eyes show close correlations to axial eye length. To form functional optical systems, lens refractive properties must match eye axial length to focus light onto the retina. Close correlation of these two parameters suggests lens nuclei centralization is required for a functional optical system. Our findings suggest a conserved evolutionary mechanism for vision development in some aquatic species. Understanding key mechanisms regulating crosstalk between eye optics and eye axial length will aid in discovering therapies to prevent or delay formation of diseases resulting in a mismatch between these two properties, as occurs in short-sightedness, when an eye grows too long for its optics. This work is supported by SDB CD Fellowship (KG), NIH EY031587 (IV), and NIH GM146672 (KT).

Poster 30

Jade Ghanayem[1], J. Raul Perez-Estrada[1,2], Caroline Kennelly[1], Lehua Hoops[1], Katia Del Rio-Tsonis[1,2]

Unraveling the FGF Signaling Cascade in RPE Reprogramming

[1]Department of Biology, Miami University, Oxford, OH, US; [2]Center for Visual Sciences, Miami University, Oxford, OH, US

Retinal degeneration leads to irreversible vision loss, significantly impacting quality of life. Approximately 19.83 million Americans over 40 have age-related macular degeneration (AMD), a leading cause of vision loss and retinal damage. Unlike humans, embryonic chicks demonstrate a remarkable ability to regenerate their neural retina by activating neural progenitors present in the ciliary margin (CM) and through cell reprogramming of the retinal pigment epithelium (RPE). However, chicken retina regeneration only occurs at day 4 of development and in the presence of fibroblast growth factor 2 (FGF2). Previous work from our lab has shown that retina regeneration depends on the interaction of FGF2 with its receptor (FGFR). This activates CM neural progenitor proliferation by triggering Mitogen-Activated Protein Kinase (MAPK) signaling, allowing retina regeneration. A similar mechanism was proposed for retina regeneration via RPE reprogramming, however, our recent findings using MAPK signaling inhibitors suggest that even though FGFR activation is necessary and activates the MAPK pathway, the activation of the MAPK pathway is not required for RPE reprogramming, both, *in vivo* and *in vitro*. Ongoing experiments are geared to show if the PI3K/AKT pathway or Src-family kinases such as JNK and PKC are activated by FGF2-FGFR interactions and required for RPE reprogramming. Altogether, our studies aim to enhance our understanding of retinal regeneration mechanisms and provide insights into potential therapeutic strategies for human retinal degenerative diseases. Funding: R01EY034980 to KDRT. KTEF Career Starter Grant to JRPE.

Poster 31

Erika Grajales-Esquivel, Carlos M Charris Dominguez, Stacy Bendezu-Sayas, Jared A Tangeman, Raul Perez-Estrada, Katia Del Rio-Tsonis

Transcriptional and Chromatin Dynamics Governing Neurogenic Competence of Retinal Pigment Epithelium

Miami University, USA

Retinal pigment epithelium (RPE) cells can be reprogrammed into neural retina (NR) in several species. In embryonic chickens, RPE cells retain neurogenic competence until embryonic day (E) 4 in the presence of fibroblast growth factor 2 (FGF2), but lose this ability by E5. To investigate the mechanisms underlying this transition, we performed single-nucleus multimodal profiling of developing eyes from E3 to E7, as well as at E4 and E5 with or without FGF2 at 6 hours post-retinectomy. Our analyses revealed dynamic transcription factor changes in RPE across the E4–E5 window, coinciding with restriction of neurogenic competence. During regeneration, we captured early molecular signatures of dedifferentiation, with altered chromatin accessibility at RPE-determining genes including OTX2, MITF, and NFIA/B. Notably, NFIA and NFIB were elevated in fate-restricted RPE even in the presence of FGF2. Injury and FGF2 responsive genes such as PLXNA4, HGF, and KIFC1 were differentially expressed at E5, while pigmentation genes (TYR, TYRP1, DCT) were inefficiently silenced. In contrast, E4 + FGF2 highlighted

distinct gene expression profiles associated with neurocompetence. In silico perturbation analyses further implicated OTX2 and NFIs as key regulators of the RPE molecular program. Ongoing loss of function experiments, targeting these transcription factors, aim to promote RPE reprogramming in the absence of FGF2. Together, these findings provide a foundational understanding of the transcriptional and chromatin landscapes that govern RPE fate, offering insights into the intrinsic and extrinsic cues that regulate the loss of neurogenic potential. Funding source: R01 EY034980 to KDRT

Poster 32

isabella herider[1,2], Imani Joshi[1], Hannah Nartker[1], Juri Lim[1], Chunyue Yin[1,3,4]

Modeling cholestasis caused by LSR deficiency in Zebrafish, Danio *rerio*

[1]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, USA; [2]Xavier University, USA; [3]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA; [4]Center for Undiagnosed and Rare Liver Disease, Cincinnati Children's Hospital Medical Center, USA

Cholestasis is characterized by failure to drain bile from the liver. Excessive bile accumulation leads to hepatocellular injury, which can progress to fibrosis, inflammation, and liver failure. Deleterious variants in the *LSR* gene are linked to infantile intrahepatic cholestasis, but the underlying mechanism is unknown. *LSR* encodes the lipolysis-stimulated lipoprotein receptor that clears lipoproteins from the blood. It is also part of the tricellular tight junction complex. Global *Lsr* knockout mice are embryonically lethal. Liver-specific knockouts have not shown cholestasis. We generated *lsr* knockout zebrafish by CRISPR/Cas9 and found that they were prematurely lethal. While mutants were grossly normal at the larval stage, they had enlarged and more spherical livers. Mutant hepatocytes formed a rosette-like architecture rather than the cord arrangement seen in wild type. We administered fluorescent lipid analog BODIPY C5:0 to 6-day-old larvae. *lsr* mutants drained the dye to gallbladder, suggesting that they retained some hepatobiliary function. In contrast, mutant hepatocytes failed to excrete fluorescent bile acid derivative CGamF into bile ducts, directly demonstrating intrahepatic cholestasis. Immunofluorescence for bile salt export pump/BSEP showed shortened, dilated bile canaliculi in mutants, consistent with the bile excretion phenotype. We generated *Tg(fabp10a: lsr-gfp)* transgenic zebrafish to investigate *Lsr* expression in hepatocytes. *Lsr*-GFP fusion protein localized to hepatocyte membrane and tight junctions, similar to *LSR* localization in human hepatocytes. This data establishes *lsr* knockout zebrafish as a valid model of cholestasis caused by *LSR* deficiency. Further research will determine whether cholestasis is attributed to disruption of LSR function in tight junction or lipoprotein clearing. This work is supported by R01DK140321, R01DK117266, and The Peter and Tommy Colucci Research Award for PFIC.

Poster 33

Tyler Hoard, Daniela Hernandez, Katie Liu, Roman Giger, Benjamin Allen

Semaphorin Receptors Antagonize Wnt Signaling Through Beta-Catenin Degradation

University of Michigan, United State of America

Morphogens are essential for proper tissue formation and organ development during embryogenesis, directing a wide range of cellular responses, including cell fate specification, cell migration, proliferation, and survival. Notably, different morphogens do not act in a vacuum, but instead often act simultaneously, sometimes in cooperation, and other times in competition with each other. One outstanding question is: what are the mechanisms that regulate the integration of simultaneous signals from multiple different morphogens in a cell? Previous studies have shown that the Semaphorin (SEMA) receptors, Neuropilins (NRPs) and Plexins (PLXNs), promote Hedgehog (HH) pathway activity. Here, we present evidence that PLXNS and NRPs also act to restrict Wnt signaling. Specifically, we find that either Nrp or Plxn expression drives Wnt pathway inhibition in both fibroblasts and epithelial cells, while Nrp1/2 deletion in fibroblasts results in a significant increase in Wnt activity. Mechanistically, we find that PLXN/NRP-mediated HH pathway promotion is primary cilia-dependent, while PLXN/NRP-mediated Wnt pathway inhibition is primary cilia-independent. Further, PLXNs and NRPs act downstream of Dishevelled (DVL) to promote proteasome-dependent destabilization of β-catenin (CTNNB1). Notably, NRP-mediated CTNNB1 destabilization is GSK3/CK1-dependent, while PLXN-mediated CTNNB1 destabilization is GSK3/CK1-independent. Overall, this work defines NRPs and PLXNs as multifunctional regulators of at least two morphogen families– NRPs/PLXNs promote HH signaling, while restraining Wnt pathway activity. Future studies include investigating potential roles for NRPs/PLXNs in the regulation of other morphogens, as well as elucidating the in vivo contributions of NRPs and PLXNs to HH and Wnt signaling. Specifically, we are generating mice that lack all Plxna family members (i.e., Plxna1, Plxna2, Plxna3, Plxna4) to assess potential contributions to HH- and Wnt-dependent neurogenesis.

Poster 34

Yilun Huang, Zhaoming Wu, Paul Iyyanar, Yu Lan, Rulang Jiang

Identification and characterization of enhancer elements regulating *Alx1* expression and function during craniofacial development

Division of Developmental Biology, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, USA

ALX1, encoding a homeodomain-containing transcription factor, is highly specifically expressed in cranial neural crest cells (CNCCs) populating the embryonic frontonasal prominence. Disruption of *ALX1* causes frontonasal dysplasia syndrome-3 (FND3), characterized by severe microphthalmia, midfacial hypoplasia, and orofacial clefting. Both human and mouse studies have highlighted *ALX1* as a key regulator of frontonasal morphogenesis and a central node in the gene regulatory network controlling craniofacial development. However, the molecular mechanisms regulating *Alx1* expression during craniofacial development are largely unknown. In this study, we have identified a 4.5kb-long distal enhancer (*Alx1*-DE1) residing in a large intron of the neighboring *Lrriq1* gene through combinatorial analyses of the chromatin architecture and epigenomic profiles. *Alx1-DE1* consists of four evolutionarily conserved

subregions (ECRs 1-4). We show that *ECR1*, marked by strong H3k27ac and Twist1 transcription factor occupancy in mouse embryonic frontonasal tissues, exhibited strong enhancer activity *in vitro* and drove transgenic reporter gene expression specifically in mouse embryonic frontonasal tissues. We show that CRISPR/Cas9-mediated deletion of the *ECR1* region caused significant reduction in *Alx1* mRNA expression in the embryonic frontonasal mesenchyme but homozygous *ECR1*-deletion mice survive postnatally without obvious craniofacial abnormalities. We further generated mice lacking the entire *Alx1-DE1* enhancer and found that $Alx1^{?DE1/?DE1}$ mice partly recapitulated frontonasal developmental defects found in $Alx1^{del/del}$ mice, including nasal cartilage hypoplasia and extraocular muscle disorganization. These results identify *Alx1-DE1* as a crucial composite enhancer regulating endogenous *Alx1* expression and function during frontonasal development. This work was supported by NIH/NIDCR grant R01DE029417 to RJ.

Poster 35

Samantha Igaz

Measures of lifespan in fruit flies exposed to environmentally relevant concentrations of bisphenols: Implications for embryology and development Samantha Igaz*, Sophia Moberly, Halie Stemple, Katie Mullin, Alexanna Taylor and Gary M. Lange Department of Biology Saginaw Valley State University

Saginaw Valley State University, USA

The fruit fly, Drosophila melanogaster, is a widely used model organism in developmental biology. With an average lifespan of ~50 days under standard conditions, longevity studies can be a beneficial tool for examining impacts of pollutants in the environment that may shape development. Bisphenols are a group of chemical agents used widely in the manufacture of plastics and resins, and the most famous bisphenol, bisphenol-A is known by the general public to be an endocrine disruptor, mimicking estrogen communication in the body of organisms. Effects of bisphenols have also been associated with both reproductive and metabolic anomalies. Bisphenol exposure has been linked to shortened lifespan in some animal studies, largely through disruption of endocrine signaling, metabolic change, and elevated stress responses. These effects appear to accelerate aging processes and increase vulnerability to age-related diseases. In this research, we present preliminary data comparing exposure to three different bisphenols and examining their impact on developing fly populations. Three specific, commercially relevant bisphenols (bisphenol-A, bisphenol-S, and bisphenol-F) were compared to control populations. We examine these populations of flies relative to their growth, development and lifespan. Exposure periods to the various bisphenols began at the start of oviposition and extended through the lifespan. Observed decreases in lifespan relative to bisphenol exposure are examined relative to potential impact on developing neurons in the brain, especially the insulin-producing cells (IPCs) in the brain. The Insulin/Insulin-like Growth Factor Signaling (IIS) pathway is a highly conserved pathway exerting key roles in growth, metabolism, reproduction, and stress resistance. Finally, we hypothesize about our planned next steps in this research. Funded in part by the Field-Spicer Research Fellowship.

Poster 36

Fariha Islam, Jeremy Lynch

Assembly and Age-Related Degeneration of the *Nasonia vitripennis* Oosome

Biological Sciences, University of Illinois at Chicago, USA

Germline specification in many insects relies on maternally provided ribonucleoprotein (RNP) granules. A unique system for studying this process is offered by the parasitoid wasp *Nasonia vitripennis*, as its germ plasm is organized into a single, massive RNP body called the oosome. The oosome is evolutionarily divergent from the well-studied polar granules of *Drosophila*, differing in its enormous size, dynamic migration, and unique protein architecture. However, fundamental questions remain about how this complex structure is assembled and how it is affected by external factors such as maternal age. To understand its formation, the spatio-temporal hierarchy of oosome assembly during oogenesis is being investigated. The precise order in which core proteins and RNAs are integrated is being mapped using super-resolution microscopy combined with fluorescence in situ hybridization and immunofluorescence. Furthermore, parental RNA interference is used to functionally dissect the roles of core components in orchestrating this process. To determine the impact of maternal age on the fidelity of oosome assembly, oosomes from young and old mothers are compared, and age-related changes in volume, internal architecture, and composition are quantified. The functional consequence of these changes is assessed by quantifying the number of pole cells specified in the resulting embryos. We show *Nasonia tudor* is critical for oosome integrity, as its knockdown results in smaller oosomes and causes the mislocalization of *barkbeetle* RNA into an ectopic shell-like structure. Despite these defects, the core *oskar* mRNA meshwork is still able to form. Strikingly, oosomes from aged mothers are found to be significantly smaller and compositionally deficient in key RNAs compared to those from young mothers. This research provides a high-resolution analysis of the assembly of this unique RNP granule and establishes a direct link between maternal age and the structural integrity of the germ plasm.

Poster 37

Paul Iyyanar[1], Logan Willeke[1], Jesus M. Leal[1], Russell Chuah[1], Katarina Dunn[1], Rolf W. Stottmann[1,2]

An ENU Forward Genetic Screen Identifies Novel Craniofacial Genes

[1]Steve and Cindy Rasmussen Institute for Genomic Medicine, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH 43205, USA; [2]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH 43210, USA

Congenital birth defects affect 5% of the population and account for twice as many deaths as childhood cancers annually. Craniofacial malformations represent a significant proportion of these defects, yet their genetic causes remain largely unknown. While large-scale human patient sequencing efforts have resulted in a rapidly growing number of "variants of unknown significance", research has been largely biased toward well-studied genes and pathways. This underscores the need for an unbiased approach to discover new causal genes in craniofacial development. Here, we employed N-ethyl-N-nitrosourea (ENU) to introduce heritable single-base-pair changes in the mouse genome. This forward genetics approach allows us to recover novel craniofacial phenotypes and identify the causal variants through whole exome sequencing. We also utilized a *Patched1-lacZ* knock-in allele as a sensitizing allele, genetically

predisposing the embryos to craniofacial malformations. In our initial screen, we analyzed 85 pedigrees and identified 23 phenotypes of interest. We have cloned the causal variant in four of these lines and have strong candidate genes in seven additional lines. We also observed two lines with phenotypes dependent on the *Ptch1*-sensitizing allele: one with cleft palate and another with polydactyly. Our initial sequencing analysis has identified genes such as *Wnt7b*, *Igf1r*, and *Slc39a8*, which were not previously implicated in craniofacial development, as well as *Pik3ca*, where loss-of-function mutants were previously found to be early embryonic lethal. These data highlight the power of our unbiased approach, which is not only effective in identifying new genes and pathways essential for craniofacial development but also provides crucial functional data to enhance the interpretation of human genetic testing results. Funding for this project comes from Abigail Wexner Research Institute at Nationwide Children's Hospital recruitment funds (R.W.S.).

Poster 38

Praise Joel[1], Charlotte Meyer-Gerards[2], Cally Xiao[2], Marta Grzonka[2]

Dissecting The Mechanism of The Mitotic Surveillance Pathway

[1]University Of Michigan, United States; [2]CECAD Cluster of Excellence, University of Cologne, Germany

Cell cycle checkpoints are essential surveillance mechanisms that ensure faithful cell division and chromosome segregation in developing embryos. We and others have described a p53-dependent mitotic surveillance pathway (MSP) that eliminates cells that undergo prolonged mitosis, triggered by centrosome loss of function or pharmacological arrest. Centrosomes, composed of centrioles and pericentriolar material, organize mitotic spindles. We have shown that mutations in mouse *Sas-4* or *Sass6* genes, which are required for centriole formation, cause centrosome loss, p53-dependent cell death, and embryonic arrest at mid-gestation (E9). MSP activation in centrosome mutant embryos begins at gastrulation (E7), aligning with centrosome maturation, when p53 is upregulated by the MSP upstream players 53BP1 and USP28. To investigate MSP *in vitro*, we derived *Sas-4*$^{-/-}$ mouse embryonic stem cells (mESC) which lack centrosomes and display a p53-dependent proliferation defect. We used a drug-based protocol to induce MSP in mESC. Prolonged mitosis triggered the formation of membrane less foci containing known pathway components, dependent on 53BP1 but not USP28 or p53. Live-cell imaging of p53-eGFP and 53BP1-HcRed knock-in mESC shows that the foci first appear as small cytosolic puncta near DNA, coalescing into larger structures as mitosis persists, cells with foci upregulated p53 after release into G1. Proximity labeling with a p53-TurboID knock-in mESC line further identified the mitotic kinase PLK1 as a regulator, which localized to these foci. Together, our findings define the developmental timing and molecular requirements of MSP activation *in vivo* and establish an *in vitro* system to dissect how centrosome loss or mitotic delay promotes 53BP1-dependent foci assembly, leading to MSP initiation and p53 stabilization. Funding Sources: Center for Molecular Medicine, Cologne, Germany; Startup funds from Cell & Developmental Biology, University of Michigan

Poster 39

Jinwen Kang, Peiyao Liu, Shoko Ichimura, Lauryn Cook, Zhiyuan Chen

Polycomb Repressive-Deubiquitinase Complex Safeguards Oocyte Epigenome and Female Fertility by Restraining Polycomb Activity

Cincinnati Children's Hospital Medical Center, USA

Two key modifications, histone H2A lysine 119 (H2AK119) monoubiquitylation and histone H3 lysine 27 (H3K27) trimethylation are hallmarks of heterochromatin and essential for Polycomb-mediated gene silencing. Although it is well established that the Polycomb group proteins establish these histone marks during oogenesis, it remains unknown which factor(s) counteract Polycomb activity to prevent excessive H2AK119ub1 and H3K27me3. Here, we report that BAP1-mediated H2A deubiquitylation in oocytes is crucial to constrain pervasive H2AK119ub1 and prevent ectopic H3K27me3 domains in oocytes. Loss of maternal BAP1 disrupts the maternal-to-zygotic transition and leads to severe preimplantation defects. To study its function in oogenesis, we conditionally depleted *Bap1* using a *Gdf9-iCre, Bap1$^{flox/flox}$* mouse model. We found that BAP1 depletion does not affect folliculogenesis. However, immunofluorescence and CUT&RUN analyses indicated that H2AK119ub1 level in KO FGOs was remarkably higher than that in control oocytes. In contrast to H2AK119ub1, increase of H3K27me3 is modest in KO oocytes. RNA-seq revealed 618 genes significantly downregulated in KO FG oocytes, while only 83 genes were upregulated, consistent with BAP1's role as a transcriptional activator. To assess developmental consequences, we fertilized BAP1 KO oocytes with wild-type sperm. The maternal KO embryos exhibited developmental delay around 4- and 8-cell stages. RNA-seq analyses at late 1-cell, early and late 2-cell revealed defects in both maternal decay and zygotic genome activation. Notably, 455 (73%) out of the 618 down-regulated genes in KO FGOs were down-regulated in late 1-cell embryos, suggesting that aberrant transcriptomes in KO FGOs are inherited in zygotes. Together, these findings reveal a critical role for PR-DUB in safeguarding the oocyte epigenome by protecting euchromatin from ectopic Polycomb activity, rather than enforcing transcriptional repression.

Poster 40

Hariharan Karthikeyan[1], Nicole Edwards[1], Lu Han[1], Steve Vokes[2], Sumeda Nandadasa[3], Aaron Zorn[1]

Mechanisms of Hedgehog signaling-dependent GLI signaling in Tracheoesophageal Development

[1]Cincinnati Children's Hospital and Medical Center , USA; [2]University of Texas at Austin, USA; [3]UMass Chan Medical School, USA

The Hedgehog signaling pathway plays a crucial role in the development of vertebrates, influencing key processes in the cardiopulmonary system. The spatiotemporal expression of SHH controls the processing of the GLI transcription factors into their activator and repressor forms. During early embryonic development, in the tracheoesophageal (TE) region, the balance between these forms of GLI at the nuclear level dictates cellular mechanisms, such as dorsal-ventral foregut patterning and mesenchymal medial constriction. Disrupting HH/GLI activity results in defects ranging from esophageal atresia/tracheoesophageal fistula to laryngotracheoesophageal clefts. However, the precise gene regulatory network and spatiotemporal expression patterns established by GLI signaling in mediating the cellular processes between the endoderm and mesoderm remain unclear. We hypothesize that the

HH/GLI signaling pathway influences cellular parameters such as localized extracellular matrix remodeling, cell migration, adhesion and tissue morphogenesis in the splanchnic mesenchymal cells, contributing to tracheoesophageal separation. By overlapping *Gli2/3* loss-of-function mutant bulk RNA sequencing data, $Gli3^{3xFlag}$ ChIP sequencing data and publicly available data from $Shh^{-/-}$ foregut mesenchyme, we identified 23 differentially expressed genes regulated by SHH-mediated GLI3 signaling of which *Adamts20* is a key target. Loss-of-function *Adamts20* mutants exhibit tracheoesophageal clefts, highlighting its importance in this process. We propose to perform CUT&RUN to identify GLI transcriptional targets and multiome sequencing with *Gli2;Gli3* loss-of-function mutants to uncover the gene regulatory network established by GLIs. To understand the effect of perturbed gene dosage of GLIs on cell behavior, we will perform live imaging on slice culture of foreguts. This work is funded by **NICHD P01 HD093363**.

Poster 41

Hank Kern, Christoph Lepper, Kedryn Baskin

Med12 is Essential for Skeletal Muscle Development

Department of Physiology and Cell Biology, The Ohio State University, United States

Skeletal muscle development is essential for movement and metabolic health, requiring tight gene expression control during muscle cell differentiation. Mediator is a multisubunit protein complex with dynamic interfaces that link basal transcriptional machinery and enhancers to regulate transcription of target genes. MED12, a kinase submodule subunit of Mediator, is integral to proper regulation of eukaryotic transcription. *MED12* mutations are linked to hypotonia and heart defects, but its role in muscle development is unclear. We hypothesize that MED12 coordinates muscle differentiation and development by recruiting transcription factors to Mediator at the Pre-Initiation Complex. To test this, we generated conditional skeletal muscle-specific *Med12* knockout mice. *Med12mKO* mice have a runted phenotype, smaller and disorganized muscles, and suffer premature death, compared to littermate controls. RNA-seq of day 1 *Med12mKO* and control quadriceps muscles revealed that MED12 deletion dysregulates metabolic and structural gene expression, including myosins. MED12 is a transcriptional regulator that interacts with transcription factors (TFs) without binding DNA directly. To identify putative TFs that MED12 could be working with, we performed Upstream regulator analysis (URA) of dysregulated genes in *MED12mKO* muscle. Multiple TFs were predicted to have decreased activity in the absence of MED12, including SRF, which is known to regulate the expression of many myosin genes. *Med12* knockdown in C2C12 myoblasts diminished myotube size and reduced expression of myosin genes, mirroring *Med12mKO* phenotypes. Using these *in vivo* and *in vitro* models, we are investigating mechanisms through which MED12 regulates structural and metabolic gene expression during myogenesis. Future work will clarify MED12's role in muscle gene regulation during myogenesis.

Poster 42

Rosa Kwon

Determining the Effects of *mecr-1* Gene Deletion on the *Caenorhabditis elegans* Germ Line Through Expression Analysis of FOG-3 and LIN-41

Kenyon College, USA

Mitochondrial Enoyl CoA reductase Protein-associated Neurodegeneration (MEPAN Syndrome) is a rare, autosomal recessive mitochondrial disease that is caused by mutations in the MECR (Mitochondrial Enoyl CoA Reductase) gene. MECR encodes the catalyst for the final step of mitochondrial fatty acid synthesis (mtFAS), a pathway responsible for producing lipoic acids and long acyl chains that are integral to mitochondrial function and respiration. As a result, defects in mtFAS can lead to diverse physiological symptoms such as basal ganglia abnormalities, muscular atrophy, and childhood-onset optic atrophy. However, the exact mechanisms behind this genotype-phenotype relationship are largely unknown. In order to investigate the role of MECR, we used the hermaphroditic nematode Caenorhabditis elegans and knocked out its MECR ortholog, mecr-1. The mutants, mecr-1(av238), are sterile with an absence of oocytes possibly due to defects in the switch from spermatogenesis to oogenesis near adulthood. To test this possibility, we used immunofluorescence analysis to assess the spatiotemporal expression of FOG-3, a pro-spermatogenic protein that inhibits oogenesis. While the expression was comparably faint, FOG-3 was localized in the same gonadal region as in normal, wild-type (WT) C. elegans, suggesting that spermatogenesis does terminate. To analyze defects in oogenesis, we also examined the expression of LIN-41, a pro-oogenic protein. LIN-41 expression was noticeably more faint and mislocalized when compared to WT, indicating that the sterility of mecr-1(av238) can largely be attributed to defects in oogenesis rather than in spermatogenesis. While further investigation is needed using other oocyte markers, these results are promising steps into uncovering the functions of mecr-1, and by extension MECR, and the different regulatory, developmental protein networks that they are involved in to understand how symptoms of MEPAN occur. Funding was provided by the Kenyon Research Fund.

Poster 43

Avelina Lee, Samantha A. Brugmann

Investigating the role of *ESRP2* in craniofacial development using the *cleft primary palate* (*cpp*) avian model

Cincinnati Children's Hospital Medical Center, USA

Craniofacial abnormalities (CFAs) make up about one-third of congenital abnormalities, with those affecting the midface being among the most difficult to treat. The frontonasal prominence (FNP) is the embryonic precursor of the midface, which gives rise to the forehead, bridge and tip of the nose, the philtrum, and the primary palate in humans and the upper beak and primary palate in avians. Impaired FNP development results in CFAs, including facial clefting, which affects basic functions including mastication, respiration, and speech. Despite the moniker, the avian *cleft primary palate (cpp)* mutant does not merely present with a cleft, rather it is characterized by a complete loss of the FNP. Recently, the pathogenic variant responsible for this phenotype was identified as a 1-bp deletion in *Epithelial Splicing Regulatory Protein 2 (ESRP2)*. *ESRP2* is expressed in craniofacial signaling centers that contribute

to FNP development including the frontonasal ectodermal zone, forebrain neuroectoderm, oral epithelium, olfactory epithelium, and midbrain-hindbrain boundary. *cpp* embryos exhibit agenesis of the FNP as well as ectopic glandular tissue in the roof plate. BaseScope analysis revealed impaired splicing of *FGFR2*, resulting in a loss of the epithelial-specific *FGFR2b* isoform, while mesenchymal-specific *FGFR2c* isoform expression was maintained. Furthermore, bulk RNA sequencing revealed differentially expressed genes related to cranial neural crest cell (CNCC) migration, specification, and differentiation, including *SEMA3F*, *BMP4*, and *OVOL2*, suggesting that *ESRP2* regulates these CNCC processes either directly or indirectly through signaling like the FGF pathway. Future studies using the *cpp* will examine the mechanisms by which *ESRP2* regulates these processes and downstream FGF signaling to advance understanding of temporospatial signaling required for proper formation and outgrowth of the FNP and subsequent midface. This project is funded by NIH-R35DE027557 to SAB.

Poster 44

Lauren Levesque, Kara Braunreiter, Susan Cole

Notch-dependent mRNA decay of the transcriptional repressor *Hes7* during somitogenesis

Molecular Genetics, The Ohio State University, USA

Somitogenesis produces the embryonic precursors of ribs and vertebrae. In mammals, this process is regulated by oscillation of the transcriptional repressor HES7 in the presomitic mesoderm (PSM). Notch signaling activates *Hes7* transcription, while HES7 protein represses its own transcription, creating a negative feedback loop essential for cyclic expression. In Notch-deficient PSMs, *Hes7* transcription is reduced and *Hes7* mRNA is stabilized, suggesting Notch also promotes *Hes7* mRNA decay through an unknown mechanism. The mouse PSM produces three *Hes7* isoforms with different 3'UTR lengths. qRT-PCR shows all isoforms are stabilized in Notch-deficient PSMs, though the extent is difficult to quantify since Notch impacts both transcription and stability. To isolate Notch's role in mRNA turnover, I transiently expressed *Hes7* isoforms under an SV40 promoter in C2C12 cells. Increased Notch had no effect on steady-state transcript levels. Further testing of known Notch targets showed that Notch was insufficient to induce some targets but unnecessary for others, leading to a model where Notch fails to upregulate a trans-factor required for *Hes7* mRNA decay in C2C12 cells.To identify Notch-dependent factors promoting *Hes7* decay, I analyzed public transcriptomic and ChIP-seq data for genes involved in RNA processing that bind *Hes7*, cycle in the PSM, and/or are Notch targets. This yielded 59 candidate trans-factors. I will knock down moderately to highly expressed candidates in C2C12 cells using siRNAs and perform a CRISPRa mini screen on lowly expressed candidates. In both assays, GFP and mCherry-HES7 will be co-expressed from a bidirectional promoter to assess post-transcriptional effects on *mCherry-Hes7* expression. Once a candidate is identified, I will test its effect on each *Hes7* isoform and characterize its expression in wild-type and Notch-deficient PSMs. This work was supported by NIH T32 GM141955 and Pelotonia.

Poster 45

Bo Li

Foxa2-dependent uterine glandular cell differentiation is essential for successful implantation

Cincinnati Children's Hospital Medical Center, USA

Pregnancy is a tightly regulated process involving implantation, decidualization, placentation, and parturition. Implantation marks the beginning of a successful pregnancy and relies on the dialogue between an implantation-competent blastocyst and a receptive uterus. Uterine receptivity is thus essential, yet reliable markers to evaluate it are lacking. Uterine glands play an indispensable role in establishing receptivity, but their functional and morphological changes approaching this state remain incompletely understood. The uterus consists of endometrium and myometrium. The endometrial luminal epithelium gives rise to glandular epithelium, which forms coiled tubular glands extending toward the myometrium. In both mice and humans, glands secrete critical factors for uterine receptivity and embryo development. One such factor is leukemia inhibitory factor (LIF), secreted on day 4 of pregnancy in mice. LIF deficiency results in implantation failure and exogenous LIF rescues implantation in delayed pregnancy models. FOXA2, a transcription factor specifically expressed in glandular epithelium, is required for LIF production. Its deletion in the uterus leads to implantation failure, indicating its essential role in gland function. We hypothesize that gland readiness for estrogen stimulation is key to receptivity. Our study shows that uterine glands undergo FOXA2-dependent differentiation, with expanded branching during the preimplantation period. Three-dimensional imaging of whole-mount uteri reveals dynamic glandular morphogenesis. Single-cell RNA profiling identifies a LIF-producing *Prss29*$^{+}$ glandular subgroup on day 4, which develops prior to estrogen stimulation. This subgroup is absent in *Foxa2*-deficient glands, which also lack branching. We define this FOXA2-dependent maturation as a “transitional phase” that precedes and predicts uterine receptivity—an important conceptual advance in implantation biology.

Poster 46

Ramon Macias[1,2], Rolf Stottmann[1,2]

Functional Redundancy of *Tubb2a* and *Tubb2b* in Embryonic Brain Development

[1]The Ohio State University, United States; [2]Nationwide Children's Hospital, United States

Microtubules are fundamental components of the cytoskeleton, built from repeating alpha and beta tubulin subunits. Humans express nine genes for each alpha-tubulin and beta-tubulin but little is known about their specific developmental requirements. During embryogenesis, microtubules are essential for brain development and pathogenic variants in *TUBB2A* and *TUBB2B* are linked to severe congenital cortical malformations in humans. High sequence similarity among tubulin genes prevents construction of effective antibodies and confounds RNA expression analysis. To overcome these limitations in studying tubulin biology, we generated novel transgenic mice with epitope tags in the endogenous *Tubb2a* and *Tubb2b* loci, enabling precise expression analysis. We previously showed that deletion of either *Tubb2a* or *Tubb2b* have no gross brain phenotype. To test the hypothesize this is due to functionally redundancy, we created a conditional *Tubb2a* allele in combination with a *Tubb2b* null allele. We are ablating both genes in both the forebrain and throughout the embryo. Strikingly, most whole embryo double

knockouts die postnatally, yet their brains appear grossly normal. Transcriptomic data from these mutants reveal potential compensatory changes in multiple other tubulin genes. The current understanding of tubulinopathies is that *de novo* missense mutations have dominant negative effects on tubulin genes. Knowing this, we seek to ablate *Tubb2b* in the *Tubb2b-brdp* mouse missense mutant to explore therapeutic treatment strategies for tubulinopathies. This presents a potential therapeutic strategy for humans with beta-tubulin missense variants otherwise destined to have significant cortical malformations. Funding for this work from the Abigail Wexner Research Institute at Nationwide Children's Hospital and R56NS134119 (R.W.S.). R.M. was supported by T32GM141955.

Poster 47

Elizabeth Manning

Investigating the Role of MECR-1 on Spermatogenesis in *C. elegans*

Kenyon College, USA

MEPAN Syndrome is an autosomal recessive disease caused by loss of the last enzyme of the mitochondrial fatty acid synthesis II (mtFASII) pathway: the mitochondrial enoyl-coA reductase (MECR). In humans with this disorder, symptoms include muscular dystrophy, basal ganglia deterioration, and childhood-onset optic atrophy. In the nematode *C. elegans*, mutants with a complete deletion of the orthologous *mecr-1* gene are sterile due to a complete lack of oocyte production. Interestingly, however, the gonads still seem to produce viable sperm. Although the lack of oocytes appears to be an oocyte-specific phenotype, we have also observed precocious germ cell differentiation during spermatogenesis. This study aims to examine the progression of spermatogenesis in the *mecr-1Δ* mutants in order to elucidate both the process of spermatogenesis termination and the state of the sperm produced. To do this, we used immunofluorescence imaging to track the expression of the spermatogenic marker Major Sperm Protein (MSP). Since MSP is first expressed in primary spermatocytes, we expected that expression would be limited to germ cells of the sperm lineage. However, we found that the *mecr-1Δ* mutants have MSP expression in transition zone germ cells, before germ cell specification occurs. Since the transition zone is when germ cells enter meiosis, this finding could indicate a broader phenotype of mecr-1 being essential for both the entry into, and progression through, meiosis. Therefore, analysis of these images shows unexpected results that require further investigation. Analysis of upstream spermatogenic regulators such as SPE-44 and NHR-23 will give insight into the observed meiotic dysfunction in *mecr-1Δ* mutants. Funding is provided by Kenyon College.

Poster 48

Ella Markley[1], Hannah Schrader Dear[1], Nicole Franks[1], Alexander Holtz[2], Jane Song[3], Craig Johnson[1], Paola Medina-Cabrera[1], Daniela Hernandez[1], Praise Joel[1], Marina Pasca di Magliano[1,4,5], Deneen Wellik[6], Benjamin Allen[1]

Developing a Novel Mouse Model to Investigate GLI Localization during Hedgehog-Dependent Neural Patterning

[1]Department of Cell & Developmental Biology, University of Michigan, Ann Arbor, MI 48109, United States ; [2]Center for Regenerative Medicine, Boston Medical Center, Boston, MA 02118, United States; [3]Genentech, South San Francisco, CA 94080, United States; [4]Department of Surgery, University of Michigan, Ann Arbor, MI 48109, United States; [5]Rogel Cancer Center, University of Michigan, Ann Arbor, MI 48109, United States; [6]Department of Cell and Regenerative Biology, University of Wisconsin, Madison, WI 53705, United States

Hedgehog (HH) signaling is essential for neural tube patterning. Specifically, notochord-derived, secreted Sonic HH (SHH) directs ventral cell fate specification, ensuring normal spinal cord development. Notably, SHH acts in a time- and concentration-dependent manner to pattern distinct cell fates (e.g., interneurons vs. motor neurons). GLIs (GLI1-3) are the transcriptional effectors of SHH. However, a lack of robust tools has limited our ability to visualize potentially dynamic changes in GLI localization during HH-dependent neural patterning. To address this, we generated endogenous epitope-tagged alleles for each *Gli* (*Gli1*^{FLAG}, *Gli2*^{HA}, *Gli3*^{V5}), establishing a novel mouse model, *Gli1*$^{FLAG/FLAG}$;*Gli2*$^{HA/HA}$;*Gli3*$^{V5/V5}$ (referred to as *Gli*^{FHV}), which are viable and fertile with no overt phenotypes. Examination of neural patterning in E10.5 and E11.5 *Gli*^{FHV} embryos compared to wildtype embryos revealed no significant changes in HH-dependent neural patterning, suggesting that HH signaling is unperturbed in this tissue over time. We have also investigated GLI localization in *Gli*^{FHV} embryos to assess potential changes in GLI distribution between cell types exposed to different durations and levels of HH signaling, as well as in the same cell types at different axial levels (e.g., forelimb vs. hindlimb), and at different developmental stages (e.g., E10.5 vs. E11.5). Further, we are breeding *Gli*^{FHV} animals with animals carrying mutations in HH pathway components to assess potential alterations in GLI localization. Specifically, we are crossing *Gli*^{FHV} animals with mice carrying a mutant *Gas1* allele, which encodes for Growth arrest-specific 1 (GAS1), an essential HH co-receptor. Prior studies have demonstrated that *Gas1* null embryos exhibit defects in ventral neural tube patterning. We will use *Gas1*$^{-/-}$;*Gli*^{FHV} embryos to study the effects of *Gas1* deletion on GLI localization, providing mechanistic insight into GLI dynamics during HH-dependent neural patterning.

Poster 49

Sarah McLeod[1], Raisa Bailon-Zambrano[2], Sandhya Paudel[1], Melissa Scott-Preusse[1], James Nichols[2], Lindsey Barske[1]

Exploring Midline Appendage Evolution Through Ectopic Fin Development in pax9 Mutant Zebrafish

[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Colorado Anschutz Medical Campus, USA

Zebrafish median fins are a valuable model for studying vertebrate appendage evolution, yet the genetic mechanisms regulating their placement and initiation along the body axis remain poorly understood. We

investigate these questions using the pax9 mutant, which develops a continuous anal-to-caudal ventral midline fin lacking the gap typical to most extant fishes. To examine the developmental and genetic basis of this phenotype, we used skeletal staining, developmental staging, tissue clearing, and single-cell RNA sequencing. A developmental series from 8 to 21 days post-fertilization (dpf) revealed that anterior caudal fin structures are mispatterned from the onset of cartilage condensation, with ectopic fin segments emerging in the interfin region shortly thereafter. Clearing of fully ossified 30 dpf+ fish confirmed fused and malformed skeletal elements within the caudal fin and spine. Single-cell transcriptomic analysis at 3 dpf identified three mesenchymal subpopulations, all derived from the sclerotome compartment of the somite: skeletogenic mesenchyme, fin fold fibroblasts, and a hybrid population. pax9 mutants exhibited increases in skeletogenic mesenchyme and hybrid cells at the expense of fin fold fibroblasts. These findings suggest that pax9 regulates sclerotomal cell fate and fin patterning, and its disruption enables expansion of mesodermal derivatives into the gap between anal and caudal fins. Given pax9's role in limb and vertebral patterning in other vertebrates, this mutant model offers novel insight into developmental and evolutionary mechanisms driving median fin innovation. Changes in pax9 expression may be associated with the ancestral median fin loss and appendage patterning that occurred as vertebrates moved from sea to land. Funding for this study provided by a Research Innovation & Pilot grant from the Cincinnati Children's Research Foundation.

Poster 50

Kelli Johnson[1], Xiangning Dong[1], Yu-Hwai Tsai[1], Angeline Wu[1], Sydney Clark[2], Sha Huang[1], Rachel Zwick[3,4], Ian Glass[5], Katherine Walton[1], Ophir Klein[4,6], Jason Spence[1,7,8]

Mapping mesenchymal diversity in the developing human intestine and organoids

[1]Department of Internal Medicine, Division of Gastroenterolgy, University of Michigan Medical School, USA; [2]Department of Cellular and Molecular Biology, University of Michigan Medical School, USA; [3]Department of Cell Biology, NYU Grossman School of Medicine, USA; [4]Program in Craniofacial Biology and Department of Orofacial Sciences, University of California, San Francisco, USA; [5]Department of Pediatrics, Genetic Medicine, University of Washington, USA; [6]Department of Pediatrics, Cedars-Sinai Guerin Children's, USA; [7]Department of Cell and Developmental Biology, University of Michigan Medical School, USA; [8]Department of Biomedical Engineering, University of Michigan and University of Michigan College of Engineering, USA

The organization of diverse mesenchymal populations during human intestinal development is critical for tissue architecture and function yet remains poorly defined. To construct a comprehensive, tissue-scale map of the developing human small intestine, we leveraged single-cell RNA-sequencing data to build a custom Xenium spatial transcriptomics gene panel covering the diversity of cell types in the human intestine. Analysis was focused on the developing mesenchyme populations (also referred to as fibroblasts or stroma) given the lack of spatiotemporal information about these cell populations. We defined 5 broad mesenchymal populations occupying discrete locations within the lamina propria and submucosa – the subepithelial cells (SEC), lamina propria fibroblasts (LPF), submucosal fibroblasts (SMF), smooth muscle cells (SMC), and *CXCL13*+ fibroblasts. Our data reveal dynamic spatial remodeling of fibroblast communities during development and establish molecular markers to distinguish these

populations. We leverage this high-resolution atlas to benchmark pluripotent stem cell-derived human intestinal organoids and to demonstrate how this foundational resource can be used to dissect intestinal stromal signaling in a spatial manner, with broad implications for modeling development, regeneration, and disease. Funding: This work was supported in part by: 2019-002440 (Seed Network) and 2021-237566 (Pediatric Network) from the Chan Zuckerberg Initiative DAF to J.R.S., the Intestinal Stem Cell Consortium (NIDDK and NIAID, U01DK103141 to J.R.S), the NIDDK (R01DK137806 to J.R.S; RC2DK140862 to J.R.S and O.K; R01DK121166 to K.D.W; F32DK138694 to K.J), and the University of Michigan Center for Gastrointestinal Research (NIDDK 5P30DK034933). I.G. and the University of Washington Laboratory of Developmental Biology was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD- 5R24HD000836).

Poster Session 2

Poster 51

Amartya Mitra, Shubham Rathore, Aaron Stahl, Elke Buschbeck

Establishing Parallels Between Eye Types

University of Cincinnati, USA

Eyes are among the most complex animal sensory organs and have thus been the subject of extensive evo-devo research. What remains understudied in this area however, is how one elaborate eye-type, such as a compound eye, might evolve into another, such as a camera-type eye. This is a phenomenon observed repeatedly in arthropods and exemplified by stemmata, the camera-type eyes of larval holometabolous insects. A particular gene of interest in this system is Cut, a homeodomain transcription factor restricted in its expression to compound eye Semper cells, which are support cells that develop immediately after photoreceptors. *cut*RNAi results in disruption of the ommatidial array and fusion of neighbouring ommatidia in the structurally and optically distinct adult compound eyes of Thermonectus marmoratus diving beetles and D. melanogaster flies. As ommatidial fusion is a proposed pathway for the development of certain stemmata, we posit that the regulation of Cut expression may play an important role in mediating fusion during the evolutionary transition from compound to camera eyes. Here we explore the expression and function of Cut during the embryonic development of eyes in T. marmoratus. Aligning with our expectations, in T. marmoratus larval camera-type eyes, Cut is expressed in the proximal corneagenous cells and preliminary data shows that embryonic knockdown leads to the fusion of neighbouring eye units. We expect these results to shed light on the ontogeny and evolution of arthropod camera-type eyes while providing novel insights into organogenesis. This work was funded by the National Science Foundation under grant I0S-1856241

Poster 52

Sophia Moberly, Azalee Almond, Aayushi Gandhi, Xsandrie Guimba, Nathaniel James, Katie Mullin, Alexanna Taylor, Gary Lange

Developmental effects of environmentally relevant exposure to bisphenol-S in the juvenile period impacts responses of rats (Rattus norvegicus): Comparisons to bisphenol-A

Saginaw Valley State University , USA

Use of plastic has become so abundant that it represents one of the most pervasive and persistent sources of pollution on Earth. Increased use of plastics since the 1980s has transformed our work and home environments. The chemical composition of plastics degrades over time and these breakdown products enter our environment resulting in concerns about exposure to these compounds in terms of health and wellness. Bisphenol-S is in many plastics used in everyday life. Used to create plastics with flexibility and clarity, it is widely used in food handling. Bisphenol-S is now a common replacement bisphenol for Bisphenol-A, since the latter (also widely used) has become recognized as a chemical of concern by the broad general public. While Bisphenol-S is technically NOT Bisphenol-A, it exhibits a similar chemical structure, and it too can act as an endocrine disruptor impacting development and neuroendocrine function. Bisphenol-S leaches from plastics and is inadvertently ingested by organisms. Research on the physiological impact of Bisphenol-S ingestion is limited, especially related to development. However, current evidence suggests it exhibits estrogenic properties and also may serve as a mimic for metabolic hormones. We theorize that juvenile exposure to bisphenol-S may specifically influence behavior by reshaping organization and function of the mammalian central nervous system during development just prior to the onset of puberty, resulting in changes developmentally affecting behavior. In our research, exposure to bisphenol- S occurred post weaning in rats at environmentally relevant levels. At maturity, neuromuscular development was assessed. Measures related to stress responses and reproductive behavior were additionally measured. The potential sexually dimorphic effects of environmentally relevant exposure of Bisphenol-S on the development of the nervous system warrants additional study. Funded in part by the Field-Spicer Research Fellowship.

Poster 53

Brendan Moran

Role of LOTUS domain and DEAD-box proteins in condensate assembly and small RNA production

The Ohio State University, USA

Germ granules are membraneless organelles present in animal germlines that promote fertility through small RNA biogenesis (Dobrynin et al., 2022). In C. elegans, germ granules are composed of multiple sub-compartments, among these, Mutator foci lie at the nexus of RNAi response by producing small interfering RNA (siRNA) (Sundby et al., 2021). Mutator foci are nucleated by the intrinsically disordered protein MUT-16 (Phillips et al., 2012), yet the mechanism by which these foci are assembled remains poorly understood. Previous studies have shown that LOTUS domain-containing proteins control the assembly of germ granule sub-compartment by regulating the helicase activity of DEAD-box (DDX) proteins (Price et al., 2021; Cipriani et al., 2021). Guided by AlphaFold structural predictions, we identified a putative LOTUS domain within MUT-16. Here we show that mutations to this domain abolish

recruitment of the DDX protein, MUT-14, to Mutator foci and ablate RNAi within the germline. Our preliminary data indicate that loss of MUT-14 recruitment, as well as mutations affecting its catalytic activity, alters the morphology of Mutator foci. Further, ectopic expressions of MUT-16 and MUT-14 in muscle cells result in the formation of Mutator-like foci. These findings support the hypothesis that the LOTUS domain of MUT-16 stimulates the activity of MUT-14 to promote Mutator foci assembly and siRNA production. Moving forward, I aim to utilize biochemical assays and genetic approaches to characterize how MUT-16 regulates the activity of MUT-14 and uncover the mechanistic role of MUT-14 within Mutator foci. This research is funded by NIH T32 training grant 5T32GM141955-05 to B.J.M.

Poster 54

Peyton Morrow[1], J. Raul Perez-Estrada[1,2], Katia Del Rio-Tsonis[1,2]

Salamander Eye Morphogenesis

[1]Department of Biology, Miami University, Oxford, OH, US; [2]Center for Visual Sciences, Miami University, Oxford, OH, US

Eye tissue regeneration after injury is present in a limited number of vertebrates, such as zebrafish and salamanders. In this regard, the salamander *Pleurodeles waltl* (*P. waltl*) can regenerate its retina and lens by cell reprogramming of the retina pigment epithelium (RPE) and iris pigment epithelial cells, respectively. Interestingly, it has been observed that during lens regeneration, a molecular dorsoventral axis program is present in the iris, a phenomenon that is usually observed only during eye development. On the other hand, it is known that the RPE and the neural retina have a common embryonic origin: the optic vesicle. This suggests that the RPE neural potential could be associated with its embryonic origin. Eye morphogenesis has not been studied in *P. walt*, and it is unclear whether the molecular and cellular mechanisms that control eye development are also essential for its eye regeneration capabilities. As a first step, we characterized *P. waltl*eye morphogenesis and compared the process in vertebrates that are incompetent for eye regeneration. Using histology, immunostaining, HCR RNA-FISH, and RT-qPCR, we analyzed eye development from 4 to 12 days post-fertilization (dpf). We observed that eye morphogenesis in *P. waltl* occurs similar to that observed in other vertebrates, starting with the formation of optic vesicles by 4 dpf. We found that the lens is well defined at 5 dpf, and the RPE is detected at 5 dpf, and well-defined by 6 dpf. Our data indicate that overall eye morphogenesis in salamanders occurs as in other vertebrates. However, the spontaneous capacity of the RPE to reprogram into neural retina after retina removal suggests that RPE differentiation during embryogenesis might be different from what happens in nonregenerative species. Funding R01EY035325 to KDRT. KTEF Career Starter Grant to JRPE.

Poster 55

Cimran Nakum, Brandi Bull, Sunitha Yarlagadda, Shalini Indugula, Meredith Schuh

Perinatal exposures during preterm birth and susceptibility to acute and chronic kidney disease

Cincinnati Children's Hospital and Medical Center, USA

Preterm infants continue nephrogenesis postnatally and are often exposed to nephrotoxins like gentamicin (gent). Mothers at risk of preterm birth are given steroids such as betamethasone (beta) to accelerate lung development, but its impact on kidney development and injury susceptibility is not well characterized. We utilized a neonatal rat model to evaluate the impact of beta and gent during postnatal kidney development. In this model, nephrogenesis continues until postnatal day 5 (p5), with tubular maturation until p10. Pregnant dams received beta (0.25 mg/kg IM) or saline (sal) at embryonic day 17 & 18. After birth, rats received gent x5 days (100mg/kg IP) or sal during nephrogenesis (p0-4, “early”) or tubular maturation (p6-10, “late”). Kidneys were analyzed three hours (AKI), 2 weeks (failed repair), and 3 months (CKD) post last gent dose. Immunofluorescence (IF) and RT-qPCR assessed for KIM1. Sox9 and CDH6 IF evaluated failed repair. At 3 months, blood urea nitrogen (BUN) was measured and fibrosis was assessed (via Trichrome staining). Nephron quantification was performed using lectin labeling and imaged on the Bruker LCS SPIM Light Sheet. **AKI**: In those without prenatal steroids, late-exposed rats had higher Kim1 expression than early-exposed rats ($p = 0.002$). However, late exposed rats with prenatal beta (beta-late) had even higher Kim1 expression compared to late-exposed rats without prenatal beta (beta-sal) ($p=0.041$). **Failed repair**: Two weeks after injury, both beta-late and sal-late had increased Kim1, Sox9, and Cdh6 IF compared to early-exposed. At 3 months, beta-late had highest levels of BUN ($p=0.0071$) and fibrosis. Nephron quantification is ongoing. This study suggests that nephrotoxin exposure during tubular maturation is more harmful than during ongoing nephrogenesis, and that prenatal corticosteroids worsen the degree of AKI and CKD during this exposure period. This work was supported in part by the Procter Scholar award

Poster 56

Elysa Ng

Sticking to the Plan: Mechanisms of Floorplate Patterning in Zebrafish Spinal Cord Morphogenesis

Washington University at St Louis, USA

During spinal cord morphogenesis, the medial floorplate (MFP) forms a morphologically distinct singular row of cells along the ventral midline, right above the notochord. The mechanisms causing this precise patterning remain unclear. We found that MFP patterning is disrupted in zebrafish flh and ntl mutant embryos, where the notochord is lost, suggesting a critical role for the notochord in MFP patterning. Notochord is a known signaling center for Sonic hedgehog (Shh) signaling. However, inhibition of Shh signaling does not disrupt MFP pattern, implying that the notochord does not mediate MFP patterning through its signaling role. We therefore hypothesize that notochord facilitates MFP patterning through inter-tissue adhesion. Using single-cell transcriptomics, we found two adhesion genes enriched in the MFP cells, a cell-cell adhesion gene cadherin 6 (cdh6) and a cell-extracellular matrix (ECM) adhesion gene integrin alpha 6a (itga6a). Cdh6 mutant embryos showed normal MFP patterns. In contrast, some itga6a F0 CRISPR mutants showed disrupted MFP patterns by splitting into clusters. We are currently

generating itga6a mutants to further investigate its function in MFP patterning. Our study will provide valuable insights in the contribution of cell-ECM adhesion to floor plate organization, critical for understanding morphogenesis and developmental robustness. This research was funded by NIGMS R35 GM150759.

Poster 57

Timothy Nguyen[1], Fan Shao[1], Jennyfer Mitchell[2], J.C. Thomas[1], Jaclyn Olberding[1], Erliang Zeng[1], Huojun Cao[1], James Nichols[2], Brad Amendt[1], Colin Kenny[1], Eric Van Otterloo[1]

A mouse model for dissecting gene regulation networks underlying midface development an dysplasia

[1]University of Iowa, United States; [2]University of Colorado, Unites States

Cranial neural crest cells form the bulk of the craniofacial skeleton thanks to their ability to take on positional identities. These positional identities are controlled primarily by transcription factors connected into regulatory networks, which are conserved and often disrupted to give rise to the high prevalence of craniofacial disorders. While the gene regulation pathways controlling the neural crest jaw and neck identities are well-characterized, those underlying the midface identity (forehead, nose, cheeks, upper lip) are poorly understood. Here, we present evidence from a mouse model for addressing this knowledge gap, where known AP-2 transcription factor paralogs AP-2α and AP-2β are conditionally removed in the neural crest. These mouse embryos present a fully penetrance midfacial cleft, while gene expression profiling revealed decreased expression of frontonasal dysplasia-associated ALX transcription factor genes. Transcription factor mapping (CUT&RUN), chromatin profiling (ATAC-seq), and genetic interaction studies support a model where AP-2α and AP-2β cooperatively regulate ALX genes by maintaining a permissive epigenome after the cranial neural crest have acquired their positional identities. Using this mouse model alongside our epigenome datasets, we identify candidate transcription factors cooperating with, and functioning independently of, AP-2. These observations begin disentangling the gene regulation networks underlying midfacial development and dysplasia. Training and research support were provided by the NIH/NIDCR (F31DE032881, F32DE029995, R01DE030448, R01DE034668, R01DE033009, R00DE026823) and University of Iowa.

Poster 58

Hoang Nguyen, Daniel Buchholz

Synergistically regulated genes by two hormones during frog metamorphosis

University of Cincinnati, United States

During metamorphosis of Xenopus tropicalis, hormones are key regulators in physiological changes. Out of many hormones, thyroid and glucocorticoids, acting mainly through their respective nuclear receptor thyroid hormone receptor (THR), and glucocorticoid receptor (GR), are the 2 hormones that have the most profound effect in the stage transition of frogs. Although individual effects of each hormone are well-recorded, synergistic effects of these two hormones at the gene level are not well understood. To provide insights into how these hormones interact, we have conducted a genome-wide transcriptomic analysis using RNA-Seq with tadpoles treated with glucocorticoid and thyroid to better understand the

dynamic between these hormones in metamorphosis. Out of 1172 differentially expressed genes, a majority of them (46%) required both hormones. Furthermore, there are many genes that are upregulated to a greater degree (i.e. synergistically), when there are both hormones presented. By doing this experiment, we hope to translate the findings to expand our knowledge of human perinatal endocrinology. Work supported by NSF IOS 2410732 to DB.

Poster 59

Daniel Paluh

Evolution and development of the frog mouth: from tadpole keratinized mouthparts to adult teeth

University of Dayton, USA

The frog feeding apparatus is a complex organ system that undergoes dynamic reorganization during the life cycle of anurans and displays remarkable morphological and functional diversity across species. The components of this system include the stomodeum of embryos, the oral disk of tadpoles, and the jaws, mineralized teeth, and tongue of postmetamorphic frogs. A subset of these components, including the unique jaw cartilages and keratinized mouthparts of tadpoles, represent poorly studied evolutionary novelties. Mineralized teeth form after hatching, during metamorphosis, unlike other vertebrates in which tooth development occurs in the embryo and coincides with primary mouth formation. Frog teeth are evolutionarily labile, being repeatedly lost in over 20 lineages. Using an evo-devo framework, we are characterizing the developmental-genetic basis for the formation of tadpole and frog feeding structures across species and exploring the underlying mechanisms that may be responsible for the origin of novelties and convergent trait loss. We assessed 1) if the genes underlying odontogenesis are evolutionarily conserved in the late-forming teeth of frogs and 2) if the unique keratinized mouthparts of tadpoles impede or induce tooth induction. We demonstrate that the genes underlying odontogenic competence (*Pitx2*, *Shh*, *Sox2*) are conserved in the frog upper jaw, comparable to those of other vertebrates. Adult teeth emerge before larval mouthparts degenerate, but their location may be spatially constrained by keratin. Keratinized mouthparts and teeth unexpectedly express a similar complement of developmental genes. We hypothesize that the novel mouthparts of tadpoles, which we characterize as ectodermal appendages, may have originated by partially co-opting the developmental program that typically mediates mineralized tooth formation. This work was supported by the U.S. National Science Foundation (DBI-2109344).

Poster 60

Hana Pan, Brian Basinski, Charukesi Sivakumar, Qiang Li, Jing Xu, Rajesh Rao

Investigating PRDM13 and its Protein Interactors in their Contribution to CNS Disorders

University of Michigan - Ann Arbor, United States of America

Mutations in PRDM13 are implicated in CNS disorders such as cerebellar hypoplasia and North Carolina Macular Dystrophy, leaving questions regarding the role of PRDM13 in regulating transcription of developmental genes within the nucleus. Fractionation experiments show that inactivating zinc fingers 1 and 2 of PRDM13 in HEK293T cells leads to a significant reduction in nuclear localization, indicating that

they are vital in PRDM13's mechanism. However, how PRDM13 is able to translocate is unknown. To further explore its functionality, we are interested in discovering the protein-protein interactions (PPIs) PRDM13 exhibits. We hypothesize that PRDM13 interacts with nuclear transport protein KPNA2 through its zinc finger domains to localize into the nucleus. Pull-down of FLAG-tagged PRDM13 along with its protein interactors was performed and processed by mass spectrometry. This resulted in a list of 3445 identified proteins, which were then filtered on cellular properties to a list of 11 candidate proteins. Of note, KPNA2, a protein known for its role in nuclear transport was identified. There was a reduction in the complex's AlphaFold3 (AF3) prediction strength with truncation of PRDM13's zinc finger domains. Moving forward, co-immunoprecipitation experiments will be conducted to validate the interaction between KPNA2 and PRDM13. Further analysis via AF3 will be performed to identify specific interacting regions. Overall, these experiments will provide invaluable insights towards our understanding of various CNS disorders and the development of treatment. This work is supported by Research to Prevent Blindness (RPB), the Beatrice & Reymont Paul Foundation, March Hoops to Beat Blindness, Leonard G. Miller Endowed Professorship and Ophthalmic Research Fund at the Kellogg Eye Center, LSA Honors Summer Fellowship, Grossman, Elaine Sandman, Marek and Maria Spatz (endowed fund), Greenspon, Dunn, Avers, Boustikakis, Sweiden, and Terauchi research funds.

Poster 61

Meet Patel

Elucidating the molecular role of prom1b in photoreceptors using zebrafish

University of Kentucky, USA

Mutations in PROM1, a cholesterol-interacting pentaspanin protein, lead to cone-rod dystrophy (CRD). Studies in mice indicate that PROM1 interacts with CDHR1, a photoreceptor-specific cadherin required for outer segment (OS) - calyceal process (CP) connections associated with cone OS stability. However, the molecular function of PROM1 underlying pathogenesis and how it interacts with CDHR1 remains unknown. Using a zebrafish model, we explored how loss of prom1b function leads to CRD and whether it impacts cdhr1a to better understand its function. To establish a prom1b mutant zebrafish model, we generated a CRISPR-mediated knockout of prom1b (prom1b^{D64}). Prom1b^{D64} maternal zygotes at 5 days post fertilization (dpf), 15dpf, 30dpf, 90dpf, and 1ypf were analyzed for retinal dystrophy using confocal microscopy. Prph2 staining was used to demarcate all OS morphology, while peanut and wheat germ agglutinins were used to distinguish between cones and rods. To examine the subcellular localization of Cdhr1 and CPs, cdhr1a, actin were visualized. Imaging studies revealed thatprom1b mutants exhibit gross cone OS morphology defects starting at 5dpf and severe cone OS disruption by 15dpf, where majority of cone OSs have degenerated. Conversely, rod OS were enlarged so significantly that they appear to occupy the entire retinal OS space by 30dpf. Interestingly, cdhr1a OS localization appeared to be significantly impaired, appearing short and disorganized compared to wildtype. CP morphology using actin staining revealed truncation and overall disorganization of cone but not rod CPs. In conclusion, our prom1b^{D64} CRD model recapitulates some aspects of CRD, primarily the significant loss of cone cells. However, our model also exhibits enlarged rod OS, suggesting a dispensable or different role of prom1b

in rod OS function in zebrafish. Lastly, cdhr1a localization was significantly impaired in the prom1b mutant, suggesting it may impact its ability to connect the OS with CPs.

Poster 62

David Paulding[1], Simon Han[1], Jonathan Timmons[1], Michelle Caye[1], Alexa Riedel[1], Samantha Brugmann[1,2], Lindsey Barske[1,2]

Early requirement for NR2F nuclear receptors in the mouse cranial neural crest

[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati, USA

Recent research has shown that nuclear receptors are iteratively deployed during neural crest development, from pre-induction through differentiation stages. NR2F1 and NR2F2 in particular have been proposed as broad regulators of neural crest gene expression, but the timing, extent, and redundancy of their developmental requirement in mammals were unknown. Here we show expression of Nr2f1 and Nr2f2 in the mouse cranial neural crest from specification through post-migratory stages. Conditional knockout of Nr2f1 and Nr2f2 in the neural crest with Wnt1-Cre or the alternative Pax3Cre caused severe midfacial clefting and major reduction of all facial skeletal elements except the distal mandible. Loss of skeletal elements of the upper jaw and skull was traced to embryonic day 8.75 where we identified loss of the trailing first arch migratory stream. This loss does not appear to be caused by apoptosis. RNAseq revealed downregulation of many migratory crest genes, including a possible direct target, phospholipase Plcg2. These findings place an essential requirement for mammalian NR2F1/2 within the later-forming cranial neural crest. NIDCR 5R00DE026239 (LB) F31DE032261 (DP)

Poster 63

Nicole Pek[1,2], Konrad Thorner[1], Hee-Wong Lim[1,2], Darrell Kotton[3], Robert Rottier[4], Aaron Zorn[1,2], Mingxia Gu[5,6]

Uncovering Mechanisms Underlying Capillary Maldevelopment Caused By FOXF1 Mutations Using A 3D In Vitro Vascular Model System

[1]Cincinnati Children's Hospital Medical Center, USA; [2]College of Medicine, University of Cincinnati, USA; [3]Center for Regenerative Medicine, Boston University, USA; [4]Erasmus University Medical Center, Netherlands; [5]David Geffen School of Medicine, UCLA, USA; [6]Broad Stem Cell Research Center, USA

Mutations in the Foxhead box F1 (FOXF1) gene cause a rare and severe congenital lung disorder called Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV). ACDMPV is characterized by vasculo-pulmonary growth defects including immature lobular development and a sizeable decrease in the number of capillaries in alveolar walls. Individuals with ACDMPV typically die within the first few weeks of life due to respiratory failure. At present, it is unclear how mutations in FOXF1 cause alveolar capillary malformations that ultimately lead to abnormal alveologenesis in humans. The lack of therapeutic or pharmaceutical interventions for ACDMPV is perpetuated by this gap in knowledge. Here, we report the derivation of vessel organoids (VOs) with iPSCs generated from ACDMPV patients with different FOXF1 mutations (ACD-VOs). Differentiating ACD-VOs exhibited clear developmental aberrations and altered FOXF1 expression. Also, ACD-VOs displayed distinct vascular defects such as a

significant reduction in endothelial cell population, poorly formed vascular networks that lack lumen structures, and heightened levels of hypoxia, akin to phenotypes observed in ACDMPV patients. 3D gastruloids formed from ACD-iPSCs were less elongated compared to controls suggesting impaired symmetry breakage and expression of key mesodermal markers was significantly reduced. Hence, FOXF1 mutations clearly resulted in dysregulated mesodermal differentiation, thereby disrupting proper cellular patterning. By employing scMultiome-seq, coupled with FOXF1 CUT&RUN, at key stages of VO differentiation, we were able to discern molecular mechanisms underlying capillary maldevelopment caused by mutant FOXF1 proteins. Overall, we present a strategic approach to carefully decipher the role of FOXF1 in capillary development. N.P. is supported by the American Heart Association Pre-doctoral Fellowship and the Albert J. Ryan Fellowship. M.X. is funded by 5R01HL166283.

Poster 64

Alyssa Pennell, Geremy Lerma, Kamorah Ryhlick, Joseph Beljan, Olivia Carraher, Gavin Muniz, Sharon Amacher

Investigating the impact of adamts17 on Duchenne muscular dystrophy in zebrafish

Department of Molecular Genetics, The Ohio State University, Columbus, OH, USA

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease for which there is no cure. There is a critical need for additional therapeutics. Human genome-wide association studies have identified putative genetic modifiers of DMD that could serve as potential therapeutic targets. One putative modifier identified was ADAMTS19. Using a CRISPR-based screen, we provided evidence that supports adamts17, a closely related sister gene of ADAMTS19, is a bona fide DMD modifier in zebrafish. However, It is unclear how adamts17 modulates disease severity. Previous studies have suggested that Adamts17 may regulate Fibrillin-1 and -2 localization which play important roles in regulating TGFb signaling through LTBP4. Additionally, our work has shown that both mutation of LTBP4 or treatment with TGFb inhibitor SB431542 ameliorates DMD severity. To elucidate the mechanism by which adamts17 mutation ameliorates DMD severity we are investigating the expression levels of transcripts associated with inflammation, fibrosis, and muscle regeneration. We show that expression of transcripts associated with inflammation (iL1b) and fibrosis (col1a1, tgfb1a, acta2, and mmp9) are elevated at both 7- (mid) and 10-dpf (late) in dmd zebrafish, while myf5 expression is decreased. These data provide the framework to being to functionally assess the impact of adamts17 mutation on the DMD phenotype. This work is supported by NIH grant R01GM11764, an Ohio State University President's Research Excellence Accelerator Award, a sub-contract of NIH grant R01NS085238, and a Thomas J. Beyers Memorial Scholarship.

Poster 65

Laurence A. Lemaire[1,2], Josefina Perez[1], Shili Wu[1], Wei Lin[1], Michael Levine[2]

Role of Lmx1 in Neural Tube Morphogenesis in the invertebrate chordate *Ciona*

[1]Saint Louis University, USA; [2]Princeton University, USA

Neural tube closure is a critical developmental process, essential to the proper formation of the vertebrate nervous system. This process starts with the invagination of neural plate cells. Its borders then converge, leading to the closure of the neural tube, propagating like a zipper. Afterwards, cell intercalation and proliferation allow the tube to elongate. Neural tube closure involves thousands of cells in vertebrates. However, the closest invertebrates to vertebrates, the tunicates such as *Ciona*, form a hollow neural tube with just eight cells on each side of the neural plate border. This minimal model makes it easier to study the mechanisms of this intricated process. In *Ciona*, the transcription factor Lmx1 is expressed in the dorsal-most cells of the developing neural tube, like its vertebrate orthologs. In vertebrates, Lmx1 paralogs are involved in neural tube patterning. However, no function related to morphogenesis has been uncovered. Here, we explore *Ciona* Lmx1 roles during neural tube closure. *Lmx1* Knockdown leads to slight defects in neural tube closure. To further study its function, we overexpressed a dominant negative form of this transcription factor. Overexpression prevents the proper intercalation of the dorsal neural tube cells, impeding the anterior progression of the zipper. Furthermore, the study of *Lmx1* regulatory sequences indicates that Pax3/7, ZicL, and BMP signaling may regulate its transcription. These transcription factors and the pathway are present in the vertebrate neural plate border, suggesting that the regulation of *Lmx1* is conserved across vertebrates. Therefore, our study might highlight an unrecognized role of Lmx1 during vertebrate neural tube morphogenesis.
This work was supported by a grant (NS076542) from the NINDS of the NIH. It is currently funded by the Office of Vice President for Research and the Provost Office of Saint Louis University (RI award 01978 and grant 001654).

Poster 66

Quentin Phillips

SOX factors modulate WNT/Beta-Catenin-mediated transcription during embryonic lung development

Cincinnati Children's Hospital Medical Center, USA

WNT signaling regulates numerous transcription programs in development and homeostasis, but when disrupted may cause developmental anomalies and diseases. Canonical WNTs bind FZD/LRP co-receptors to initiate an intracellular cascade to translocate beta-catenin (BCAT) to the nucleus where it interacts with one of four TCF/LEF transcription factors on WNT-responsive enhancers (WRE) to activate WNT-responsive transcription. But how TCFs, having nearly identical DNA-binding specificity, control cell-specific gene programs is unknown. Recent evidence indicates that lineage-specific SOX transcription factors also bind BCAT on WREs to differentially enhance or repress WNT-responsive transcription, but the mechanisms by which SOXs, BCAT and/or TCFs interact on chromatin to regulate WNT activity remains unclear. Using genomic analysis and human stem cell (hSC) models, I will define the genomic mechanisms by which SOX2 restrains WNT activity as developing foregut endoderm (FG) is patterned into an esophagus or respiratory fate. During FG patterning, SOX2 is broadly expressed but

downregulated ventrally to permit WNT activation of respiratory genes, like NKX2-1. Loss of SOX2 in mouse FG and human FG organoids results in ectopic NKX2-1 expression in esophagus tissues, suggesting SOX2 suppresses WNT-driven transcription. Additionally, published data shows that chromatin architecture of WNT-responsive genes varies in a SOX2 concentration-dependent manner and data from our lab demonstrated that SOX2, TCFs and BCAT co-bind a subset of WREs in hSC-derived neuromesodermal cells. Based on this, I hypothesize that SOX2 renders esophagus and respiratory enhancers accessible to BCAT/TCFs in FG but then co-occupies and represses TCF/BCAT-bound WREs. We posit that these mechanisms contribute to the transcriptional specificity of WNT signaling and helps establish the esophagus-respiratory transcriptional boundary. T32HL007752 Pulmonary Development and Disease Pathogenesis Training Program.

Poster 67

Jordyn Mensh[1], Bonnie Wu[1], Darren Bridgewater[2], Thomas Drysdale[3], Timothy Plageman[1]

Junctional contraction of outer limiting membrane is required for normal postnatal retinal development

[1]The Ohio State University, College of Optometry, USA; [2]Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Canada; [3]Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, Western University, Canada

Congenital retinal dystrophies have a variety of genetic causes, many of which are due to disruptions in genes that regulate photoreceptor morphogenesis or the junctional integrity of the outer limiting membrane (OLM) during retinal development. For example, Crb1 is enriched within OLM and mutations in the encoding gene in humans or animal models cause retinal rosette formation and retinal degeneration due to the disruption of the cell junctions among photoreceptors and Muller glial cells. We have recently determined that the F-actin and Rho-kinase-binding cytoskeletal protein Shroom3 is strongly localized to the OLM of the developing retina. Because both Crb1 and Shroom3 are associated with regulating apical junctional contractility, we hypothesized that OLM development may similarly depend on Shroom3. The absence of Shroom3 in the neural retina causes the formation of rosettes that begin between postnatal day 2 and postnatal day 6. In addition, a significant disruption in the morphogenesis of the inner and outer segments of developing photoreceptors are observed in regions within and away from the retinal rosettes. Discontinuous localization of several adherens junctional proteins as well as disturbances to the junctional structure are also noted. Viewing the OLM *en face* revealed that a striking enlargement of the apical junctional cross-sectional area also occurs throughout the developing retina in *Shroom3* mutants. Importantly, a similar apical junctional phenotype is also observed in the *Crb1*$^{Rd8/Rd8}$mice. These data support the notion that Shroom3-dependent apical junctional contractility within the cells of the OLM is important to maintain retinal structure. Furthermore, retinal dystrophies, such as those caused by mutations in CRB1, may partially be caused by a disruption to the function of Shroom3 and the contraction of the actomyosin cytoskeleton that is closely associated with the OLM adherens junctions. *Funding: NEI (R01EY02691) and OLERF*

Poster 68

Kurt Reynolds[1], Rolf Stottmann[1,2]

Collagen processing deficiencies underlie skeletal defects and clefting in P4hb mutant mouse models

[1]Nationwide Children's Hospital, USA; [2]The Ohio State University, USA

Orofacial clefts are collectively the most common human congenital anomaly worldwide and are influenced by complex genetic and environmental risk factors. Mouse genetics provides a powerful approach for discovering and validating novel orofacial clefting genes. Through a forward genetic screen in mice, we have identified a recessive variant in P4hb which contributes to cleft palate. We further find the penetrance of the cleft palate presentation in this model is dependent on mouse genetic background, and subject to multiple modifier loci, modeling common gene-phenotype relationships in human disease. Additionally, we have generated a conditional loss-of-function P4hb model in neural crest which also exhibits cleft palate, along with excessive bruising and lack of skeletal elements in the facial tissues. Previously, the only human genetic findings are P4HB gain-of-function variants which cause a rare form of osteogenesis imperfecta called Cole-Carpenter syndrome. P4HB is an ER chaperone and catalyzes the hydroxylation of proline residues in procollagens to facilitate their helical assembly. We have begun to examine the molecular basis for the P4hb mouse phenotypes and have evidence for increased ER stress, as well as increased collagen deposition with reduced hydroxy-proline content. These results likely reflect a feedback-dependent upregulation due to altered collagen signaling resulting from incomplete processing in these mutants. Altogether, there results identify a new form of orofacial clefting caused by recessive variants in P4hb. This work is supported by a lab start up package from Nationwide Children's Hospital.

Poster 69

Matthew Riccetti[1], Patrick Woller[2,3], Amy Riesenberg[1], Avelina Lee[1,4], Matthew Revilla[3], Laura Tweedie[1,4,5], Steven Crone[2,3], Kenneth Campbell[1,4,5]

Gsx2 is essential for the neurogenesis and function of the dorsal respiratory group neurons

[1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]Neuroscience Graduate Program, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [3]Division of Neurosurgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [4]DSRM Graduate Program, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [5]MSTP Program, University of Cincinnati College of Medicine, Cincinnati, OH, USA

The dorsal respiratory group (dRG) of the hindbrain integrates sensory input to drive adaptive breathing and airway protection. Disruptions in dRG components, namely the nucleus tractus solitarius (nTS) and the developmentally related A1/C1 A2/C2 catecholamine neurons, have been implicated in post-mortem Sudden Infant Death Syndrome (SIDS); however, the molecular mechanisms involved in dRG neurogenesis remain poorly understood. In mice, homozygous loss of the homeobox transcription factor Gsx2 results in basal ganglia dysgenesis and a "hypocellular" nTS, and neonates become cyanotic and die shortly after birth. Recently, three different homozygous GSX2 variants were reported in patients who display basal ganglia dysgenesis together with dystonia. All of these patients suffer from feeding/swallowing difficulties, and two died of respiratory failure by age two, suggesting a potentially

conserved role in humans. We generated a mouse model (Gsx2Q252R) of one of these pathological human variants (GSX2Q251R). Here, we identified a nearly complete loss of glutamatergic nTS and catecholaminergic A1/C1, A2/C2 dRG neurons in Gsx2 nulls (Gsx2RA/EGFP), and a 50% reduction in these neurons in Gsx2Q252R/GFP (i.e. human variant) mice which survive into adulthood. Gsx2 is highly expressed in "dA3" progenitors of the glutamatergic/catecholaminergic dRG neurons and is essential for their specification during neurogenesis. Gsx2 null neonates display fewer apneas and aspirate milk when fed. When exposed to hypoxia, neonatal Gsx2 nulls fail to adapt respiration, while neonatal and adult Gsx2Q252R/GFP animals demonstrate a blunted initial response and fail to enter tachypneic breathing during long-term exposure. We provide mechanistic insight into how genetic disruption of dA3 neurogenesis and dRG neural circuit formation may contribute to perinatal respiratory failure and SIDS vulnerability. Funding provided by R01NS124660 (KC) and F32HD118646 (MRR).

Poster 70

Lizza Roman[1], Daniel O. Kechele[1], J Guillermo Sanchez[1], Kentaro Tominaga[2], James Wells[1,3,4]

Using human intestinal organoids to study the development of tissue-resident macrophages

[1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [2]Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan; [3]Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; [4]Center for Stem Cell and Organoid Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH,, USA

Significant advances have been made in understanding human gastrointestinal (GI) organ development, yet the role of immune cells in organogenesis remains incompletely understood. Tissue-resident macrophages (TRMs) in organs such as the brain and liver regulate immunity, maintain tissue homeostasis, and mediate responses to injury, but how TRMs acquire tissue-specific signatures and interact with other cells in developing GI tissues is unclear. Traditional organoid models lacked immune components, limiting study of these interactions. Recent studies have incorporated human pluripotent stem cell (hPSC)-derived myeloid cells into hPSC-derived intestinal organoid (HIO) cultures, enabling modeling of immune-epithelial interactions during organ development. We hypothesize that the microenvironment in developing GI tissues drives monocyte-like cells to differentiate into TRMs, performing organ-specific functions. To test this, we compared published human fetal single-cell RNA sequencing datasets with hPSC-derived monocytes/macrophages incorporated into HIOs. After two or more weeks of co-culture, organoids were subjected to transcriptional profiling and functional characterization. These macrophages exhibited transcriptional signatures resembling TRMs from corresponding fetal tissues, indicating acquisition of tissue-specific features. CellChat analyses were used to infer signaling interactions between myeloid and other intestinal cells and compare them to organoid data, enabling assessment of how closely hPSC-derived macrophages recapitulate fetal-like signaling within the intestinal niche. This organoid system provides a powerful platform to investigate molecular mechanisms of TRM development and their role in GI health and disease, including inflammatory bowel disease. This research was supported by NIH grants R01 CA272903, P01 HD093363, the Shipley Foundation, the Allen Foundation, and the Digestive Disease Research Center (P30 DK078392).

Poster 71

Nicholas Russell[1,2], Sarah Trovillion[1,2], Natalia Bottasso-Arias[1,2], Daniel Swarr[1,2,3], William Zacharias[1,2,3], Debora Sinner[1,2,3]

Defining the Role of Chromatin Remodeling in Respiratory Tract Development.

[1]Perinatal Institute, Cincinnati Children's Hospital and Medical Center, USA; [2]Division of Pulmonary Biology, Cincinnati Children's Hospital and Medical Center, USA; [3]Department of Pediatrics, University of Cincinnati College of Medicine, USA

The chromatin remodeling complex BAF, is essential for regulating gene expression by altering chromatin accessibility. Two mutually exclusive subunits of this complex are ARID1A and ARID1B, which are pivotal for pluripotency, differentiation, and tissue development. *Arid1a*'s role in pulmonary alveolar type I cell differentiation is irreplaceable and the deletion of *Arid1a/Arid1b* from the respiratory endoderm (dcKO) leads to a halt in lung and tracheal formation and cell specification. Our goal was to determine the molecular mechanisms by which ARID1A and ARID1B promote differentiation of epithelial and mesenchymal lineages in the mouse respiratory tract. Typically, *Nkx2.1* is expressed in the trachea and lung epithelium. In dcKO, it is drastically reduced throughout the epithelium, along with mislocalization of TRP63, indicating disrupted epithelial cell specification and patterning. The trachea and esophagus do not separate, and epithelium appears dorsalized with preserved ventral identity of the mesenchyme in the TE region based on SOX9-positive cells. Interestingly, tracheal smooth muscle was absent. To define transcriptional profiles and signaling pathways affected after deletion of *Arid1a/Arid1b*, we performed RNA-seq of E10.5 control and dcKO foreguts. Through Gene Set Enrichment Analysis, we identified enriched signatures related to chromatin remodeling activity, epithelial cell differentiation, cell-cell adhesion, semaphorin-plexin signaling and β-catenin binding. Validation of the RNA-seq determined changes in Wnt targets and ligands, adhesion molecules and Bmp ligands. In conclusion, *Arid1a/Arid1b* are indispensable for TE resolution and lung branching morphogenesis. At the molecular level, the BAF complex regulates the expression of *Nkx2-1* and critical signaling pathways, including Wnt and Bmp, necessary for respiratory epithelial and mesenchymal cell differentiation. Partially supported by NIH HL156860, HL144774 and the Schmidlapp Program.

Poster 72

Kristina Sattler[1], Christoph Lepper[1], Kelly Crowe[2]

Evaluating lectin biomarkers for GNE Myopathy gene therapy

[1]The Ohio State University, USA; [2]Xavier University, USA

GNE myopathy (GNEM) is a rare, autosomal recessive disease characterized by progressive skeletal muscle weakness and wasting. GNEM is caused by biallelic mutations in the UDP-N-acetylglucosamine(GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase (*GNE*) gene, which encodes a bifunctional enzyme that catalyzes two consecutive steps of sialic acid (SA) biosynthesis. SA is the terminal sugar in sugar chains attached to glycolipids and glycoproteins on the extracellular surface of membrane proteins, and functions in cell communication and cell-cell adhesion. Mutations in the *GNE* gene significantly decrease SA production, leading to skeletal muscle pathology through an unclear mechanism. Gene therapy for GNEM is in development, which would provide a corrected copy of the

GNE gene to the muscle. Although it may be difficult to readily determine clinical efficacy of such a therapy due to the slow progression of GNEM, a robust molecular biomarker could allow detection of molecular changes in SA levels that may precede signs of clinical efficacy of gene therapy. Immunostaining with lectins, enzymes which bind glycans in a linkage-specific manner, have individually shown altered binding in GNEM models and patient samples; however, they have not been directly compared across multiple *GNE* gene mutations and GNEM models to determine the most efficient therapeutic biomarker. Here, we characterize a panel of SA- and subterminal glycan-binding lectins to determine which is most robustly changed in conditions of altered sialylation, such as in GNEM. We determined a subset of lectins that show consistent, significant differences in binding in conditions of altered sialylation, which could be suitable biomarkers to reflect restoration of sialylation that would occur in skeletal muscle after *GNE* gene therapy. This work was supported by the Neuromuscular Disease Foundation (NDF).

Poster 73

Kiera Savage, Ian McCrary, Amy Ralston, Ph.D.

Investigating the mechanisms of primitive endoderm lineage specification *in vitro* using mouse primitive endoderm stem cells

Michigan State University, USA

During the earliest stages of embryonic development, cells undergo a series of lineage decisions that determine their developmental fate. These decisions are controlled by a complex gene regulatory network, and investigation of these pathways is crucial to understanding the mechanisms of early development as well as the origins of developmental abnormalities. When the embryo reaches the blastocyst stage, three distinct stem cell progenitor lineages are present: (i) the epiblast, which gives rise to the fetus, (ii) the trophectoderm, which becomes the placenta, and (iii) the primitive endoderm (PE), which develops into the yolk sac. To accurately model early embryogenesis *in vitro*, the ability to derive and maintain each of these stem cell lineages in culture is essential. While sophisticated *in vitro* models of all three lineages exist, widely used models of the primitive endoderm - such as extraembryonic endoderm (XEN) cells - appear to represent later stage PE derivatives, and exhibit important differences in gene expression and differentiation potential compared to newly specified PE cells within the embryo (Kunath et al., 2005). However, a recently established stem cell line known as primitive endoderm stem cells (PrESCs) has been shown to more closely represent the early PE as it exists *in vivo* (Ohinata et al., 2022), providing a novel platform to study the regulatory events underlying initial PE lineage specification. In this project, I aim to derive PrESCs from mouse blastocysts of various genetic backgrounds and use them to investigate the gene regulatory mechanisms that drive commitment to the PE lineage. Gaining insight into this vital cell fate decision will enhance our understanding of early development and may inform future strategies in reproductive health and regenerative medicine. This project is funded by the National Institutes of Health award R35 GM131759 to AR.

Poster 74

Nour Sayed[1], Jacob Weaver[1], Brad Wagner[1], Milan Jamrich[2], Paul Overbeek[2], Anil Upreti[3], Michael Robinson[1]

Functional studies of mice with novel Foxe3 mutations

[1]Miami, USA; [2]Baylor College of Medecine, USA; [3]Harvard Medical School, USA

FOXE3 is a key transcription factor for lens development. Mutations in mice and humans cause severe ocular defects including microphthalmia, aphakia, and cataracts. While FOXE3 contains a conserved Forkhead domain, the functions of its N and C-terminal domains remain unknown. This study aims to investigate these domains. CRISPR/CAS9 genome editing was used to create a deletion of 12 bp, including the start codon which deletes the entire N-terminal FOXE3 domain due to the utilization of a downstream start codon. In addition, several unique deletions leading to frameshift mutations in the C-terminal domain of FOXE3 were created. Finally, the insertion of an mCherry coding sequence replaced the endogenous FOXE3 start codon to create a null Foxe3 allele. Mutant mice were bred to homozygosity, and lens phenotype, transcript levels (ddPCR), and protein expression (Western blot) were analyzed. Mice lacking the N-terminal domain developed normal embryonic lenses but postnatal cataracts. Foxe3 null (mCherry) mice displayed severe microphthalmia and cataracts, with absent FOXE3 protein and reduced transcripts. Three frameshift mutations were made in the C-terminal domain resulting in the loss of either the last 43 (MLR69), 47aa (MLR68 and MLR70) of the FOXE3 protein. MLR68 exhibited congenital cataracts and microphthalmia, MLR69 led to postnatal cataracts, and MLR70 maintained clear lenses >1 year. ddPCR showed reduced transcripts in all mutants (MLR68 lowest, MLR69 intermediate, MLR70 near wild type). Western blot confirmed corresponding protein reductions. Differences between MLR68 and MLR70 may be explained by the deletion of the transcriptional terminator, a novel 3'UTR as well as two additional genomic insertions in MLR68. Neither the N-terminal domain nor the last 47 aa of the C-terminal domain are essential for embryonic lens development. The unexplained reduction in transcript in Foxe3 null mutants suggests potential autoregulation. NEI R21 EY033471

Poster 75

Vered Shacham-Silverberg[1,2], Daniel O. Kechele[1,2], Mansa Krishnamurthy[3], James M. Wells[1,2,3]

Modeling Human Esophageal Development and Disease Using iPSC-Derived Epithelial-Mesenchymal Raft Cultures

[1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA; [2]Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA; [3]Division of Endocrinology, Cincinnati Children's Hospital Medical Center, USA

Insights into human esophagus development remain limited due to the scarcity of embryonic tissue and the substantial divergence between human and commonly studied animal models. To overcome this barrier, we developed a human *in vitro* platform using induced pluripotent stem cell (iPSC)-derived three-dimensional esophageal raft cultures (ERCs) that incorporate both epithelial and mesenchymal lineages. By co-differentiating both lineages in a shared culture system, we established self-organizing structures that recapitulate hallmark features of the developing human esophagus. These cultures

exhibit stratified squamous epithelium and underlying mesenchyme, express canonical markers of esophageal lineage commitment, and closely resemble human fetal esophageal tissue. We leveraged this platform to interrogate the developmental consequences of genetic and environmental perturbations. ERCs derived from iPSCs of a patient with a heterozygous RFX6 mutation recapitulated some of the *in vivo* phenotype, including ectopic expression of gastric markers within the esophageal epithelium. Additionally, we modeled Barrett's esophagus by exposing ERCs to acidic conditions, inducing metaplastic changes reflective of the disease. Together, these data highlight the utility of this human-specific model in elucidating esophageal development and establishing a foundation for studying congenital anomalies and acquired diseases of the esophagus. This work is funded by NIH/NICHD P01 HD093363, NIH/NCI RO1 CA272903-03 and NIH/NIDDK P30DK078392.

Poster 76

Emily Shifley

Involving developmental biology students in science education outreach

Northern Kentucky University, USA

Developmental biology classes provide many engaging and accessible examples of core biological principles such as evolution, cellular and molecular biology, and the relationship between structure and function, which can be shared with a wide audience. At Northern Kentucky University, undergraduate students in a developmental biology course were assigned a science education outreach project to design and lead hands-on activities for 4th grade students visiting campus for a STEM day. These activities included crafts or games that helped illustrate concepts such as insect life cycles, evolution of bird beaks, genetic control of traits, and organ formation and function. This project supported a departmental goal to foster strong communication skills in our undergraduates while also offering local elementary students an opportunity to explore developmental biology concepts aligned with Kentucky academic science standards.

Poster 77

Charukesi Sivakumar, Qiang Li, Jing Xu, Hana Pan, Rajesh Rao

Elucidating the role of MOF in retinal development and disease

University of Michigan - Ann Arbor, United States of America

Males absent on the first (MOF) is a lysine acetyltransferase, catalyzing the acetylation of histone H4 at lysine 16 (H4K16ac) which remodels chromatin, promoting an active state of gene expression. In 2020, MOF was implicated in a congenital disease, LIGOWS, wherein patients with missense mutations in both domains of *Mof* present with developmental delays as well as vision defects. Previous work in the lab shows that the conditional knockout of *Mof* using *Dkk3*-Cre results in the loss of retinal ganglion cells at embryonic day 11.5/12.5. However, the molecular mechanisms by which MOF regulates eye field and RPC development and how it affects the terminal differentiation of all retinal cell types remain unknown. Our central hypothesis is that MOF drives retinal differentiation through: 1) regulating cell proliferation and cell death during eye development and 2) the direct activation of eye field transcription factors

(EFTF) and retinal specification genes. Through generation of a *Mof* KO model in mouse embryonic stem cells (mESCs), we have shown that retinal differentiation to mouse retinal organoids (mRO) is impaired. qPCR analysis in *Mof* KO mROs shows that key EFTF mRNA expression is repressed. Further experiments exploring the global changes in gene expression and localization patterns of MOF and H4K16Ac on chromatin will be performed on KO mROs. Overall, these studies suggest MOF is essential for early retinal development, and future work will provide additional insight of the molecular mechanisms MOF in the eye. This work is supported by UM Rackham Student Research Grant, Research to Prevent Blindness (RPB), the Beatrice & Reymont Paul Foundation, March Hoops to Beat Blindness, Leonard G. Miller Endowed Professorship and Ophthalmic Research Fund at the Kellogg Eye Center, Grossman, Elaine Sandman, Marek and Maria Spatz (endowed fund), Greenspon, Dunn, Avers, Boustikakis, Sweiden, and Terauchi research funds.

Poster 78

Morgan Smith

COPB2 and Corticogenesis: How Vesicle Trafficking Shapes Brain Development

Nationwide Children's Hospital, USA; The Ohio State University, USA

Autosomal recessive primary microcephaly (MCPH) is a congenital disorder characterized by a pronounced reduction in brain size, mild to severe cognitive impairment, and seizures. The developing mammalian brain relies on proper proliferation and differentiation of neural progenitor cells to produce the correct number of mature neurons. Disturbances in a variety of cellular processes can compromise this process, ultimately leading to fewer neurons and a drastic decrease in overall brain size. A homozygous missense variant in the *Coatomer Protein Complex, Subunit Beta 2* (*COPB2*) gene has been identified as a pathogenic mutation associated with primary microcephaly. *COPB2* encodes the β'-COP subunit of the COPI coatomer complex and is essential for Golgi-ER intracellular trafficking. Identification of this variant highlights the critical role of vesicle trafficking in proper neuronal development. While coatomer complexes have been well established, the individual role of each subunit in specific cell types, such as neurons, and their cargo specificity remains unresolved. Our study aims to investigate the distinct involvement of *Copb2* in neurogenesis and assess how mutations in the protein disrupt cortical development. We hypothesize that COPB2 directs the trafficking of proteins essential for neural progenitor proliferation and differentiation, and that alterations in its binding domain compromise trafficking efficiency, thereby reducing neuronal output. Our approach employs a novel allelic series in mice to define COPB2's role in mammalian corticogenesis and early neurogenesis through both standard and conditional knockout models. We will present evidence that the β' subunit of the COPI coatomer complex is indispensable for embryogenesis and for maintaining neural progenitor cell survival. This research is funded by the Cellular and Molecular Biology T32 6218701 (to M.E.S.)

Poster 79

Anna Statsenko[1], Vishista Vulpala[2], Caiden Duskey[1], Lucy Bailey[1], Elke Buschbeck[3], Mark Charlton-Perkins[1]

Studying Genetic Forms of High Myopia in Developing Zebrafish Larvae

[1]Miami University, USA; [2]William Mason High School , United States; [3]University of Cincinnati , United States

High myopia is a severe, early-onset form of nearsightedness that is typically diagnosed in children by the age of two and is strongly linked to genetic factors. Unlike low or moderate myopia, which can be influenced by environmental conditions, high myopia is most often caused by mutations that disrupt the normal developmental growth of the eye. Children with high myopia are at significantly increased risk for vision-threatening complications, including cataracts, glaucoma, and retinal detachment, making it an urgent pediatric health concern. We developed novel zebrafish models to investigate the developmental mechanisms underlying high myopia. Zebrafish provide distinct advantages for this work: their eyes develop rapidly and transparently, enabling direct imaging of ocular growth, and their genetic tractability allows rapid modeling of human disease alleles. Importantly, zebrafish undergo axial elongation of the eye, a conserved developmental process that is disrupted in high myopia. Using CRISPR-Cas9 mutagenesis, we targeted three human genes associated with high myopia: Lrpap1, expressed in the choroid and linked to TGF-β signaling; Opn1lw1, a cone opsin with no established role in myopia; and Znf644, a transcription factor expressed in the retina and RPE whose function in eye development has not been studied. Mutations in each gene produced robust myopic phenotypes in zebrafish. To capture these developmental changes, we employed optical coherence tomography (OCT) alongside a newly adapted fluorescence-based micro-ophthalmoscopy technique, which provided sensitive, non-invasive measurements of refractive error in vivo. This zebrafish platform offers a unique developmental system to dissect the genetic pathways driving high myopia and to reveal how disruptions in early ocular growth lead to pediatric vision disorders.

Poster 80

Matthew Stocker[1,2], Lee Halevi[3], Lisa Gutzwiller[1], Olfri Axelrod[3], David Sprinzak[3], Brian Gebelein[1]

Quantitative analysis of the Notch transcriptional response using synthetic biology and *Drosophila* genetics

[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati, USA; [3]Tel Aviv University , Israel

Notch signaling is crucial for development as Notch pathway haploinsufficiency can cause syndromes that impact multiple tissues and organs. While Notch is known to convert extracellular interactions into changes in gene expression, why some target genes are highly sensitive to gene dose is not well understood. Notch signaling is initiated by ligand-induced cleavage of Notch, releasing the Notch intracellular domain (NICD) to transit into the nucleus and activate transcription in complex with the CSL transcription factor. The CSL-NICD complex activates genes via two types of DNA sites: monomer sites and cooperative dimer sites. However, CSL can also directly antagonize Notch target gene expression by recruiting a co-repressor (co-R). Unlike the CSL-NICD activator, the CSL-coR complex only binds DNA via a

non-cooperative mechanism. While these complexes equally compete for monomer sites, CSL-NICD cooperativity provides a ~20-fold greater probability for dimer sites to bind the activator complex than the repressor complex. Here, we created a series of *Drosophila* GFP reporter lines that only differ in the type and number of DNA sites and use genetics to manipulate levels of Notch and the Hairless corepressor. These studies revealed: (1) A distinct threshold of cooperative versus non-cooperative DNA binding sites is required for gene activation. (2) Reporter strength is dependent upon the number and type of DNA sites. (3) Reporters with non-cooperative sites are much more sensitive to Notch and Hairless haploinsufficiency than reporters with cooperative sites. These data were used to build a statistical mechanics model of Notch transcription that fits closely to experimental results, and ongoing studies are focused on testing this model using new synthetic reporters. Future studies will focus on testing the hypothesis that the most Notch dose sensitive target genes will be preferentially regulated via enhancers with non-cooperative sites. NSF/MCB-BSF:2114950 to BG and DS

Poster 81

Nicholas Strash[1], Abigail Vallie[1], Sha Huang[1], Marisa Patsy[2], Nenad Bursac[2], Jason Spence[1]

Scalable Engineered Human iPSC-derived Intestinal Tissues with Uniaxial Tension

[1]University of Michigan, United States; [2]Duke University, United States

The Spence lab has developed a versatile iPSC-derived human intestinal organoid (HIO) platform for studying how various perturbations affect intestinal development and maturation. This 3D culture system recapitulates key aspects of human intestinal development in vitro and can be transplanted into mice for advanced tissue maturation. My research focuses on improving the reproducibility and scalability of HIO production, as well as applying uniaxial tension in vitro to orient cells, both of which we hypothesize will enhance HIO maturation and overall utility. To do this, monolayers from HIO differentiations are dissociated and encapsulated in a fibrin-Matrigel hydrogel which is then dispensed into either cylindrical or square sheet configurations both supported by thin nylon frames to try to promote anisotropic and isotropic cellular orientation, respectively. These free-floating constructs are then cultured for up to six weeks, by which time we have found the tissues to contain the same cell types we expect to see in HIOs: intestinal epithelium, mesenchyme/stroma, endothelium, enteric neurons, smooth muscle, and serosal mesothelium. Optimization of this system has included testing different starting cell densities, culture durations, hydrogel stiffness via fibrin concentration, and supplementation with additional smooth muscle cells differentiated in parallel. Future work will include more comparative analysis between these constructs and our conventional HIOs and exploration into how uniaxial tension in the cylindrical tissues may influence HIO maturation. Beyond that, this platform offers a way to scale-up HIO size and incorporate cells produced separately from the HIO differentiation, both of which we can leverage in future studies. This work was supported by NIH grants U01DK103141, R01DK137806, and RC2DK140862 and grant CZF2019-002440 from the Chan Zuckerberg Initiative to J.R.S.

Poster 82

Mariya Kibtiya Sumiya, Elizabeth M. Duncan

Exploring the Functional Conservation of shoc2 in ERK-mediated Tissue Homeostasis and Regeneration of *Schmidtea mediterranea*

University of Kentucky, USA, United States

The ERK1/2 (Extracellular Signal Regulatory Kinase 1/2) signaling pathway is an critical regulator of cell proliferation, differentiation, wound healing and regeneration across diverse organisms, including mammals, fish, hydra, and planarian. In regenerative spiny mice, sustained ERK activation promotes tissue regeneration instead of scarring, but the mechanisms remain unclear. SHOC2, a conserved scaffolding protein, regulates ERK1/2 activation and has key roles across species from Noonan syndrome in humans, to neural crest development and hematopoiesis in zebrafish, and embryonic viability in mice highlighting its broad biological significance. Our work identified a planarian shoc2 transcript upregulated in the outer epithelium after injury, suggesting a role in regeneration. Further BLAST analysis revealed the presence of three putative SHOC2 homologs, now named A, B, and C. Single-cell RNA-seq data showed distinct expression patterns: shoc2A is broadly expressed in parenchymal, neuronal, muscle, late epithelial progeny; shoc2B is enriched in muscle, neuronal and parenchymal cells and shco2C has clear expression in parenchymal and pigmented cells. Functional knockdown studies revealed that *shoc2A(RNAi)* caused asymmetrical blastema formation, *shoc2B(RNAi)* led to loss of eyespots and feeding defects while *shoc2C(RNAi)* produced no obvious phenotype. To test the functional conservation of these homologs with vertebrate SHOC2, I am using immunoblotting to detect the activation of ERK in protein extracts from shoc2A, shoc2B, and shoc2C knockdown worms compared to controls after injury. Collectively, this will allow me to assess whether any of these putative planarian SHOC2 homologs share conserved molecular roles, i.e., ERK pathway activation, or if they operate as scaffolds of other signaling pathways. This work is funded by NIH grant R35GM142679.

Poster 83

Thomas Taylor, Matthew Riccetti, Jenna Green, Kimberly Wagner, Cheng-Lun Na, Anne-Karina Perl

Homeobox Transcription Factor MEOX2 functions as a key regulator of the developmental alveolar niche

Cincinnati Children's Hospital Medical Center, USA

Premature birth disrupts the final process of embryonic lung development (alveolarization), a complication that eventually develops into bronchopulmonary dysplasia (BPD). During alveolarization, three types of functionally distinct PDGFRα+ fibroblasts define the alveolar space: matrix fibroblasts, secondary crest myofibroblasts (SCMF), and lipofibroblasts. The first phase of alveolarization is defined by the contraction of the SCMFs, creating secondary septa that greatly increase lung surface area. The second phase is characterized by SCMF apoptosis and microvasculature remodeling that results in a mature capillary network underneath AT1 epithelial cells. Mesenchyme homeobox 2 (MEOX2) is signature transcription factor of lung matrix fibroblasts. Existing literature and preliminary data focused on FGF receptors in the lung identified MEOX2 as a primary candidate for cell identity in alveolar fibroblasts, so we generated $Pdgfra^{CreER}/Meox2^{Flx/Flx}$ mice to delete *Meox2* postnatally in pulmonary

mesenchyme. Histology and transmission electron microscopy show that *Meox2*$^{\Delta/\Delta}$ lungs undergo severe alveolar simplification and develop an impaired blood-air barrier, morphological traits that correspond to BPD. Single-cell RNA sequencing revealed extreme differences localized to the AF1 celltype (a subtype of matrix fibroblasts), which shift towards a myofibroblast-like identity. Epithelial differentiation programs halt at P3, an effect that is recapitulated in our primary-cell organoid system. *Cxcl14*, encoding a ligand competitive to angiogenesis-driving chemokine CXCL12, was the most upregulated gene and presents itself as a promising candidate for the manipulation of an endothelial differentiation defect identified in the scRNA-sequencing, and it further supports the developing idea of MEOX2+ fibroblasts as key regulators of the developmental alveolar niche. This study was supported by NIH/NHLBI grants R01 HL167030, R01 HL164418, and F31 HL162470.

Poster 84

James "Kenny" Triplett[1,2], Xiaowen Zhong[1,2], Arman Esshaghi Bayat[2], Dazhuan Xin[2], Eva Nicholson[2], Nong Chen[2], Richard Lu[1,2]

Native Histone H3.3K27M Mutation on Developing Brain and Diffused Midline Glioma (DIPG/DMG)

[1]University of Cincinnati , United States; [2]Cincinnati Children's Hospital, United States

The H3.3K27M oncohistone defines a majority of diffuse midline gliomas (DMGs), including diffuse intrinsic pontine gliomas (DIPGs), yet how this mutation reprograms neurodevelopment to create a glioma-susceptible cellular ecosystem remains unresolved. Here, we develop a Cre-inducible knock-in mouse model expressing untagged H3.3K27M to interrogate its developmental impact across the cortex, cerebellum, and pons. In contrast to prior overexpression systems, our model preserves native histone structure, revealing that H3.3K27M expression in neural progenitors elicits widespread brain malformations, including cortical dyslamination, cerebellar hypoplasia, and motor deficits. Mechanistically, H3.3K27M promotes expansion of progenitor populations, impairs neuronal and glial differentiation, and disrupts regional transcriptional programs governing pontine patterning. These developmental defects are accompanied by global loss of H3K27me3, highlighting a failure of epigenetic repression that locks cells in a proliferative, stem-like state. Our findings position H3.3K27M as both an epigenetic disruptor and developmental regulator, establishing a molecular and cellular framework for glioma initiation. This work uncovers critical windows of vulnerability in brain development that may be leveraged for early therapeutic intervention in pediatric high-grade gliomas.

Poster 85

Esther Ushuhuda[1], Jenniluyn Nguyen[2], Natalie Pfaltzgraff[1], Matthew Kofron[1], Maria Mikedis[1,2]

MEIOC prevents continued mitotic cycling and promotes meiotic entry during mouse oogenesis

[1]Cincinnati Children's Hospital , USA; [2]Whitehead Institute, United States

In multicellular organisms, germ cells' transformation into haploid gametes requires that they transition from mitosis to meiosis, whereby they stop mitotic cycling and enter the meiotic cell cycle. In mammals, transcriptional activator STRA8-MEIOSIN mediates the decision to enter the meiotic cell cycle by triggering the G1-to-meiotic S phase transition. However, the molecular basis by which mammalian germ

cells prevent continued mitotic cycling before entering the meiotic cell cycle remains unclear. Here, we investigate MEIOC's role in the mitosis-to-meiosis transition during mouse oogenesis by analyzing proliferation, cell cycle transcriptomics, and cell cycle-associated protein expression. MEIOC was previously shown to destabilize mRNA and repress the mitotic program after meiotic entry. Here, we demonstrate that MEIOC prevents continued mitotic cycling prior to meiotic entry in oogenic cells. We find that the mitosis-to-meiosis transition involves the repression of G1/S cyclin CCNA2 at the transcript and protein levels, and that MEIOC downregulates CCNA2 protein expression. In addition, MEIOC promotes entry into meiotic S phase by increasing Meiosin transcript abundance and consequently activating the STRA8-MEIOSIN transcription factor. Given that STRA8-MEIOSIN upregulates Meioc expression, MEIOC and STRA8-MEIOSIN form a positive feedback loop to reinforce timely meiotic initiation. We also demonstrate that BMP signaling halts mitotic cycling and promotes meiotic entry by upregulating MEIOC. We conclude that, in mouse oogenic cells, the transition from mitosis to meiosis occurs as two molecularly regulated steps– (i) halt of mitotic cycling and (ii) entry into the meiotic cell cycle – and that MEIOC modifies the cell cycle program to facilitate both steps in this transition. This work was supported by NICHD award R00HD097285.

Poster 86

Madison Velez, Abigale Wisenbach, Katie Mullin

Developmental shaping by the dual effects of elevated ambient temperature and Bisphenol-S exposure examined in fruit flies (*Drosophila melanogaster*)

Saginaw Valley State University, USA

In this research, we examine dual impacts of non-lethal, higher ambient environmental temperatures simultaneously with Bisphenol-S on the shaping of behavioral and physiological responses of fruit flies. We present data on larval travel distances, pupation location measures, and on locomotor behavior of two strains of this species. This work highlights potential impacts of global warming, through elevated environmental temperatures on *Drosophila melanogaster.* Flies are ectothermic and exhibit sensitivity to thermal changes. Developmental temperatures experienced impact locomotor activity in flies, meaning ambient temperature experienced during development influences the thermal performance curves for locomotor activity in adult flies. Development at elevated temperatures is also associated with lowered overall activity levels, suggesting early high thermal environments exert lasting impacts on adult physiology and behavior. Variations in developmental temperatures also are associated with shaping behavior in flies. At cooler temperatures, flies develop more neural connections and display heightened attraction to certain odors, indicating temperature during development imparts lasting effects on sensory processing. Climate change, exacerbated by use of carbon-based fossil fuels leading to the emission of greenhouse gases therefore has the potential to impact development of this species. Chemical pollutants in the environment also shape organismal development. Many plastics of daily life incorporate a chemical compound, Bisphenol-S, imbued in their makeup. Various bisphenols are recognized as chemicals of concern as they are potential endocrine disrupting pollutants. Bisphenol-S leaches from plastics and is inadvertently ingested by organisms. We examine potential synergistic

effects of environmentally elevated temperatures with simultaneous endocrine disruption on organisms. Funded in part by the Field-Spicer Research Fellowship.

Poster 87

Isha Verma

Developmental biology teaching lessons on regeneration and stem cells using the Planarian model system

University of Michigan, Ann Arbor, USA

Planarians have been adopted as a model system for studying regeneration and stem cell biology because they can regenerate an entire organism from virtually any portion of their bodies in a relatively short period of time. Planarians provide an excellent model system for studying the underlying mechanisms and the evolutionary aspects of regeneration. Additionally, planarians can be easily maintained and are affordable. We performed a teaching activity focused on regeneration and stem cells using the Planarian as the model system. This activity was performed as part of the Developing Future Biologist (DFB) program at the University of Michigan, Ann Arbor. DFB organizes a week-long summer short course to teach undergraduate students fundamental concepts in developmental biology. The learning goals of this activity were to understand the regulation of regeneration in planarians and its relation to human physiology, learn the function of multipotent stem cells and dedifferentiation in regeneration and how this differs from wound healing, and explore how research using these model organisms can help us better understand human physiology and pathology. Planarians were shipped to students' homes. The students cultured them and performed different types of cuts, including bisection, down the midline, between the head, between bottom, and trisection. Students took photos and videos every day to record regeneration. After a few days, planarians regenerate their lost body parts. Complete regeneration took around 2-3 weeks. Students were further encouraged to look into the roles of various signaling pathways, such as BMP, Hedgehog, and retinoic acid, in regeneration. Overall, planarians provide a low-cost and accessible model system for studying various aspects of the regeneration response. (Funding: University of Michigan, Ann Arbor).

Poster 88

Bennett Andrassy, Maximos McCune, Beija Waltmunson, Sarah Petersen

Co-Migration of Peripheral Axons and Neural Crest Cells Requires *tcf15/paraxis*

Kenyon College, USA

Development of peripheral nerves requires coordinated migration of nascent axons and neural crest cells (NCCs), which aid axonal guidance and stabilization. NCCs differentiate into Schwann cells (SCs), the myelinating glia of the peripheral nervous system (PNS) which facilitate signal transmission along axons. In zebrafish with mutations in the bHLH transcription factor *tcf15*, the mechanosensory posterior lateral line nerve (PLLn) shows mispatterning of both axons and glia (Limbach *et al.* 2022). We therefore hypothesized *tcf15* is required for axon/NCC co-migration in early neurodevelopment. To test this, we utilized in vivo time-lapse to image axons (*nbt:dsRed*) and NCCs (*sox10:GFP*) in wild-type sibling (WT) and

mutant zebrafish larvae. We found that NCCs failed to remain associated with the PLLn in mutants. In the anterior, NCCs cluster adjacent to the PLLn ganglion, as do melanocytes that share NCC precursors with SCs. However, NCCs in mutants remain in a migratory state without elongation along axons, suggesting defects in directed migration and contact-mediated differentiation into SC precursors. Quantification of NCCs in *tcf15* mutants was similar to WT, showing that *tcf15* influences NCC migration, adhesion, and differentiation more so than proliferation or survival. These findings indicate that *tcf15* regulates PNS development by promoting NCC adhesion and co-migration between NCCs and developing nerves. As *tcf15* is predominantly expressed in developing muscle, we hypothesized that *tcf15* non-cell autonomously promotes ErbB signaling in NCCs, allowing proper PNS development. We are currently investigating ErbB expression and signaling in *tcf15* mutants via HCR. Our work characterizes genetic determinants of peripheral neurodevelopment, contributing to an increased understanding of mechanisms underlying human demyelinating disorders. This work was supported by NSF BIO-IOS #1941664 and Kenyon College Summer Science Scholars fellowships.

Poster 89

Chin Wang[1,2,3], Hannah Lathrop[2,4], Wen Tang[1,2,3,4]

Determining the function of human TDRD7 in RNA granule formation

[1]Department of Biological Chemistry and Pharmacology, The Ohio State University (OSU), United States; [2]Center for RNA Biology, OSU, United States; [3]Molecular, Cellular and Developmental Biology Program, OSU, United States; [4]Ohio State Biochemistry Program, OSU, United States

Ribonucleoprotein condensates are membraneless organelles assembled by proteins and RNA. Human Tudor domain-containing 7 (TDRD7) is a key component of cytoplasmic RNA granules (RGs) and is critical for lens and spermatogenic cell differentiation. Deficiency in TDRD7 causes congenital cataracts and male sterility. Previous studies and my unpublished work suggest that TDRD7 associates with DDX4 in germ cells and is critical for RG assembly, yet the mechanisms by which TDRD7-RGs function in lens and sperm cell development remain poorly understood. Preliminary data show that deletion of both mLOTUS domains results in cytoplasmic diffusion, whereas removal of all three Tudor domains shifts TDRD7 to the nucleus. Using AlphaFold multimers prediction and mass spectrometry of TDRD7 immunoprecipitation from cow eye lysates, I identified several candidate DEAD-box proteins (DDX). Co-expression experiments in U2OS cells revealed a specific interaction between TDRD7 and DDX19A. These findings support the hypothesis that distinct TDRD7 domains determine its subcellular localization and that TDRD7 interacts with DDX19A to form cytoplasmic RGs during eye development. My ongoing experiments, utilizing genetic, biochemical, and quantitative cell biology approaches, aim to further characterize TDRD7 localization and dynamics to uncover its mechanistic role in RG assembly. This work was supported by grants from the National Science Foundation and the Center for RNA Biology at The Ohio State University.

Poster 90

Bailey Weatherbee[1], Scott Rankin[1], Yann Audic[2], Agnes Mereau[2], Luc Paillard[2], Shreyasi Mukherjee[3], Hannah Truong[1], Aaron Zorn[1]

Multifunctional RNA-binding Transcription Factors Coordinate Cell States in Development

[1]Cincinnati Children's Hospital Medical Center, USA; [2]Institute Genetics & Development of Rennes, France; [3]Harvard Medical School, USA

Gene expression requires the coordinated regulation of multiple molecular events, including chromatin state changes, transcription, RNA processing, and ultimately, translation of proteins. Precise control over these processes is particularly important during development when pluripotent cells make a series of fate decisions, which, when disrupted, can result in pregnancy loss and congenital disease. Most studies have focused on transcription factors' interaction with chromatin, with much less attention on post-transcriptional regulation. Emerging evidence indicates that some DNA-binding transcription factors may regulate RNA, but very little is known about the biological impact, underlying mechanisms, or the prevalence of this phenomenon. Here, we hypothesize that some **lineage-defining transcription factors also bind RNAs and regulate post-transcriptional events to coordinate differentiation**. SOX factors are candidate RNA-binding transcription factors critical for embryonic development. During gastrulation, SOX17 is required for definitive endoderm differentiation (early digestive system precursor). Subsequently, the foregut endoderm (precursor to stomach, lungs, etc.), will re-express pluripotency-factor SOX2, promoting organogenesis. By combining the advantages of human stem cell models and in vivo functional genomics in Xenopus, we have identified target SOX2 and SOX17 RNAs, begun interrogating the developmental significance of the SOX RNA-binding domain, and defined the partner RNA-binding proteins mediating SOX-RNA regulation. Broadly, this work will define new mechanisms for the coordination of transcriptional and post-transcriptional regulation essential for cell differentiation. Several congenital abnormalities and cancers are associated with variants in SOX RNA-binding regions, and we thus also anticipate this work will identify new candidates for therapeutic intervention.

Poster 91

Jacob Weaver[1], Anil Upreti[2], Jared Tangeman[3], Milan Jamrich[4], Paul Overbeek[4], Brad Wagner[1], Michael Robinson[1]

Upregulation of multiple neural transcripts in FoxE3 null lenses

[1]Miami University, USA; [2]Harvard Medical School, USA; [3]Johns Hopkins University, USA; [4]Baylor College of Medicine, USA

Purpose: FOXE3, a transcription factor (TF) expressed in the lens, is essential for lens formation. Expression begins in the lens placode shortly after Pax6. Mutations or loss of Foxe3 in humans and animal models produce severe ocular defects, including aphakia, Peters anomaly, and cataracts. This study examines how FOXE3 shapes gene expression and chromatin regulation during lens development. **Methods:** Foxe3 knock-out (KO) mice were created by CRISPR/Cas9 knock-in of mCherry at the Foxe3 locus in FVB/N mice, preventing translation of the FOXE3 protein. Lenses from wild-type (WT) and KO embryos were collected at E12.5, E15.5, and P0.5 for RNA sequencing. CUT&RUN was performed on P0.5 lenses to define FOXE3 binding sites and derive a de novo motif. **Results:** RNA-seq detected 376 DEGs at

E12.5, 1239 at E15.5, and 2553 at P0.5 (LFC > 1, Padj < 0.005). K-means clustering identified two consistent groups: 196 downregulated (cluster 1) and 279 upregulated (cluster 2). GO terms highlighted “Lens Fiber Cell Differentiation” (cluster 1) and “Nervous System Development” (cluster 2). Cluster 1 included core lens genes Bfsp1, Hsf4, Pitx3, and Maf, while cluster 2 featured neural TFs Vsx2, Otx2, Rorb, Sox4, and Sox11. CUT&RUN revealed FOXE3 occupancy near genes from both clusters, including Bfsp1, Pitx3, Sox11, Rorb, and Sox4. Motif analysis produced a FOXE3 motif similar to FOXD3 consensus. **Conclusion:** Loss of FOXE3 yields more upregulated than downregulated genes, suggesting a primary repressive role. Both RNA-seq and CUT&RUN indicate FOXE3 suppresses neural gene programs within the developing lens. Funding: NEI R21 EY033471.

Poster 92

Emma Wei

Exploring the roles of alternative polyadenylation of *elavl1a* expression and function during zebrafish embryogenesis

Oberlin College of Arts and Sciences, USA

Addition of the polyA tail occurs during the final steps of transcription and mRNA processing, which is crucial for the export, stability, and translation of a mature mRNA. For many genes, the addition of the polyA tail can occur at one of multiple locations within the 3' untranslated region (3'UTR), or in a few cases, the coding sequence. This is known as alternative polyadenylation (APA), which can result in the production of mRNAs with varying 3'UTR lengths or alternative coding sequences. Since 3'UTRs often contain many different binding sites for RNA binding proteins and miRNAs, the production of different lengths can result in the presence of absence of these binding sites. Consequently, APA can alter the stability, localization, and/or translation of the mRNA. In our lab, we use zebrafish as a model organism to study how APA influences post-transcriptional gene regulation, and the impact of APA on the determination of the identities and functions of cells during embryonic development. We are currently investigating APA of the RNA binding protein *elavl1a*, which has been shown to bind to and stabilize *gata1a*, a transcription factor that is essential for the proliferation of red blood cells in zebrafish embryos. Consistent with previous studies using SAPAS (Sequencing of Alternative Polyadenylation Sites) on zebrafish embryos at different timepoints of development, we used 3' Rapid Amplification of cDNA Ends to show that *elavl1a* undergoes 3'UTR lengthening between 24hpf and 48hpf. Preliminary qPCR data shows that longer isoforms are expressed at low levels at somitogenesis to 24-28hpf stages but increase by 36hpf. 3'UTR lengthening has been described as a hallmark of cell differentiation, as this can impose more "fine-tuned" regulation of genes that tend to promote proliferation. Our work aims to investigate the effect of alternative polyadenylation on post-transcriptional regulation of *elavl1a* during embryonic development.

Poster 93

Shelbie Wenner[1,2], Natalie Pfaltzgraff[2], Esther Ushuhuda[2], Maria Mikedis[2,3]

MEIOC Regulates HSPA2 to Coordinate the Timing of Early Meiosis

[1]Medical Scientist Training Program, University of Cincinnati College of Medicine, USA; [2]Reproductive Sciences Center, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA; [3]Department of Pediatrics, University of Cincinnati College of Medicine, USA

Meiosis is a specialized cellular division that occurs in the gonads to produce mature gametes for reproduction. Meiosis begins with meiotic DNA replication and progresses to prophase I, during which homologous chromosomes undergo pairing, synapsis, and recombination. Failure to complete the chromosomal events of meiotic prophase I results in apoptosis or chromosomal missegregation. Fertility depends on the success of meiosis, with aberrant meiosis being a common cause of infertility and birth defects. It remains unclear how germ cells establish the cell cycle program that coordinates meiotic prophase I. MEIOC is a germline-specific protein that acts in a complex with YTHDC2 and RBM46 proteins to upregulate the meiotic cell cycle program. When Meioc is knocked out in mice, germ cells are delayed in entering meiotic prophase I and fail to complete prophase I. Knockouts display either early apoptosis or an abnormal premature metaphase. It is unknown if this metaphase is a continuation of the mitotic cell cycle or an advancement of the meiotic one. We discovered that MEIOC represses HSPA2, a protein required for progression from prophase I to metaphase I. In wildtype spermatogenic cells, HSPA2 is first detected during late prophase I. In Meioc knockout cells, HSPA2 is expressed in early prophase I. Here, we hypothesized that MEIOC's regulation of HSPA2 protein expression impacts the timing of early meiosis. We use a genetic approach to dissect how spermatogenic cells' progression through meiotic prophase I is regulated by MEIOC and HSPA2. Our studies will molecularly define how MEIOC regulates HSPA2 to determine the timing of meiotic prophase I. These studies will expand our understanding of the regulation of key events in the meiotic cell cycle and may provide opportunities to facilitate in vitro spermatogenesis, a novel regenerative medicine-based treatment for infertility. This work is supported by NICHD Award Number R00HD097285 (M.M.M).

Poster 94

Rachel Wiley, Sarah Paramore, Victoria Prince

The Role of the Fat1a/Lar Polarity Module in Zebrafish Pronephric Collective Cell Migration

University of Chicago, USA

Collective cell migration (CCM) drives organ formation and tissue homeostasis in diverse animals ranging from *Drosophila* to humans. However, much of our understanding of CCM comes from studies performed in vitro. In the Prince Lab, we leverage the genetic tractability of the zebrafish (*Danio rerio*) embryo to study CCM in the context of a developing organ, the pronephros, which consists of two ducts analogous to the mammalian nephron. In the pronephros, epithelial cells undergo an anteriorly-directed CCM, driven by basally-localized lamellipodia (Vasilyev et al., *PLoS Biology*, 2009). This CCM is required for the stereotyped convolution of the pronephric proximal tubule. While lumenal fluid flow is the upstream regulator of pronephric CCM (cells migrate against flow), the molecular players guiding this polarized migration are unknown. However, pronephric CCM shares remarkable similarities with the

well-understood CCM observed in the *Drosophila* egg chamber, where Lar and Fat2 proteins guide the formation of polarized lamellipodia. We hypothesize that LarA/B and Fat1a, the zebrafish homologs of these proteins, function as a polarity module orienting lamellipodia in pronephric CCM. We first confirmed that LarA/B (*ptprfa/b*) and *fat1a*, are expressed in the pronephros via hybridization chain reaction. Furthermore, our data indicate that loss of *fat1a* disrupts pronephric morphogenesis, leading to a failure of convolution of the proximal tubule. Now, our work focuses on assessing if Fat1a is required for cell migration. To this end, we are performing time-lapse confocal imaging of pronephric migration in *fat1a* mutant embryos. These data will reveal whether Fat1a is directly required for pronephric CCM. Together, these experiments will not only determine the role of Fat1a and Lar proteins in pronephric migration but may also identify these proteins as a conserved polarity module that functions in vertebrate organogenesis. Funding: UChicago Undergrad Summer Fellowships.

Poster 95

Zach Wright, Hannah Rice, Dan Bergstralh

Fas2 Mediated Cell Reintegration

University of Missouri, USA

The term "popped out" refers to the apical positioning of a follicle cell outside of the epithelial layer. The process of pulling the cell back into the epithelial layer is known as reintegration. Reintegration is driven at least in part by three cell-cell adhesion proteins of the Immunoglobulin Cell Adhesion Molecule (IgCAM) family; Fasciclin 2 (Fas2), Fasciclin 3 (Fas3), and Neuroglian (Nrg). Whereas Fas3 plays a relatively minor, supplemental role in reintegration, genetic disruption of either Nrg or Fas2 causes the appearance of popped out cells. In this study we aim to understand the molecular mechanism that explains the contribution of Fas2 to reintegration. There are seven isoforms of Fas2; five are transmembrane proteins with intracellular domains and the other two are anchored to the plasma membrane by lipid (GPI) modification. We set out to determine which of these isoform types drives reintegration, acknowledging the additional possibility being that both types are required. To address this question, we obtained two different “protein trap” lines in which the Fas2 gene is combined with a fluorescent tag (either GFP397 or YFP483) at the endogenous locus. Unexpectedly, western blot results revealed that the while the GFP397 line expresses proteins that are size-consistent with at least one transmembrane and one GPI-linked isoform, the YFP483 flies demonstrate only one band, size-consistent with a GPI-linked isoform. We explored this further using microscopy and found that YFP483 line expresses at least one transmembrane isoform, but it is not tagged. We also find that these two isoform types are not always spatiotemporally linked. Although both types are observed in an early, proliferative epithelium, only the transmembrane type is seen at a later developmental stage. This separation suggests that GPI and transmembrane isoforms could have specific functions. Funded by the NIH, NSF, and Mizzou forward.

Poster 96

Manqi Wu[1], Danielle Becker[2], Sha Huang[2], Ansley Conchola[2], Yusoo Lee[2], Mengkun Yang[2], Michael Downey[2], Ian Glass[3,4], Gail Deutsch[5,6], Peggy Hsu[7,8], Tristan Frum[2], Jason Spence[1,2,8,9]

Elucidating Mechanisms Regulating Alveolar Type 2 (AT2) Differentiation using iPSC-Derived Bud Tip Organoids

[1]Department of Cell and Developmental Biology, University of Michigan Medical School, USA; [2]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan Medical School, USA; [3]Department of Pediatrics, Division of Developmental Medicine, University of Washington, Seattle Children's Hospital, USA; [4]Division of Genetic Medicine, University of Washington, Seattle Children's Hospital, USA; [5]Center for Respiratory Biology and Therapeutics, Seattle Children's Research Institute, USA; [6]Department of Laboratory Medicine and Pathology, University Children's Hospital, USA; [7]Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan Medical School, USA; [8]Rogel Cancer Center, University of Michigan Medical School, USA; [9]Department of Biomedical Engineering, University of Michigan College of Engineering, USA

Alveolar type II (AT2) cells are essential for lung homeostasis, repair, and regeneration. Loss or dysfunction of AT2 cells contributes to diseases such as bronchopulmonary dysplasia (BPD) and idiopathic pulmonary fibrosis (IPF). During human lung development, bud tip progenitors (BTPs) give rise to AT2 cells. However, the mechanisms regulating BTP-to-AT2 differentiation and AT2 maturation remain poorly understood due to limited access to fetal tissue. Human induced pluripotent stem cells (iPSCs) provide a scalable platform to model lung development, but existing methods often yield immature AT2-like cells with limited functionality. To address this gap, we are analyzing single-cell RNA sequencing datasets spanning fetal to adult lung development, enabling us to map transcriptional trajectories from BTPs to AT2 cells and to identify key signaling pathways and regulatory factors that control differentiation and maturation. In parallel, we are developing an optimized iPSC differentiation method to model human alveolar development in vitro. This approach is designed to incorporate an intermediate bud tip progenitor (BTP)-like state to better recapitulate alveolar development and to promote the generation of more mature, functional, and transplantable iPSC-derived AT2 (iAT2) cells. Together, this work will provide new insights into the molecular mechanisms of human alveolar development and establish an iPSC-based platform for lung disease modeling and regenerative medicine. Funding for this project was provided by NIH (NHLBI 5R01HL166139).

Poster 97

Shili Wu, Liam Rodman, Laurence Lemaire

Remodeling of the *Ciona intestinalis* central nervous system

Saint Louis University, USA

The invertebrate chordate *Ciona intestinalis* belongs to the tunicate, the closest living relative to vertebrates. Its free-swimming larva metamorphoses into a sessile, filter-feeding adult. During this, most of the larval central nervous system (CNS) undergoes apoptosis, while neural progenitors proliferate and differentiate to form the adult CNS. Understanding CNS remodeling mechanisms during *Ciona* metamorphosis may improve neural regeneration in mammals. Studying its CNS formation and

composition might highlight the evolutionary steps leading to the vertebrate nervous system. This research focuses on studying the formation of the juvenile CNS and from the formation of neural progenitors to their differentiation, exploring the role of specific cell types and maps their gene regulatory network. Putative larval neural progenitor cell types were identified in the single-cell transcriptomic atlas of *Ciona* development from gastrula stage to swimming larva, including *Foxd+* cells, nerve cord (b), and trunk nerve cord (A). To localize these cell populations, I generated nuclear and membrane fluorescent reporters specific to these cell types and performed in situ hybridization chain reaction to confirm their precise location. My results indicate that the trunk nerve cord (A) cells are in the neck region of the nervous system and could be neural progenitors, while *Foxd+* cells are in the motor ganglion and likely correspond to the ascending motor ganglion (AMG) neurons. Based on their transcriptomic signature and location, these AMG neurons may be homologous to the V1 interneurons of the vertebrate spinal cord. Further work will map the contribution of the putative neural progenitor to the juvenile CNS and confirm the evolutionary relationship of the AMG neurons and V1 interneurons by mapping their gene regulatory network. This work is funded by the Office of Vice President for Research and the Provost Office of Saint Louis University (RI award 01978 and grant 001654).

Poster 98

Jingyue Xu[1], Han Liu[1], Mohammed Sayed[1], Yilun Huang[1], Yu Lan[1,2], Hee Woong Lim[1], Rulang Jiang[1,2]

Foxd1 regulates musculoskeletal development and integration during palatogenesis

[1]Cincinnati Children's Hospital, USA; [2]University of Cincinnati College of Medicine, USA

The mammalian palate consists of the anterior bony hard palate and posterior muscular soft palate. The palatal muscles connect to the hard palate bones through the palatine aponeurosis, a fan-like dense connective tissue comprising the core of the soft palate. Essential soft palate functions in directing air/food/liquid traffic at the oropharynx during breathing, swallowing, and speech depends on properly coordinated development and integration of the mesoderm-derived palatal muscles with cranial neural crest-derived skeletal and other connective tissues. Abnormal attachment of palatal muscles is a common birth defect associated with palatal dysfunction, but little is known about how palatal muscle development and integration is regulated. We show here that the Foxd1 transcription factor is highly specifically expressed in the embryonic soft palate mesenchyme cells and plays critical roles in palatal connective tissue morphogenesis and palatal muscle patterning in mice. *Foxd1*$^{-/-}$ mutant embryos exhibited reduced soft palate mesenchyme proliferation, resulting in shortened soft palate at birth. Using single-cell RNA-seq and data analysis together with *in vivo* genetic mouse models, we found that Foxd1 is required for activation of BMP signaling and Sox9-mediated morphogenesis of the medial pterygoid process of the sphenoid bone. Furthermore, loss of *Foxd1* caused imbalance in the differentiation of perimysial connective tissues in the soft palate, resulting in mis-insertion of the tensor veli palatini muscles directly into the pterygoid process and disruption of the palatine aponeurosis. Together these results identify Foxd1 as a key regulator of, and provide novel insight into the molecular mechanisms controlling, palatal musculoskeletal development and integration. This work was supported by NIH/NIDCR grant R01DE033890.

Poster 99

Inah Yang, Xuyao Chang, Joel Blair, Jason Tchieu

Human Embryonic Stem Cell-Derived Olfactory Epithelium Model

Cincinnati Children's Hospital Medical Center, United States

The olfactory epithelium (OE) arises from specialized ectodermal progenitors, named cranial placode, and serves as the sensory interface between the environment and the central nervous system. While rodent models have provided much insight into OE development, fundamental differences between in receptor gene number, regenerative ability and sensitivity highlight the need for human-specific OE models. Here, we show our efforts in developing an in vitro human OE model derived from human pluripotent stem cells. First, we found that WNT signaling plays a critical role in anterior placode identity and these progenitors can be further specified to produce cranial placode progenitors with axial identity. Secondly, we find that in 3D organoid-like cultures, these cranial placodes can generate epithelial-like structures that express canonical OE markers such as PAX6, TFAP2A and FOXG1. Furthermore, other OE component cell markers such as ASCL1, CK18 are expressed in 3D organoid model. After 60 days of culture, we observed the expression of neuronal populations such as TUJ1 and OMP suggestive of olfactory neuron development. Together, these findings demonstrate that human pluripotent stem cells can be directed toward OE-like identity, providing a tractable model to study not only the developmental emergence, but to ultimately investigate the vulnerabilities in neurodegenerative disease ultimately.

Supported by Alternatives Research & Developmental Foundation.

Poster 100

Juli Liu, Sheng Liu, Jun Wan, Lei Yang

Decoding human-specific mechanisms in the regulatory of early heart development

IU School of Medicine, USA

Background: Previous studies of early heart development were focused on conserved gene regulatory mechanisms. However, compared to rodents, human embryos exhibit unique dynamics and properties of cardiogenesis, suggesting the existence of human-specific genetic regulatory programs underlying early human heart development. Due to lack of in vivo model, study of human-specific mechanisms in early cardiogenesis still remains as a challenge. **Results:** Modeling human cardiogenesis with human pluripotent stem cells (hPSCs), we identified a novel human-specific long non-coding RNAs (lncRNA), Heart Brake LncRNA 1 (HBL1), which plays essential roles in regulating human early heart development. HBL1 expresses in both cytoplasm and nucleus of hPSCs and represses cardiomyocyte differentiation from hPSCs. Cytosolic and nuclear HBL1 employ different molecular mechanisms, which crosstalk to orchestrate cardiogenesis. Cytosolic HBL1 modulates cardiac development by counteracting microRNA-1. Nuclear HBL1 interacts with two PRC2 subunits, JARID2 and EED to recruit PRC2 epigenetic complex. HBL1 deficiency disrupts genome-wide PRC2 occupancy and H3K27me3 chromatin modification on essential cardiogenic genes. Hence, HBL1-depletion significantly and broadly enhances cardiogenic gene transcription in hPSCs and during hPSC differentiation. Additionally, ChIP-seq reveals reduced PRC2-occupancy on overlapped cardiogenic genes in HBL1$^{-/-}$ and JARID2$^{-/-}$ hPSCs, indicating both HBL1 and JARID2 are required for PRC2 deposition to set up the epigenetic state of critical cardiogenic

genes in undifferentiated hPSCs. Furthermore, the cytosolic and nuclear fractions of HBL1 could crosstalk via a conserved "microRNA-1-JARID2" axis to modulate the transcription of cardiogenic genes. **Conclusion:** Overall, our findings delineate the indispensable role of human-specific mechanisms by which cytosolic and nuclear portions of HBL1 coordinate to precisely control human early heart development.

Poster 101

Mateo Zevallos, Rejenae Dockery, Carson McNulty, Tessa Zecchino, Reece Ratermann, Richard White, Aaron Goldman

The roles of translation initiation factor homologs during zebrafish regeneration

The Ohio State University, USA

Heart disease is the leading cause of death due to the human heart's poor ability to replenish cardiomyocytes (CMs). In contrast, zebrafish fully regenerate heart tissue through CM proliferation by reactivating developmental programs, though the exact mechanisms remain unclear. While transcriptional regulation is well studied, we examined differences in translational control via the translation initiation factor eIF4E that is expressed in all eukaryotes. eIF4E initiates translation by binding the 5' mRNA cap, recruiting transcripts to the ribosome. Notably, zebrafish possess a unique family of eIF4E, called *eif4e1c*, while also containing two paralogs of the canonical variant, *eif4ea* and *eif4eb*. Using CRISPR, we deleted *eif4e1c* to investigate and found mutant fish had decreased survival, smaller bodies, fewer CMs, and reduced CM proliferation following injury. While intriguing, these results could have been due to a general reduction in translation initiation or due to a unique phenotype associated with *eif4e1c.* Here, we explore these possibilities by deleting both canonical eIF4E genes to test if the mutants adopt *eif4e1c*-like phenotypes. Deletion of the canonical *eif4ea* and *eif4eb* genes had no impact on survival or size. This suggests that *eif4e1c* has unique functions, only partially compensated for by canonical factors in the *eif4e1c* mutant. Even double (*eif4ea* and *eif4eb*) deletion mutants survive and grow normally demonstrating that *eif4e1c* alone fully compensates for canonical function. Unexpectedly canonical mutants have *increased* cardiomyocyte proliferation in contrast to *eif4e1c* mutants. In addition, only canonical mutants and not *eif4e1c* mutants exhibited impaired fin regeneration. These results indicate that canonical eIF4E and fish-specific *eif4e1c* serve distinct functions during different forms of regeneration. We thank the Department of Biological Chemistry and Pharmacology, College of Medicine, Ohio State University Medical Center for funding.

Poster 102

Alex Zhang, Michael Wen, Victoria Prince

Investigating zebrafish neural crest cell fate restriction by imaging transcription in vivo

University of Chicago, USA

The neural crest (NC) is a multipotent stem cell-like cell population found in developing vertebrate embryos. Neural crest cells (NCCs) give rise to a large array of tissues and cell types including craniofacial cartilage and bone, neurons and glia of the peripheral nervous system, pigment cells, and

cardiomyocytes. Although NC differentiation has been well-studied over the past several decades, the mechanism by which these cells commit to a single fate is still unclear. In 2021, Robert Kelsh and colleagues proposed the cyclical fate restriction (CFR) model, which reconciles conflicting evidence underpinning the classic hypotheses. CFR posits that NCCs cycle through different 'substates' that are primed for specification signals for a single fate. If the relevant signals are present at sufficient strength and duration, the NCC will adopt that fate; otherwise, it will transition into a different substate. Switching between substates is thought to depend on levels of transcription factors necessary for differentiation into certain fates (e.g., *sox10* for neuronal fates, *foxd3* for glial fates). To test CFR, we are applying the PP7/MS2 RNA labeling systems to enable live imaging of transcriptional dynamics for *sox10* and *foxd3* in individual neural crest cells. We have generated transgenic zebrafish lines expressing fluorescently labeled PP7 coat protein (PCP) and MS2 coat protein (MCP). Crossing these fish with a transgenic line labeling *sox10* and *foxd3* with MS2 and PP7 stem loops, respectively, will allow us to quantify transcriptional activity and test for cycling of these transcription factors, in accordance with the cycling substates of the CFR model. Applying this system in a vertebrate model offers a new way to study differentiation that may resolve the debate of fate restriction mechanisms within the neural crest. This work is supported by the University of Chicago Biological Sciences Collegiate Division.

Poster 103

Barbara Zhao[1,2], Helen Warheit-Niemi[1], Kathleen Cook[1], Andrea Toth[1,2], Satish Madala[2], William Zacharias[1,2]

Stressed transitional epithelial cells differentiate into alveolar-basal intermediates during fibrotic lung remodeling

[1]Cincinnati Children's Hospital Medical Center, United States; [2]University of Cincinnati College of Medicine, United States

RATIONALE: In respiratory disease, an intermediate cell state termed the "stressed transitional epithelium (STE)" is acquired during alveolar epithelial cell differentiation and characterized by DNA damage, cellular senescence, and enhanced Keratin 8 expression. Deletion of Nkx2.1 in alveolar epithelial progenitors (AEPs) leads to irreversible transition of AEPs to STE, providing a tractable *in vivo* model to study STE biology. Morphologically, STE are distinctly elongated and express high levels of intercellular junction markers. We hypothesize that STE accumulation disrupts cell adhesion in the alveolar niche and promotes fibrotic remodeling. Defining the cell fate and alveolar niche generated by the STE is critical to identifying key mechanisms that promote functional alveolar regeneration. METHODS: We used silica-induced lung injury to evaluate the effect of fibrotic lung injury on our adult murine AEP-specific knockout of Nkx2.1 (Nkx2.1KO). RESULTS: STE preferentially cluster around areas of lung injury and enhance blood-air barrier disruption. Nkx2.1KO lungs present with increased collagen deposition, indicating heightened lung fibrosis severity. Flow cytometry reveals decreased alveolar cell fate acquisition and increased airway cell fate acquisition, as well as increased frequency of alveolar macrophages that are not monocyte-derived, in Nkx2.1KO. scRNA-seq and IHC demonstrate that STE express basaloid markers (i.e., Krt19, Krt7), upregulate expression of integrin b6, and move toward a unique inflammatory cell state following silica-induced lung injury. CONCLUSIONS:

STE promote a pro-fibrotic alveolar milieu and preferentially localize to areas of severe lung injury. We conclude that STE differentiate into alveolar-basal intermediates and take on a unique inflammatory state in response to fibrotic signaling. STE enhance blood-air barrier disruption during fibrotic lung injury, which likely leads to the proliferation of tissue-resident alveolar macrophages.

Poster 104

Andrew Vontell[1,2], Jack Kucinski[1,2], Alexi Tallan[1,2], Katherine Silvius[2], Cenny Taslim[2], Benjamin Stanton[1,2,3,4], Genevieve Kendall[1,2,3]

Developmental consequences of DNA binding by a rhabdomyosarcoma fusion oncoprotein *in vivo*.

[1]Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, OH, USA, USA; [2]Center for Childhood Cancer Research, The Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA, USA; [3]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA, USA; [4]Department of Biological Chemistry and Pharmacology, The Ohio State University College of Medicine, Columbus, OH, USA, USA

Rhabdomyosarcoma is a pediatric soft-tissue sarcoma with features of arrested myogenesis. The most aggressive subtype is driven by the fusion of transcription factors PAX3 and FOXO1. The resulting PAX3::FOXO1 oncoprotein retains the DNA binding domains of PAX3, consisting of a paired domain and a homeodomain, coupled with the transactivation domain of FOXO1. This chimeric protein acts as an aberrant transcription factor, and we have demonstrated that it functions as a pioneer factor across cellular contexts. We developed an embryonic zebrafish PAX3::FOXO1 mRNA injection model to understand PAX3::FOXO1 epigenetic and transcriptional activity in a developmental context. We found that *in vivo* PAX3::FOXO1 binds inaccessible chromatin through partial homeobox motif recognition and activates targets through composite paired box/homeobox motif recognition. Interestingly, a conserved activity of PAX3::FOXO1 in zebrafish and in patient tumors is the activation of neural pathways. We hypothesize that the pioneering of these neural programs is critical for tumorigenesis. Here, we probed the structure-function relationship of PAX3::FOXO1's paired and homeobox domains in the pioneering of this neural signature. We observed that deletion of either domain reduces the extent to which PAX3::FOXO1 can activate neural pathways through different mechanisms. Both the paired and homeodomain deletions result in differential embryonic survival outcomes and less severe developmental phenotypes compared to the full-length fusion protein, demonstrating *in vivo* consequences for the alteration of the neural signature. Overall, our approach supports functionally evaluating the requirements for pioneering function of PAX3::FOXO1 to better understand its role in tumorigenesis. This work was supported by NIH/NCI R01 CA272872, Alex's Lemonade Stand A Award, V Foundation for Cancer Research V Scholar Award and Nationwide Children's Hospital startup funds.