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Graduate Hotel, 151 Goodman Dr., Cincinnati, OH 45219

Talk Abstracts:

S1T1: Keynote 1

Deneen Wellik

Hox11 regulates injury-induced fibrogenesis and responsiveness to Hh signaling in fracture repair

University of Wisconsin-Madison, USA

Hoxa11 and *Hoxd11* encode spatially restricted transcription factors that pattern the forelimb zeugopod. Prior work has established that these *Hox11*-expressing cells are a skeletal stem/progenitor population that gives rise to osteoblasts, chondrocytes and adipocytes and function from embryonic development through adult homeostasis. Herein, we utilize an ulnar fracture model to interrogate *Hox11* function in response to fracture injury. Adult conditional deletion of *Hoxd11* in the background of *Hoxa11* null mutants results in an inability to appropriately repair the fracture and a loss of osteoblast differentiation in the fracture callus. Single cell transcriptomic analyses of *Hoxa11*-lineage labeled cells from control and *Hox11* mutant fracture calluses reveal similar numbers and expression profiles of skeletal stem/progenitor cells (SSPCs). Control and mutant SSPCs differentiate to inflammatory fibrogenic cells, however, *Hox11* mutant cells are blocked at this stage while control cells continue progressing to differentiating fibrogenic cells then into the osteochondral lineage. Here, we report *Hox11* mutant fibrogenic cells are signaling deficient and, critically, fail to express *Gli1* in response to Ihh signaling from the chondrocytes, resulting in loss of osteoblast differentiation. Examination of *Hoxa11/Hoxd11* mutant limbs at embryonic stages show that loss of *Gli1* signaling in Hox-expressing cells is a conserved response. This work was supported by National Institutes of Health R37: AR061402 (DMW).

S2T1:

Nicole Pek[1], Konrad Thorner[1], Minzhe Guo[1,2], Hayden Dennison[2], Tharushi Rajaguru[2], George Stan[2], Keishi Kishimoto[1], Robbert Rottier[3], Darrell Kotton[4], Aaron Zorn[1,2], Mingxia Gu[5]
Vessel Organoids Reveal FOXF1 Variant-Specific Regulation of Mesoderm and Capillary Development
[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati, USA; [3]Erasmus MC and Eramus MC-Sophia Childrens' Hospital, The Netherlands; [4]Boston University and Boston Medical Center, USA; [5]University of California, Los Angeles, USA

Transcription factor Forkhead Box F1 (FOXF1) is a critical regulator of mesoderm and vascular development. More than 50 FOXF1 allelic variants have been identified in patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV), a lethal and incurable congenital lung disease characterized by severe loss of alveolar capillaries. How disease-causing FOXF1 variants affect early mesoderm and capillary development in human patients remains unknown. Here, we generated vessel organoids (VOs) from three human induced pluripotent stem cell (hiPSC) lines, each harboring a unique FOXF1 variant, and showed that heterozygous FOXF1 variants result in capillary maldevelopment of varying severity. Single-nucleus multiomic analysis uncovered variant-specific mechanisms – 'severe' c.253T>A (p.F85I) FOXF1 variant perturbed differentiation of nascent to lateral plate mesoderm differentiation, disrupted the FOXF1-MECOM regulatory axis in endothelial progenitors, and significantly reduced the population of collagen-producing mural progenitors. 'Moderate' variants, c.166C>G (p.L56V) and 16q24.1del, however, permit mesoderm development, but significantly rewired vascular progenitor cell states and function. Molecular dynamics simulations performed on F85I and L56V FOXF1 proteins revealed differential DNA-binding and stability profiles. Lastly, we found that variant- and developmental-stage-dependent restoration of wild-type FOXF1 via lipid nanoparticle (LNP)-mediated mRNA delivery promoted capillary formation in the diseased VOs. Our study reveals the effects of distinct FOXF1 variants on mesoderm-to-vascular developmental trajectories, demonstrates the potential of LNP-FOXF1 to restore capillary development. N.P.: American Heart Association Pre-doctoral Fellowship (121890), Albert J. Ryan Fellowship, and the Graduate Student Government Research Fellowship. M.X.: R01HL166283.

S2T2:

George Roy, Shujun Ou
Characteristics of sRNA-Driven Transposon Methylation in Plant Zygotic Genome Activation
The Ohio State University, USA

One of the most important steps in plant development is zygotic genome activation (ZGA), during which fertilized zygotes shift from utilizing maternal mRNA to expressing their own genome. Previous ZGA research has identified approaches for improving crop yield and resilience. Particular focus has been placed on developmental RNA-directed DNA methylation (RdDM), during which sRNA from vegetative cells is proposed to methylate corresponding genomic regions in gametes. This methylation is believed to suppress the deleterious expression of transposons during ZGA. However, several aspects of RdDM, including the classes and superfamilies of transposons targeted by it, as well as the methylation status of vegetative loci used to produce sRNA, are poorly characterized. To better understand the

dynamics of transposon targeting in RdDM, we integrate published results from Kim et al., 2019 and Li et al., 2020 describing methylation and sRNA expression in rice gametes, vegetative cells, and shoot cells. We use the transposon annotation tool EDTA to identify and categorize transposons overlapping reads. By analyzing transposons differentially methylated between cell types, we identify properties of transposons regulated during development. We supplement this approach by identifying patterns of transposon sRNA expression and methylation of RdDM-relevant genes. In particular, by comparing 21- and 24-nt sRNA reads, we characterize the relative contributions of canonical and non-canonical RdDM to transposon regulation during ZGA. Our findings provide a more comprehensive depiction of RdDM's components and its relevance to plant development as a whole. This work is supported by funding from The Ohio State University, through its Graduate School and Cellular, Biochemical, and Molecular Sciences Training Program fellowships.

S2T3:

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

Müller Glia Ion Homeostasis Preserves Retinal Integrity and Protects Retinal Ganglion Cells

Miami University, USA

Retinal neuroinflammation is a primary driver of retinal ganglion cell (RGC) degeneration and irreversible vision loss across numerous ocular pathologies. Müller glia (MG) are the primary architectural and homeostatic cells of the vertebrate retina that provide structural and physiological support to the retinal neurons. In the nervous system, glia have the ability to rapidly alter their cellular state in response to stress, a process known as reactive gliosis. This process is known to be a fundamental biological transition that drives inflammatory retinal pathologies. Under homeostatic conditions, MG regulate local ionic balance primarily through Kcnj10a-encoded inwardly rectifying potassium channels, which we and others have shown are highly expressed in MG from early development. To investigate the epigenetic and functional networks governing MG plasticity, we utilized the genetically tractable zebrafish model and a novel dCas9-KRAB system for MG-specific Kcnj10a silencing in zebrafish. Functionally, this targeted depletion disrupted early vision, characterized by reduced optomotor responses and altered electroretinogram (ERG) b-wave signals. Histologically, the loss of Kcnj10a in MG triggers severe reactive gliosis, increased microglial invasion, and a significant, selective loss of retinal ganglion cells (RGCs). Strikingly, we discovered that administering the psychedelic compound psilocybin to Kcnj10a-deficient embryos significantly reduced this inflammation. Ultimately, this research defines the epigenetic mechanisms controlling glial cellular plasticity and evaluates whether targeting these glial support mechanisms via psilocybin can nullify inflammation, reverse pathological chromatin shifts, and promote RGC survival. Funding sources: Marie Sklodowska-Curie Actions, Miami University.

S2T4:

Blair Leach[1,2], Courtney Stockman[1], Thomas Taylor[1,3], Joseph Finks[1], Kimberly Wagner[1], Jessica Babros[1], Jenna Green[1], Anne-Karina Perl[1,4]

Fibroblast-Derived FOXF1 Coordinates Alveolar Development and Capillary Maturation

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Background: Alveologenesis is a coordinated process that establishes the blood-air barrier through capillary development. Alveolar fibroblasts (AFs) play a vital role in this process, and loss of AF identity delays capillary maturation. FOXF1, a transcription factor expressed by AFs and capillaries, is critical for capillary development, where mutations in *Foxf1* lead to alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV). However, studies focusing on endothelial FOXF1 do not mirror all aspects of ACDMPV, indicating that FOXF1$^+$ alveolar fibroblasts contribute to disease pathology. **Methods:** We selectively deleted *Foxf1* from AFs using Meox2CreERT2 during defined stages of alveologenesis. **Results:** Fibroblast-specific deletion of *Foxf1* delayed the transition from the saccular to alveolar stage, resulting in persistent extension of alveolar ducts into postnatal alveologenesis. This developmental delay was accompanied by persistent extracellular matrix deposition and retention of secondary crest myofibroblasts beyond their normal developmental window. Notably, venous-like endothelial cells emerged, indicating impaired capillary maturation. While no overt epithelial abnormalities were observed, altered capillary identity coincided with an increased immune response. **Conclusions:** These findings demonstrate that FOXF1-dependent alveolar fibroblasts are required to maintain capillary endothelial identity and coordinate postnatal alveolar development. Despite preserved FOXF1 expression within endothelial cells, fibroblast-specific loss was sufficient to disrupt capillary identity. Together, these findings establish an unrecognized role for fibroblast-derived FOXF1 in capillary identity and highlight alveolar fibroblasts as critical regulators of lung development. This study was supported by NIH/NHLBI grants R01 HL167030 and R01 HL164418 (AKP). We thank CCHMC Transgenic Animal and Genome Editing Core for design and generation of the Meox2CreERT2 mouse.

S3T1:

Shelbie Wenner[1,2], Omodasola Ola[2], Natalie Pfaltzgraff[3], Esther Ushuhuda[3], Maria Mikedis[3,4]

HSPA2 Regulates Translation During Meiosis

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Fertility depends on the success of meiosis. Germ cells undergo meiosis in a specialized cellular division to produce mature haploid gametes. Several highly regulated chromosomal events occur during meiotic prophase I, including homologous chromosome pairing, synapsis, and recombination. Germ cells in early meiotic prophase I undergo transcriptional silencing, followed by a transcriptional and translational burst in mid-prophase I. The specialized machinery required for chromosomal events of prophase I must be expressed prior to prophase

I or at the transcriptional burst in mid-prophase I. Failure to complete the events of prophase I results in apoptosis or chromosomal missegregation, both common causes of infertility or birth defects. HSPA2 is a heat-shock protein required for meiosis in mammals. It is expressed independently of heat shock at the transcriptional and translational burst in mid-prophase I. HSPA2 knockout male mice are infertile and display spermatocytes that stall and apoptose in late prophase I. However, whether HSPA2 can regulate translation, similar to homolog HSP70, remains poorly defined. Here, we present a novel role for HSPA2 in supporting the translational burst in mid-prophase I during mouse spermatogenesis. Through immunoprecipitation and mass spectrometry, we confirm that HSPA2 interacts with heat shock-associated cellular machinery. Using polysome profiling and fractionation, we show HSPA2 directly interacts with actively translating ribosomes. Loss of HSPA2 causes a decrease in polysome number and an increase in monosomes, suggesting a global reduction in active translation. Future work will identify specific transcripts whose translation is impacted by HSPA2. Our studies will define how HSPA2 supports transcription and protein synthesis during prophase I. These studies will expand our understanding of how translation is regulated during the meiotic cell cycle. This work is funded by NICHD R00HD097285 (MMM) and NIGMS R35GM156830 (MMM).

S3T2:

Josy Joseph[1], Douglas Rusch[2], Andrew Zelhof[1]
Identifying the molecular mechanism of beetle horn formation
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Development of new morphological features or novel traits are key in the evolution of life. These features or traits are the combination of both internal and external factors. Identification of these factors remains a big question in developmental biology. An elegant system to identify the internal and external factors is the horns in beetles. Beetle horns display immense diversity in their size, shape, point of origin, and can be sexually dimorphic. Studies to date have identified some of the pathways; including insulin signaling and limb-patterning, responsible for cell proliferation and outgrowth upon the specification of the horn. RNAi studies in horned beetles also helped to identify some of the genes involved in the formation. However, the precise molecular mechanisms for the specification and development of horns are yet to be elucidated. We are addressing this knowledge gap using a mutation in *Tribolium castaneum*, *Lucifer* (*Lu*), that leads to the appearance of horns in a beetle species that normally lacks horns. The striking appearance of horns in *Tribolium* supports the idea of a possible genetic switch that either produces or suppresses horn development, prevailing in the field. We hypothesize that *Lu* represents a genetic switch to produce horns, and we are testing this hypothesis by mapping the mutation using linkage disequilibrium, Hi-C and whole genome sequencing. From our preliminary analysis we have identified that the *Lu* phenotype is due to multiple genetic rearrangements. By determining how *Lu* mutants develop horns, our research will shed light on the molecular signature behind horn formation in beetles and origin of novel morphological traits.

S3T3:

Anthony Treichel[1], Gabriel da Silva Pescador[1], Christopher Wood[1], Dan Bradford[1], Jennifer Gardner[1], Amanda Lawlor[1], Kate Hall[1], Michael Peterson[1], Rhonda Egidy[1], Gopal Kushawah[1], Huzaifa Hassan[1], Maria Blanco Salazar[1], Anoja Perera[1], Ariel Bazzini[1,2]

Streamlined mRNA targeting and phenotyping reveal non-essential roles of maternally encoded zebrafish microproteins during MZT

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A crucial early step in animal development is the switch in control from maternally provided to zygotically produced factors, known as the maternal-to-zygotic transition (MZT). During this window, loss-of-function approaches face the unique challenge that maternally provided mRNAs and proteins can mask the effects of mutations in the zygote alone (like in F0 CRISPRants). Zebrafish is an excellent vertebrate model of this transition, but generating maternal-zygotic mutants remains tedious and risky and established, morpholino-based mRNA targeting can elicit toxicity and off-target effects. Towards overcoming these barriers, our lab previously established the RNA targeting CRISPR-RfxCas13d system. Here we bolster RfxCas13d in zebrafish with 1) automated staging of 2–6 hours post-fertilization (hpf) embryos, 2) removal of problematic RfxCas13d guide RNAs, and 3) cost-minimized bulk RNA-seq. With this approach, we successfully target 29 maternally provided mRNAs that encode short proteins (< 100 amino acids) called microproteins. RfxCas13d reduces mRNA levels by >50% for over half of selected microproteins, with negligible transcriptomic perturbations at 4 hpf or organismal effects through 24 hpf. Finally, we find an extremely low frequency of off-target and non-target knockdown across all 52 unique knockdown paradigms. Overall, our work defines an improved RfxCas13d workflow and increases throughput for organismal and molecular analysis in early zebrafish development. Financial support for this work was provided by the Stowers Institute for Medical Research and the US National Institutes of Health [R01GM136849 to A.A.B., R21OD034161 to A.A.B, F31HD110268 to A.J.T.].

S3T4:

Omodasola Adekeye[1,2], Blanca de Juan Mora[2], Caramai Kamei[2], Daemon Dikeman[1], Ritu Tomar[3]

Loss of Ptprq disrupts podocyte development, structure, and function

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The glomerulus, the kidney's highly structured filtration unit, comprises specialized cells, including podocytes. Glomerular development and filtration barrier formation require complex morphogenetic processes, including cytoskeletal reorganization, cell-cell interactions, and membrane specialization, and podocytes play a central role in each. Disruption of this process causes proteinuria and drives end-stage kidney disease, showing the importance of podocyte development to glomerular morphogenesis. We identified *ptprq* as highly enriched in developing zebrafish pronephric glomeruli. PTPRQ, a lipid phosphatase receptor expressed in human podocytes and hair cells, converts PIP3 to PIP2 to regulate PIP2-dependent signaling and mediates stereocilia adhesion. We hypothesized that Ptprq regulates podocyte morphogenesis and integrity through either or both processes. Whole-mount in situ hybridization and immunohistochemistry confirmed Ptprq expression in podocytes of 4 dpf zebrafish larvae. We generated loss-of-function alleles via CRISPR/Cas9, using gRNAs targeting the N-terminal

extracellular and C-terminal catalytic domains. We assessed structural defects using confocal and electron microscopy and examined filtration barrier integrity by injecting 10 kDa and 500 kDa dextran into the vasculature. We measured podocyte calcium activity via live imaging with a transgenic GCaMP6 reporter. *ptprq* mutants developed proteinuria, indicating kidney dysfunction and impaired barrier integrity. Mutants also showed significantly reduced developmental podocyte calcium signaling, suggesting Ptprq drives calcium-dependent podocyte development, along with defects in glomerular primordia fusion, foot process effacement, and reduced foot process formation. Together, these findings show that Ptprq is crucial for podocyte morphogenesis and filtration barrier maintenance, offering insight into overall glomerular development. This work was supported by funding from the MDIBL.

S3T5:

Electra Coffman, Timothy Plageman Jr.

Adherens junctions and the spectrin-actin cytoskeleton function interdependently to maintain cortical lens transparency with age

College of Optometry, The Ohio State University, USA

The lens must remain transparent throughout a lifetime for functional vision. To do this, it overcomes increasingly difficult challenges over age such as oxidative and mechanical stress. However, the molecular systems in the lens that maintain this transparency eventually fail and cause lens opacities, or cataracts. The two most common types of cataracts are nuclear (central) and cortical (peripheral). As cortical cataracts have been less extensively studied than nuclear, why they form in two distinct locations remains a prevailing question. To answer this, we studied mice deficient in ARVCF *(Arvcf$^{-/-}$)*, an N-cadherin-binding protein in the adherens junctional complex (AJC), which develop cortical cataracts by 5 months of age. To determine why disrupted AJCs cause this age-dependent phenotype, we performed co-immunoprecipitation, western blot, mass spectroscopy, histology, and bulk RNA sequencing (RNAseq) in mouse lens tissue. While AJC protein expression was severely decreased in *Arvcf$^{-/-}$* lenses, RNAseq revealed no change in AJC mRNA expression. We then identified and validated the association of spectrin-actin cytoskeletal (SAC) proteins including ANKB, NRCAM, and SPTB with ARVCF. We therefore hypothesized that proteins in the AJC and SAC function together to maintain cortical lens transparency. To test this, we performed histology on mouse lens sections in *Arvcf$^{-/-}$* mice and found a significant localized decrease of SAC proteins in lens fiber cell radial junctions. This is remarkably the same location where aberrant cell-cell membrane fusions occur in the cortical cataract area. We further histologically examined lenses lacking *AnkB* to find increased recruitment of AJCs to tricellular junctions, implicating an interdependence between the AJC and SAC. Together, these data suggest that disruptions in membrane and cytoskeletal integrity are central mechanisms in the cortical cataract disease pathway. Funded by NIH R01EY033815.

S3T6:

Benjamin Gilbert[1,2], Sathiyanarayanan Manivannan[1], David Gordon[1,3], Peter White[1,3], Mineo Kurokawa[4], Susumu Goyama[4], Yosuke Masamoto[4], Prince Kannankeril[5], Vidu Garg[1,2,6]
Pathogenic variation in *MECOM* is associated with non-syndromic congenital heart disease
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Congenital heart disease (CHD) is the most common birth defect and conotruncal defects (CTDs), which affect the cardiac outflow tract (OT), are a considerable subset. CTDs result from defective septation and alignment of the OT during heart development. The etiology for CTDs is incompletely understood, and a substantial portion of patients have unknown genetic etiologies. We identified a family in which multiple members had CTDs. We performed exome sequencing on available family members and found that all affected members harbored a heterozygous missense variant (R393H) in MECOM (MDS1-EVI1 Complex Locus), which encodes a transcription factor implicated in murine OT development. Pathogenic variation in *MECOM* has been linked to syndromic CHD (RUSAT2 syndrome) in humans. We show *in vitro* using immunofluorescence that MECOM-R393H retains nuclear localization and in silico using AlphaFold 3 that MECOM-R393H has altered motif-specific DNA interactions, consistent with R393 being a known DNA base interacting residue. We also found in vivo using RNAscope that *Mecom* transcripts are expressed in the septating mouse OT (E10.5-E12.5) and expressed in all progenitor cell populations forming the OT: second heart field (SHF), neural crest (NC), and endocardial cells (ECs). MECOM is critical for embryonic development; utilizing a Cre-LoxP system, we conditionally deleted MECOM in the SHF, NC, and ECs and determined that MECOM is required in the SHF for septation, and functions in cardiac NCs for OT alignment. Mice with SHF-specific deletion of MECOM display a highly penetrant spectrum of CTDs, including truncus arteriosus, double-outlet right ventricle, and overriding aorta. In summary, pathogenic variation in MECOM is a cause of non-syndromic human CTD and is required in SHF progenitors for OT septation. Future work will characterize a mouse harboring a *Mecom*-R393H knock-in allele and the molecular functions of MECOM during OT septation. Funding Source: 5 T32 HL134616-09.

S4T1:

Grace Gottschamer, Lindsey Rhea, Martine Dunnwald
Periderm-like cells are present on the surface of Irf6 knockout mouse embryos.
University of Iowa, USA

The main function of the epidermis of the skin is to form an effective barrier protecting the body. The epidermis originates from the single-layered ectoderm, when cells delaminate to establish the basal and periderm layers between embryonic day (E) 9.5 and 12.5. As the epidermis stratifies (E12.5 to E17.5), the periderm stays superficial to the developing layers until it sloughs off starting at E17.5. Many molecules are required for epidermal differentiation including Interferon Regulatory Factor 6 (IRF6). Specifically, *Irf6*-null embryos show absence of terminal epidermal differentiation and of barrier function. Since the periderm is part of the epidermal differentiation process and that this process is impaired in *Irf6*-null embryos, we asked whether

the periderm is affected by *Irf6* levels. To answer this question, we analyzed the superficial layers of wildtype and *Irf6*-null embryos. At E10.5, E12.5 and E14.5, we observed that most periderm cells are polygonal in shape with bundles of microvilli marking their borders and fewer microvilli dispersed throughout their surface. Interestingly, periderm shape and abundance of microvilli vary along the cranial-caudal axis, suggesting regional influences in periderm morphology in wildtypes. In *Irf6*-null, however, microvilli are more disorganized than in the wildtype and superficial cells show indistinguishable cell borders by E14.5. They also lacked the characteristic electron-dense feature of wildtype periderm cells by transmission electron microscopy. However, despite these morphological differences, both wildtype and *Irf6*-null superficial cells show the presence of Keratin (K) 17 and K6, hallmarks of periderm cells. Collectively, although superficial cells of the *Irf6*-null exhibited distinct morphological features compared to wildtype periderm, they shared key markers of wildtype periderm, demonstrating the presence of periderm-like cells in *Irf6*-null epidermis. **NSF (MD), T32 (GG).**

S4T2:

Avelina Lee[1,2,3], Samantha Brugmann[1,2,4]

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

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The naturally occurring *cleft primary palate* (*cpp*) avian mutant was first characterized for its complete loss of the upper beak due to arrested development of the frontonasal prominence (FNP). The pathogenic variant responsible for the *cpp* phenotype was recently identified as a 1 base pair deletion in the highly conserved *Epithelial Splicing Regulatory Protein 2* (*ESRP2*), a gene that encodes an RNA-binding protein required for generating epithelial specific isoforms via alternative splicing. *ESRP2* is expressed throughout the epithelium, including in several FGF-mediated signaling centers that contribute to FNP development, like the frontonasal ectodermal zone (FEZ). *cpp* embryos showed reduced expression of *ESRP2*, loss of the epithelial-specific *FGF Receptor 2b* (*FGFR2b*), and expansion of the mesenchymal-specific *FGF Receptor 2c* (*FGFR2c*) into the ectoderm. Bulk-seq analysis of isolated control and *cpp* FNPs revealed increased *FGF18* and *DUSP5* in the *cpp,* indicating altered FGF signaling. Additionally, RNAscope assays revealed that while *FGF8* expression was maintained, *Sonic Hedgehog* (*SHH*) expression was lost in facial ectoderm of *cpp* embryos, thus eliminating the FEZ. Loss of the FEZ was accompanied by expansion of *CGA*+ and *DMBX1*+ pituitary tissue. Together, these data support a mechanism by which impaired ESRP2 function results in a loss of ectodermal *FGFR2b* signaling, rendering the ectoderm incompetent to establish key signaling centers that promote FNP survival and outgrowth. Future studies using the *cpp* will further examine this mechanism 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 and CCRF Funding to S.A.B, NIH 1F31DE035754 to A.W.L.

S4T3:

Jaclyn Olberding, Fan Shao, Timothy Nguyen, Colin Kenny, Huojun Cao, Eric Van Otterloo
Ectoderm-mediated patterning of the mandible during mouse development
University of Iowa, USA

A principle of embryonic development is the sharp partitioning of a homogenous tissue into defined domains, the disturbance of which can lead to defects in normal tissue patterning. We recently identified a functional opposing network establishing the oral-aboral axis of the mandibular epithelium. While initially diffuse and overlapping, transcription factor (TF) SOX2 forms a complementary expression domain with the TF TFAP2 (AP-2α and AP-2ß) in the ectoderm of the oral-aboral axis, respectively. Highlighting the functional significance of these opposing networks, loss of aboral TFs led to the expansion of oral programs aborally, culminating in the production of oral structures (teeth) in the otherwise skin-fated epithelium. Since previous studies of this axis have focused on mesenchymal contributions, our discovery of early ectodermal partitioning suggests an unexplored mechanism for patterning the mandible. Ongoing work includes using *in vivo* mouse genetics of mouse tissue to identify how these opposing networks are initially established, and which factors are sufficient to induce domain specification; CUT&RUN assays to assess how they influence each other's genome-wide binding; and protein-protein interaction studies to determine whether competition is mediated through direct binding. Our model provides a tractable genetic system to dissect principles of tissue partitioning. Funding: 5R01DE034668-02, 5T90DE023520-14, 5T32GM145441-05.

S4T4:

Lisa Wirtz[1,2,3], Houda Khatif[3], Ekaterina Soroka[3], Charlotte Meyer-Gerards[3], Tyler Hoard[1], Hisham Bazzi[1,2,3]
Striatin-Interacting Phosphatases and Kinases complex is an important cytoskeletal organizer during skin epidermal development
[1]Department of Cell & Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, USA; [2]Skin Research Center, University of Michigan Medical School, Ann Arbor, MI, United States; [3]Department of Cell Biology of the Skin, CECAD Research Center, University of Cologne, Cologne, Germany

Skin epidermal keratinocytes undergo cytoskeletal rearrangements and junctional remodeling to establish a functional stratified epidermal barrier by the end of embryonic development. The upstream cues mediating the dynamic cytoskeletal regulation are still not fully understood. We have previously shown that Striatin-Interacting Protein 1 (STRIP1), an essential adaptor of the Striatin-Interacting Phosphatases and Kinases (STRIPAK) complex that regulates PP2A activity, is required for actin organization-mediated cellular migration and convergent extension during mouse gastrulation. Here, we hypothesized that STRIP1 mediates the STRIPAK functions in cytoskeletal organization during skin epidermal morphogenesis. Conditional deletion of *Strip1* from the mouse skin epidermis results in compromised tight junction barrier function and perinatal death. The differentiated layers in *Strip1* mutant epidermis show abnormal accumulations of F-actin, wavy cell borders, and reduced junctional tension. Strikingly, the cortical actin organizer CTTNBP2NL is lost in the mutants, suggesting that CTTNBP2NL acts downstream of the STRIPAK complex to organize actin. Collectively, our data show that the STRIPAK regulates cytoskeletal organization to ensure epidermal morphogenesis and barrier formation, highlighting the importance of PP2A spatiotemporal regulation for dynamic adhesion remodeling during cell displacement. The work was funded by the Deutsche

Forschungsgemeinschaft (DFG, German Research Foundation), Project-ID 491326730 – BA 5810/5-1 and start-up funds from the Department of Cell and Developmental Biology, University of Michigan Medical School, to Hisham Bazzi.

S4T5: Invited Talk 1

Anna Cunningham
Optimizing Your Research with Artificial Intelligence Tools
Washington University-St. Louis, United States

Artificial intelligence tools are rapidly reshaping how researchers discover literature, generate hypotheses, and manage their scientific workflows. However, with such a vast landscape of tools, incorporating them responsibly into research practice can seem overwhelming! This interactive session will introduce a curated set of AI-powered research tools that can optimize literature discovery, citation mapping, and knowledge synthesis, alongside a practical, step-by-step workflow for integrating these tools into everyday research tasks. Emphasis will be placed on hands-on demonstration and actionable strategies that can enhance research efficiency while maintaining scientific rigor. Attendees are encouraged to bring a laptop or device to follow along with live tool demonstrations.

S5T1: Invited Talk 2

Chris Wolverton
Fractional Gravity on the ISS Uncovers a Novel Plant Gravity Sensory System
Ohio Wesleyan University, USA

Plants employ multiple sensing and signaling mechanisms to inform their growth and development in response to light quality and direction, mechanical stimulation, touch interactions, and gravity. Unlike the other signals, Earth's gravity provides a cue that is constant in force and direction, which plants can perceive and use to direct growth. Sedimentation of dense, starch-filled plastids (called statoliths) coupled with the relocalization of PIN auxin efflux carriers drives the differential transport of this growth regulator, linking stimulation angle with the rate of response. However, the persistence of gravitropism in starchless mutants, which lack sedimenting plastids and differential auxin transport, implies a second mechanism by which plants can perceive gravity. To further characterize this mechanism, we exposed seedlings to a range of fractional gravities from 0.003 g to 1 g on the International Space Station. Roots without starch required a much larger force to induce gravitropic response than wild-type roots. We used the difference in final root angle between genotypes after simultaneous application of blue light and gravity to estimate the relative contributions of the two systems to gravity sensing, demonstrating that the non-statolith mechanism produced 50% of the wild-type response. Transcriptomics across the gravity gradient showed a distinctive shift in RNA regulation coinciding with the force required for the non-statolith response. Genes involved in involved in cell-to-cell communication and extracellular signaling were highly represented in the data, and mutants at these loci showed gravity response defects. These data provide molecular evidence for both statolith-dependent and statolith-independent gravity signaling within a vascular plant as well as the molecular components used in the statolith-independent response. This work was funded by NASA grants NNX15AG55G and 80NSSC21K0585.

S5T2:

Patrizia Tornabene[1,2], Jonathan Brassard[1], Daniel O. Kechele[1,2], James M. Wells[1,2]
Modeling Cystic Fibrosis pancreatic disease using human iPSC-derived pancreatic organoids
[1]Cincinnati Children's Hospital Medical Center, USA; [2]Center for Stem Cell and Organoids Medicine (CuSTOM), USA

Cystic Fibrosis (CF) is a multisystem genetic disease characterized by progressive pancreatic dysfunction that begins early in life and contributes to exocrine insufficiency, inflammation, and CF-related diabetes. Progress in understanding CF pancreatic disease has been hindered by the lack of physiologically relevant human models that recapitulate pancreatic cellular complexity, architecture, and function. To address this need, we developed a human induced pluripotent stem cell (iPSC)-derived pancreatic organoid (hPO) platform that reproduces key structural and functional features of the human pancreas. hPOs were generated through directed differentiation of iPSCs into posterior foregut epithelium and pancreatic mesenchyme, followed by self-organization into multicellular pancreatic organoids. Single-cell RNA sequencing, immunofluorescence, and functional assays were used to assess cellular composition and function. To model disease, hPOs were generated from CF patient-derived iPSCs and analyzed for CFTR-dependent defects and pathological phenotypes. hPOs reproducibly generated pancreatic tissues containing ductal, acinar, endocrine, and mesenchymal cell populations with functional exocrine and endocrine compartments. Importantly, CF-derived hPOs recapitulated hallmark features of pancreatic disease, including luminal mucus accumulation and impaired digestive enzyme transport and retention, demonstrating CFTR-dependent dysfunction in a human-relevant tissue context. This work establishes a human pancreatic organoid model that faithfully recapitulates pancreatic architecture and function while capturing key features of CF pancreatic disease. By integrating patient-specific genetics with physiologically relevant tissue complexity, hPOs offer a powerful platform for future studies of disease pathogenesis and therapeutic intervention in CF. Research supported by grants from the Allen Foundation, the NIH and the DHC.

S5T3:

Rei Yagasaki, Tyler R. Huycke
How fluid dynamics and cellular mechanics shape colonic crypts
University of Michigan, USA

The colonic surface is organized into thousands of crypts – tissue compartments housing stem cells responsible for lifelong epithelial renewal. During development, these crypts arise as an initially flat epithelial–mesenchymal interface undergoes repeated folding to generate stereotyped tissue architecture. While crypt-like budding has been extensively studied in epithelial organoids, how crypts emerge within the distinct geometric constraints and multi-tissue context of the developing colon in vivo remains poorly understood. Using live imaging of the fetal mouse colon and functional perturbations, we identified an early phase of tissue deformation that precedes canonical myosin-dependent tissue folding and is instead associated with luminal compartment dynamics. Our preliminary observations suggest that luminal fluid mechanics generate early tissue instabilities that initiate epithelial folding, while subsequent cell-generated forces stabilize, refine, and pattern the emerging crypt architecture. Together, these findings support a model in which crypt morphogenesis is driven by a feed-forward interplay between fluid-driven deformation and cell-generated mechanical refinement, revealing how

tissue–tissue and tissue–lumen interactions transform nascent epithelial folds into stereotyped organ architecture. **Funding**: This research was supported by University of Michigan Department of Molecular, Cellular, and Developmental Biology Lab Startup Funds and Kinship Foundation Searle Scholars Program.

S5T4:

Bailey Weatherbee[1], Scott Rankin[1], Shreyasi Mukherjee[2], Samarth Kumar[1], Hannah Truong[1], Nandan Gokhale[1], Aaron Zorn[1]
Multifunctional RNA-binding transcription factors coordinate cell states in development
[1]Cincinnati Children's Hospital Medical Center, USA; [2]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. Disruption of these processes can result in disease and pregnancy loss. Most studies have focused on transcription factors' (TFs') interaction with chromatin, with much less attention on RNA processing. Emerging evidence indicates that some DNA-binding TFs bind RNA directly, but very little is known about the biological impact, underlying mechanisms, or the prevalence of this phenomenon. Here, we use the SOX family of TFs as exemplar dual DNA- and RNA-binding proteins to interrogate the mechanisms and impact and TF-RNA interaction. We hypothesize that some **SOX TFs also bind RNAs to regulate RNA processing, thereby coordinating transcription and RNA splicing of their target gene program**. Previous work has biochemically defined a distinct RNA-binding domain in several SOX family members, including SOX2 and SOX17. SOX2 and SOX17 define one of the best-characterized differentiation models: SOX2-positive pluripotency to SOX17-positive definitive endoderm. Using these cell states as a platform, our data demonstrate that **1)** SOXs bind RNA directly in cells, **2)** SOX17 associates with splicing proteins in human pluripotent stem cell-derived endoderm, **3)** loss of SOX expression leads to splicing changes, **4)** SOX17 RNA-binding domain disruption impairs in vivo endoderm organogenesis in *Xenopus*. Broadly, this work will define new mechanisms for the coordination of transcriptional and RNA processing essential for cell differentiation.

S5T5:

Sushant Bangru[1,2], Kazunori Ando[1], Rebecca Myatt[1], Pierre Gillotay[1], Stefano Di Talia[2], Kenneth Poss[1,3]
Adaptive osteoblast behaviors permitting regeneration of plucked scales from disabled progenitor pools
[1]Morgridge Institute for Research, Madison, WI, USA; [2]Department of Cell Biology, Duke University, Durham, NC, USA; [3]Department of Cell & Regenerative Biology, University of Wisconsin, Madison, WI, USA

Regeneration often involves expansion of progenitor populations to restore lost tissue. Whether and how regenerating tissues adapt to limitations in progenitor availability is incompletely understood. Here, we investigated zebrafish scale regeneration for its dependency on early osteoblast progenitors. Using a genetic approach to limit expansion of the starting osteoblast pool, we generated regenerates containing substantially fewer osteoblasts than wild-type controls. Despite a marked reduction in osteoblast number, regenerated scales recovered near-normal size and morphology. Osteoblasts within cell-limited regenerates remained organized as a coherent epithelial sheet and contributed disproportionately more to the regenerate on a per-

cell basis. Quantitative imaging and transcriptional profiling revealed that this compensatory response was accompanied by increased basal surface area, enlarged nuclear volume, and disproportionate upregulation of osteogenic and ossification-associated gene programs. To investigate the molecular basis of this compensation, we performed single-cell multi-omic profiling of regenerating scales. Analyses identify transcriptional and chromatin accessibility changes consistent with enhanced anabolic activity and cellular remodeling in compensating osteoblasts. Together, these findings reveal a previously unrecognized capacity of osteoblasts to scale their output in response to progenitor limitation, thereby preserving tissue-level regenerative outcomes. Funding NIAMS R01AR076342.

S6T1: Invited Talk 3

Kevin Cox

Building a Spatial and Transcriptomic Framework for Transcription Factor Regulation in Duckweed

Washington University in St. Louis, United States; Donald Danforth Plant Science Center, United States

Understanding how plants grow, develop, and respond to their environment requires not only identifying the genes involved, but functionally characterizing what each gene does. To understand how transcription factors regulate genes across tissues and organs, transcriptional data needs to be placed within a spatial, cellular, and organismal context. Among plants, duckweeds (*Lemnaceae*) offer unique advantages as a minimal and tractable plant system for addressing this fundamental question. These aquatic plants reproduce rapidly via clonal propagation (~2 days) and comprise the smallest flowering plants on Earth. One of these duckweed species, *Wolffia australiana*, is an attractive system due to its small body plan, fast clonal growth rates, and compact non-redundant genome. In our lab, we use high-resolution, 3D imaging technologies and transcriptomics to pinpoint the localization of transcription factors and how they globally regulate genes in a whole plant. We used X-ray microscopy to capture the 3D architecture of *W. australiana*, resolving the structure of the meristematic region and laying the groundwork for future cell segmentation and quantification. We further employed expansion microscopy, which enables nanoscale imaging beyond the diffraction limit, achieving roughly 4x physical expansion of intact *W. australiana* and enhanced resolution of nuclei and chloroplasts. Together, these imaging approaches provide a spatial and cellular scaffold for mapping transcriptional activity across the whole plant. To begin applying this framework, we examined the transcriptomic response to early salt stress by generating a bulk RNA-sequencing atlas over a 48-hour timecourse, which identified specific transcription factors and functional pathways underlying the salt stress response. These imaging and transcriptomic approaches together lay the groundwork for localizing transcription factor activity within a defined spatial and cellular context in *W. australiana*.

S6T2:

Matthew Riccetti[1], Patrick Woller[2,3], Amy Riesenberg[1], Hee-Woong Lim[4], Steven Crone[2,3], Kenneth Campbell[1]

Dorsal respiratory group neurons specified by Gsx2 drive the hypoxic ventilatory response

[1]Division of Developmental Biology, Cincinnati Children's Hopsital Medical Center, USA; [2]Neuroscience Graduate Program, University of Cincinnati College of Medicine, USA; [3]Division of Neurosurgery, Cincinnati Children's Hospital Medical Center, USA; [4]Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, USA

The dorsal respiratory group (dRG) is a key hindbrain structure that integrates sensory input to drive adaptive breathing and airway protection. Disruptions in the dRG, which includes the nucleus tractus solitarius (nTS), and the developmentally related A1/C1, A2/C2 catecholamine neurons, have been implicated in Apnea of Prematurity (AoP) and 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 a “hypocellular” nTS and perinatal respiratory failure. Recently, three different homozygous GSX2 variants were reported. All patients suffered from feeding/swallowing difficulties, and two died of respiratory failure by age two, suggesting a potentially conserved role of Gsx2 in humans. We identified a nearly complete loss of glutamatergic nTS and catecholaminergic A1/C1, A2/C2 dRG neurons in Gsx2-null mice at E18.5, but the role of Gsx2 in the development and function of these structures remained uninvestigated. Here, we demonstrate that Gsx2 is highly expressed in “dA3” dRG progenitors and is essential for their specification during neurogenesis. Loss of Gsx2 results in re-specification of dA3 progenitors into dorsal dA2 progenitors. Gsx2-null neonates display only subtle breathing alterations at rest but aspirate milk when fed, demonstrating impaired airway protection. When exposed to hypoxia, neonatal Gsx2 nulls stop breathing (i.e. apnea > 5 mins) until returned to room air. Hypoxia-induced respiratory depression in newborn babies (e.g. AoP) is offset with caffeine treatment and similarly, pretreatment of Gsx2 nulls with caffeine rescues this hypoxia-induced response. Our findings provide mechanistic insight into how genetic disruption of dA3 neurogenesis and dRG neural circuits may contribute to AoP and SIDS. Funding provided by R01NS124660 (KC) and F32HD118646 (MRR).

S6T3:

Josefina Doffo[1], Eric Ross[1], Ariel Bazzini[1,2], Kausik Si[1,2]

Regulated Intron Removal as a Post-Transcriptional Strategy in Neural Development and Disease

[1]Stowers Institute for Medical Research, United States; [2]University of Kansas School of Medicine, Department of Molecular and Integrative Physiology, United States

Post-transcriptional regulation allows cells to control protein output independently of transcription. This capacity is essential in the transcriptionally silent oocyte-to-embryo transition and in neurons, where protein synthesis must be precisely controlled in space and time. Intron retention (IR) is a form of alternative splicing in which an intron is retained within an otherwise fully processed transcript, traditionally viewed as a splicing error but increasingly recognized as a regulated, translation-restricting mechanism. How broadly this program operates and which sequence features specify it remain unknown. Using the transcriptionally silent zebrafish oocyte-to-embryo transition as a discovery platform, we identified maternally deposited transcripts

undergoing stage-dependent IR, including *fus*, an RNA-binding protein linked to motor neuron disease whose IR is conserved from invertebrates to vertebrates. We hypothesize that *fus* IR is a functional, conserved program controlling protein output during nervous system development. CRISPR-engineered zebrafish expressing constitutively spliced fus (where IR is abolished without altering transcription) will be used to define the requirement of IR in neural development via behavioral, anatomical, and single-cell transcriptomic analysis. Cross-species splicing reporters revealed that introns from closely related species splice correctly in a shared host context, while introns from more divergent species are retained, indicating a sequence-encoded, evolutionarily tunable splicing decision. Using random mutagenesis guided by *in silico* splicing prediction, we identified 13 mutations sufficient to force splicing of the normally retained *Drosophila* intron in the human context, defining a *cis*-regulatory code at nucleotide resolution. Together, this work establishes regulated IR as a conserved mechanism linking post-transcriptional control to neural development and disease. Funding: Stowers Institute and NIH R01MH101440-10.

S6T4:

Jacob Gafranek[1], Lim Hee-Woong[2], Joshua Waxman[1]

Atrial regeneration profiling reveals chamber-specific recovery dynamics within the zebrafish epicardium

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In contrast to adult mammals, which have limited regenerative capacity following cardiac injury, zebrafish have remarkable ability to regenerate their hearts. However, studies of cardiac regeneration in vertebrates have almost exclusively focused on regeneration following ventricular injury, leaving the regenerative capacity of the atrium largely unexplored. Here, we examined regeneration of the zebrafish atrial chamber following cryoinjury. We find that in contrast to ventricular cryoinjury, which can take up to 60 days to regenerate, zebrafish atria rapidly regenerate with almost complete scar resolution by 14 days. Histological characterization, complemented by bulk and single cell RNA-sequencing showed that while atrial regeneration exhibits fundamental similarities to ventricular regeneration, including atrial cardiomyocyte (AC) dedifferentiation and proliferation following injury, the initial atrial epicardial activation and epicardial-derived cell (EPDC) invasion as well as collagen deposition and subsequent resolution occurred more quickly. Heart-to-heart transplantation and in vitro migration assays demonstrated that atrial EPDCs inherently migrate more rapidly than ventricular EPDCs following wounding and thus appear more apt to rapidly respond to injury. Further, the uninjured atrial epicardium showed increased phosho-Smad3 expression at baseline and exhibited a rapid increase in both retinoic acid and TGF-β signaling following injury, while ATAC-seq uncovered an increase in chromatin accessibility at binding sites for Smad effectors. Loss of retinoic acid signaling and inhibition of TGF-β was able to delay atrial regeneration and migration of ECs, respectively. Altogether, our data demonstrate that accelerated regeneration of zebrafish atria is facilitated via potential priming and rapid deployment of TGF-β signaling in atrial ECs that influences enhanced atrial EPDC migration and collagenous scar deposition and resorption. F31HL147399.

S7T1: Keynote 2

James Wells, Jonathan Brassard, Patrizia Tornabene, Daniel Kechele, Lizza Roman, Michael Helmrath, Aaron Zorn
Endoderm organogenesis from human pluripotent stem cells: some assembly required.
Cincinnati Children's Hosp Res Fndnd, USA

Successful efforts to direct the differentiation of human embryonic and induced pluripotent stem cells (PSCs) into specific organ cell types *in vitro* have been guided by studies of embryonic organ development. Based on this, we can now direct the differentiation of human PSCs into gastrointestinal (GI) organoids including esophageal, gastric fundus, gastric antrum, small intestinal and colonic organoids. GI organoids contain complex epithelial structures and diverse cell types that are unique to their representative organ; esophageal organoids develop a stratified squamous epithelium, gastric organoids have a glandular epithelium that secrete digestive enzymes, and acid, and intestinal organoids secrete mucins and hormones and absorb nutrients. However, the first generation of GI organoids were lacking important cell types and functions that limited their utility. As such, we developed a modular approach to incorporate neurons, mesenchyme, vascular cells and immune cells into a variety of different organoids and are now able to engineer additional complexity at will. For example, we have engineered gastrointestinal organoids to have functionally innervated smooth muscle and functional immune cells. This modular approach was used to developed pancreatic organoids that form endocrine islets and a branching ductal network that end in functional acini. PSC-derived organoids are now routinely used to uncover the molecular basis of congenital malformations; to identify new pathologies in patients with diseases including inflammatory bowel diseases, diabetes, and pancreatitis; and to develop and test new therapeutics. Funding that supported this work: R01CA272903, P01HD093363, UM1TR006070, UG3DK119982, Shipley Foundation, Allen Foundation.

S8T1:

Timothy Nguyen, Kari Brown, Kim Cochran, Lylyan Pimentel, Yanfen Yang, Ching-Fang Chang, Samantha Brugmann
A BIRC5 molecular rheostat regulates cranial neural crest differentiation timing
Cincinnati Children's Hospital Medical Center, USA

Proper craniofacial development is achieved in part by fine-tuning the balance between proliferation and skeletal differentiation of cranial neural crest cells (CNCCs). Cross-species transplantation studies demonstrated that these stem/progenitor cells have the endogenous ability to control when they differentiate into craniofacial bone. However, mechanisms guiding the switch from proliferation to differentiation in CNCCs remain poorly understood. Our previous *in vitro* studies showed downregulation of the cell survival and proliferation regulator BIRC5/Survivin in CNCCs during osteogenic differentiation. Adding spatiotemporal resolution *in vivo*, we found that through development BIRC5 is robustly expressed in proliferating CNCCs while reduced in those differentiated into bone. Demonstrating this downregulation as functionally relevant, pharmacologically inhibiting BIRC5 in CNCCs accelerated *in vitro* osteogenic differentiation in a dose-dependent manner. These *in vitro* studies showed that BIRC5 inhibition compromised proliferation but not survival, hinting at the possibility of a unique role for this regulatory molecule in CNCCs compared to other tissues. Supporting its role in CNCC development *in vivo*, conditional knockout of *Birc5* resulted in dramatic hypoplasia of the murine embryonic face. Our findings raise a working model where BIRC5 dosage functions as a

rheostat to control CNCC differentiation timing, which may be tuned to generate patient-derived CNCCs and engineer skeletal tissue to treat craniofacial anomalies. Research support has been provided by the National Institute of Dental and Craniofacial Research (R01DE034357 and R35DE027557).

S8T2:

Kongju Zhu[1,2,3], Alejandra Rodríguez-delaRosa[1,2], Wenhui Tang[1,2], Shuyao Kong[1,2], Yuchuan Miao[1,2,4], Margarete Diaz-Cuadros[1,2,5], Olivier Pourquie[1,2,6]

Defective human somitogenesis in the absence of HOX genes

[1]Department of Genetics, Harvard Medical School, United States; [2]Department of Pathology, Brigham and Women's Hospital, United States; [3]Division of Genetics and Genomics, Boston Children's Hospital, United States; [4]Department of Cell Biology, Johns Hopkins University School of Medicine, United States; [5]Department of Molecular Biology, Massachusetts General Hospital, United States; [6]Harvard Stem Cell Institute, Harvard University, United States

HOX genes are key transcription factors that regionalize the vertebrate body. They are organized in four clusters and 13 paralogous groups in mammals, containing a total of 39 genes. Despite nearly half a century of research, our understanding of *HOX* genes function in vertebrate development remains limited, primarily due to the significant functional redundancy among paralogous members. So far, only partial loss of *HOX* function has been achieved in vertebrates, providing limited insights into their developmental roles. We have recently developed human pluripotent stem cell-based models that recapitulate somite development *in vitro* and exhibit the expected collinear expression of *HOX* genes. These models circumvent the lethality associated with *HOX* cluster deletions and allow experiments otherwise impossible in model organisms. We have created a series of homozygous *HOX* cluster deletions in a human pluripotent stem cell line, including a complete deletion of all *HOX* clusters (HOXKO). In HOXKO cells, the Epithelial–Mesenchymal Transition (EMT) associated to paraxial mesoderm formation occurs prematurely. HOXKO cells can form presomitic mesoderm but fail to form epithelial somites, despite a functional clock and wavefront system. This phenotype is associated with a downregulation of retinoic acid (RA) signaling and it can be rescued by re-expression of a single *HOX* gene, or by exogenous treatment with RA. Thus, our work uncovers previously unrecognized core functions of *HOX* genes in the control of RA signaling in somitogenesis. Our data suggest a critical role of *HOX* genes in regulating transitions between mesenchymal and epithelial states during development.

S8T3:

Jingyue Xu[1], Han Liu[1], Mohammed Sayed[1], In-Hee Lee[1], Yu Lan[1,2], Hee Woong Lim[1], Rulang Jiang[1,2]

Foxf2 and Twist1 acts cooperatively to pattern the embryonic palatal mesenchyme and regulate palatal musculoskeletal integration

[1]Cincinnati Children's Hospital Medical Center, USA; [2]Departments of Pediatrics and Surgery, University of Cincinnati College of Medicine, USA

The soft palate plays essential roles in daily life, including feeding, respiration, and communication. Abnormal palatal musculoskeletal development is a common birth defect associated with palatal dysfunction; however, the regulatory mechanisms underlying palatal musculoskeletal development and integration remain poorly understood. A missense mutation in *FOXF2* has been associated with shortened soft palate with misoriented palatal muscles in

patients. In developing mouse palatal mesenchyme, Foxf2 is enriched in the medial palatal progenitor cells. We show that mice with specific disruption of *Foxf2* display cleft posterior soft palate with failure of palatine aponeurosis formation and malpositioning of palatal muscles. Bulk and single cell RNA-seq analyses identified a group of perimysial mesenchyme-enriched marker genes that were ectopically up-regulated in the mesenchymal progenitor cells in *Foxf2* mutant palate. On the other hand, genes specifically expressed in the medial palatal mesenchyme, such as *Tnc*, were significantly down-regulated in *Foxf2* mutant palate. Remarkably, Sox9+ chondrogenic and Sox9+/Scx+ aponeurosis/tendon progenitors cells were dramatically reduced, resulting in disruption of morphogenesis of the medial pterygoid process and the palatine aponeurosis in the *Foxf2* mutants. Through ChIP-seq analysis, we found that Foxf2 and Twist1 co-occupy many target cis-regulatory elements in the developing palate. Furthermore, we found that *Foxf2* interacts genetically with *Twist1* to regulate soft palate morphogenesis. Together, these results indicate that Foxf2 and Twist1 act cooperatively to control palatal connective tissue differentiation and palatal musculoskeletal integration. This work was supported by NIH/NIDCR grant R01DE033890.

S8T4:

Paul Huber, Cole Milas, Betsy Schock
Modeling neural plate border differentiation using a *Xenopus* organoid system
University of Kentucky, United States

The *Xenopus* animal cap system is a powerful tool for assessing the lineage specification of vertebrate stem cells. Recently, this system has been used to pharmacologically induce animal caps into neural crest (NC) cells that differentiate into multiple cell types (chondrocytes, melanoblasts, peripheral neurons) when grafted into a host embryo. These findings suggest these cells are competent to differentiate into multiple NC lineages; however, it is unknown whether these cells have the intrinsic ability to differentiate or require an inductive environment. Here, through transcriptome analysis of pharmacologically induced NC explants cultured *ex vivo*, we determine that not only do these explants form NC-derived cell types (chondrocytes, melanoblasts, cranial nerves), but also express several markers for placodes, hatching gland, and cement gland, which are also derived from the neural plate border (NPB). Through additional *in situ* hybridization chain reaction (HCR) analysis, we determine that chondrocytes, otic placode, and cement glands self-organize into complex structures that mimic what is observed in whole embryos. Overall, our work demonstrates NPB cells generated from animal caps can differentiate into multiple cell types with the ability to self-organize in culture. We propose these NPB explants be classified as a *bona fide* organoid system that can be used to understand mechanisms governing differentiation and organization of these cell types into complex structures. This research was funded by NIH-NIDCR R00DE031825 to BS.

S8T5:

Rebecca Clements[1,2], Seble Negatu[2], Elizabeth Kennedy[2], David Song[2], Ashlyn Widmer[1], Ariana Campbell[2], Hyun Hyung An[3], Kevin Amses[2], Taylor Miller-Ensminger[2], Mary Addison[3], Laurence Eisenlohr[3], Stella Chou[3], Kellie Jurado[2]

Developmental regulation of antigen presentation machinery in human erythroid progenitors may contribute to fetal and perinatal immunity

[1]Kenyon College, United States; [2]University of Pennsylvania, United States; [3]The Children's Hospital of Philadelphia, United States

The cellular mechanisms governing human fetal and perinatal immune development remain incompletely understood. Although erythroid progenitors and precursors have recently emerged as immunomodulatory cells, their contributions to immune ontogeny remain poorly understood. Unexpectedly, we identified human erythroid progenitors as a previously unrecognized population of atypical major histocompatibility complex (MHC) class II-expressing cells. Using single cell RNA-sequencing analysis and flow cytometry, we found that committed erythroid progenitors progressively acquire MHC class II antigen processing and presentation machinery during fetal development and retain its expression into adulthood. Regardless of developmental stage, MHC II expression is lost during terminal erythroid differentiation. Erythroid progenitors also express machinery required for peptide loading and proteolytic antigen processing, and internalize and process exogenous protein antigens. Unlike conventional antigen-presenting cells, erythroid progenitors lack classical costimulatory molecules and instead express a distinct repertoire of immunoregulatory mediators. MHC II^+ erythroid progenitors localize adjacent to T cells within the human fetal liver and support MHC-dependent activation of primary $CD4^+$ T cells *in vitro*. Together, these findings identify erythroid progenitors as a developmentally regulated population of atypical MHC II-expressing cells and support a previously unrecognized contribution of erythroid cells in fetal and perinatal immune development. This work was supported by the American Heart Association, National Institutes of Health, Burroughs Wellcome Fund, and the David and Lucile Packard Foundation.

S9T1: Invited Talk 4

Noah Mitchell TBD

S9T2:

Kemal Keseroglu[1], Muhammed Simsek[2], Ertugrul Ozbudak[1]

Two distinct timers control vertebrate embryo segmentation

[1]Northwestern University, USA; [2]McMaster University, Canada

Sequential segmentation of the body axis is conserved across diverse animal phyla. The timing of somite segmentation in vertebrates is thought to be controlled solely by the period of the segmentation clock but their periods mismatch. By combing real-time imaging of the segmentation clock with pharmacological and surgical treatments, we show that the segmentation timing is governed by two sequentially acting distinct timers: while the segmentation clock controls segmental commitment, mesenchymal to epithelial transition (MET) of committed cells into a somite is regulated by an hourglass-like timer, whose duration is proportional to somite size and regulated by cytoskeletal activities. By modulating the hourglass timer, we altered the number of predetermined compartments, changed somite lengths, and

converted zebrafish segmentation from periodic to irregular, mimicking the formation of anterior somites in chick embryos. These results break the textbook dogma by showing somite lengths and segmentation timing are regulated by two distinct timers. This work was supported by NIH grant R35GM140805.

S9T3:

Peter Kropp, Ava Merickel, Rosa Kwon, Katy Spilsbury, Ellie Manning
Mitochondrial control of germ cell differentiation and death in a *C. elegans* model of MEPAN syndrome
Kenyon College, USA

Inborn errors of metabolism affect nearly 1 in 1,000 Americans, and many of these diseases are monogenic in nature. Despite understanding the genetics of these diseases in most cases, large gaps remain in our knowledge of how to connect the genetic cause to the patient phenotypes. This gap is particularly pervasive in mitochondrial diseases where tissue sensitivity and patient phenotypes are notoriously inconsistent. To close this gap for one disease, MEPAN syndrome, we generated a *Caenorhabditis elegans* model: a knockout of the causative gene, *mecr-1*. Although MEPAN syndrome is characterized by neurodegenerative phenotypes, the most prominent *C. elegans* phenotype is sterility due to a complete absence of oocytes. While the homology of these two phenotypes may seem disparate at first, many of the same regulatory pathways are employed in neuron differentiation and *C. elegans* oocyte differentiation thereby presenting us with a unique opportunity to investigate the metabolic, cellular, and tissue-level effects of *mecr-1* loss in a tractable system. Indeed, we have found that the RNA binding proteins GLD-1 (QKI in humans) and LIN-41 (TRIM71 in humans), both of which are essential for proper oogenesis, are dysregulated in our *C. elegans mecr-1* mutants. Importantly, QKI and TRIM71 are also necessary for neuronal differentiation in humans. Furthermore, we have found that germ cells undergo ferroptosis, iron-induced cell death, in the mecr-1 mutants potentially due to elevated cellular iron and/or increased fatty acids susceptible to iron-induced peroxidation. Overall, we can use the failure of oogenesis (both impaired differentiation and death) to better understand the neurodegeneration of MEPAN patients. Ongoing work seeks to clarify the mechanism of these dysregulated pathways and hopefully clarify the pathogenesis of MEPAN syndrome. This work was funded by Kenyon College.

S9T4:

Isha Verma[1], R. Keith Duncan[2], Haylie L. Miller[3], Michael Uhler[4,5]
Education, Emulation, And Ethics-Based Teaching Activity Helps Learners Appreciate The Importance Of Neurodiversity
[1]Department of Neurology, University of Michigan, Ann Arbor MI, 48109, United States; [2]Department of Otolaryngology-Head and Neck Surgery, University of Michigan, Ann Arbor MI, 48109, United States; [3]School of Kinesiology, University of Michigan, Ann Arbor MI, 48109, United States; [4]Department of Biological Chemistry, University of Michigan, Ann Arbor MI, 48109, United States; [5]Michigan Neuroscience Institute, University of Michigan, Ann Arbor MI, 48109, United States

For the inclusion of various learners in a classroom setting, it is important to destigmatize and affirm neurodiversity and promote evidence-based, equitable approaches to learning and assessment. We have designed an inclusive teaching exercise for students and instructors to explore the interface between developmental biology and genetic diversity. The students will

choose a recent publication and use it to explore the roles of specific genes characterized in autism from three main perspectives: Education, Emulation, and Ethics. For the Education part, the students will perform a thorough review of developmental neurobiology and principles of gene function within the nervous system, and for the Emulation part, they will develop a model highlighting the gene's role and function. Finally, the Ethics part will focus on how individuals should consider genetic variations. Universal design for learning and multipartiality frameworks are provided for instructors to create an inclusive learning environment. At the conclusion of the teaching exercise, the students will participate in a self-reflection exercise to facilitate their own assessment of how the teaching activity has impacted their philosophical and social perspectives on neurodiversity. Overall, this activity will encourage students to examine the mechanisms of neurodiversity within the context of autism and explore it in terms of societal functions. Funding: NINDS, NIA (NIH).

S9T5:

Timothy Plageman, Bonnie Wu

Retinal dystrophy-associated morphological defects are caused by disruption of Shroom3-dependent cell contractions within the outer limiting membrane

The Ohio State University, College of Optometry, USA

The outer limiting membrane (OLM) is composed of a dense, discrete layer of adherens junctions which form strong bonds among the photoreceptors and with their neighboring Muller-glial cells in the developing the outer retina. OLM integrity during development is among the vision-threatening disruptions caused by congenital retinal dystrophies. For example, mutations in the Crb1 gene cause retinal rosette formation and retinal degeneration in humans and animal models due to the disruption of the cell junctions within the OLM. However, what lies downstream of Crb1 or other retinal dystrophy associated genes to disrupt the OLM is relatively unexplored. We have observed that the F-actin and Rho-kinase-binding cytoskeletal protein Shroom3 is expressed within the developing photoreceptors and localized to the OLM during postnatal mouse retinal development. Therefore, it was hypothesized that OLM development may depend on Shroom3. Conditional ablation of Shroom3 in the neural retina causes the formation of rosettes that begin between postnatal day 2 and postnatal day 3. Discontinuous localization of several adherens junctional proteins as well as disturbances to the junctional ultra structure are also observed. Notably, en face observation of OLM revealed that a striking enlargement of cross-sectional area of photoceptor and Muller glial cells occurs throughout the developing retina in Shroom3 mutants. Importantly, similar disruptions are also observed in the OLM of the mouse retinal dystrophy model which disrupts the Crb1 gene. These data support the notion that Shroom3-dependent junctional contractility is important to maintain normal retinal structure and that 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 associated with OLM adherens junctions. *Funding: NEI (R01EY02691) and OLERF.*

Poster Abstracts:

Friday, September 25

6:35-9:30 pm **Poster Session 1 (Fountian Square Room)**

6:30-8:00 **Odd numbers** Posters 1-33

8:00-9:30 **Even numbers** Posters 2-34

Poster #1

Eric Wu, Idse Heemskerk, Zong-Yuan Liu, Kyoung Jo, Gauri Patel, Zhiyuan Yu, LiAng Yao, Seth Teague, Craig Johnson, Jason Spence
Endogenous FGFs drive ERK-dependent cell fate patterning in 2D human gastruloids
University of Michigan - Ann Arbor, United States

Human gastrulation is controlled by several signaling pathways, but the role of fibroblast growth factor (FGF) signaling is among the least understood. We found that FGF/ERK signaling is required for primitive streak (PS)-like formation using micropatterned human pluripotent stem cell-derived 2D gastruloids as a model of early human development, then studied how different FGF ligands regulate the PS-like formation. Single-cell RNA sequencing identified FGF4, FGF8, and FGF17 as the main FGF ligands expressed during differentiation. We found that different FGF ligands have different dynamics and spatial expression patterns. FGF4 is expressed in the PS-like region and is required for PS-like cell differentiation, while FGF17 was mainly involved in later stages such as mesoderm differentiation. In contrast, FGF8 was expressed earlier and appears within the inner boundary of the PS-like region. Reducing FGF8 expression expanded the PS-like region, suggesting FGF8 might help limit PS-like differentiation in human gastrulation, in contrast to its role in mouse. These findings show that individual FGF ligands have distinct roles during early human development and suggest FGF4- and FGF17-dependent ERK signaling to be among the key regulators of primitive streak formation and mesoderm specification, with FGF8 acting to restrict PS-like differentiation. Together, these findings highlight how individual FGF ligands coordinate early human development and provide insights for the improvement of regenerative medicine. This work was supported by the National Institute of General Medical Sciences (NIGMS R35GM138346) and the Branco Weiss Fellowship–Society in Science. K.J. was partially supported by the Michigan Pioneer Postdoctoral Fellowship and the National Institutes of Health F32 Ruth L. Kirschstein Postdoctoral National Research Service Award (5F32HD108980-02).

Poster #2

Sarah Alhilu[1], Benjamin Callaway[1], Caiden Duskey[1], Aiden Kraan[1], Diego Pareja[1], Kwangmin Choi[2], Lisa Martin[2], Carlo Prada[3], Nancy Ratner[2], Mark Charlton-Perkins[1]
Modeling NF1 Tumor Initiation and Genetic Modifier Effects in Zebrafish.
[1]Miami University, Oxford, USA; [2]CCHMC, Cincinnati, USA; [3]Lurie Children's, Chicago, USA

Neurofibromatosis type 1 (NF1) is a pediatric tumor predisposition syndrome characterized by peripheral nerve tumors and optic pathway gliomas, with substantial variability in disease severity among patients. Understanding the earliest cellular consequences of NF1 loss, as well as the genetic modifiers that shape disease

progression, remains a central challenge in the field.
To address this, we developed a glial-specific NF1 repression model in zebrafish using a CRISPR interference strategy. Under control of the GFAP enhancer, we selectively reduced NF1 function in glial progenitors of both the peripheral and central nervous systems. NF1 repression results in early Schwann cell hyperplasia and expansion of peripheral glial populations, followed by the development of peripheral nerve tumors resembling peripheral neurofibromas. As the disease progresses, animals consistently develop optic nerve thickening and optic pathway gliomas characterized by hypercellularity and expansion of GFAP-positive glia.
Building upon this platform, we are functionally testing candidate modifier genes identified through sequencing of NF1 patient cohorts. We further evaluate how recurrently altered genes influence Schwann cell expansion, peripheral tumor burden, and optic pathway glioma development.
These studies provide mechanistic insight into disease heterogeneity and establish a scalable platform for identifying pathways that may inform risk stratification and targeted therapeutic strategies in NF1. This work is supported by NIH/NINDS 1R01NS133200-01A1.

Poster #3

Maxim Shajenko, Jack Sheehan, Subbroto Saha, Chengji Zhou
Surface Ectodermal Transcriptomics of Spinal Neural Tube Closure
ADA Forsyth Institute, United States

Neural tube closure is a critical embryonic process that underpins the CNS. The transcription factor Grhl3 plays essential roles in neural tube closure, as Grhl3-KO mutants show fully penetrant spina bifida (SB). We previously revealed a unique cellular process regulated by Grhl3 for multicellular rosette formation and F-actin dynamics of the non-neural surface ectoderm (SE) encircling the closure site of the caudal neural folds (Zhou et al., 2020). However, the regulatory mechanisms of the SE dynamics remain largely unknown. A recent single-cell transcriptomics study demonstrated that Tfap2c is a key downstream target regulated by Grhl3 in SE to promote caudal neural tube closure (Deng et al., 2024). In this study, we reanalyzed their published E8.5 scRNA-seq datasets of wild type (WT) vs. Grhl3-KO alongside our unpublished E9.5 WT dataset acquired from the caudal regions of mouse embryos during spinal neurulation using a Seurat pipeline and Gene Ontology (GO) enrichment analysis. In the E8.5 samples, differentially expressed gene (DEG) analysis of the SE identified two novel SB-associated candidate targets downstream of Grhl3. Cross-referencing E9.5 WT SE featured genes with Grhl3-dependent DEGs reveals persistent expression of a new Grhl3-regulated SB gene, supporting its role in caudal neural tube closure across neurulation stages. GO enrichment analysis of WT vs. Grhl3-KO DEGs in E8.5 SE revealed significant disruption in actin filament and cytoskeletal organization, supporting our previously published findings. GO enrichment analysis of E9.5 WT highlighted gene networks in epithelial tube morphogenesis and the Wnt signaling pathway; the latter is consistent with our published works of canonical Wnt/beta-catenin signaling in caudal neural tube closure (Zhao et al., 2014 & 2022). Together, our results reveal new insights into surface ectodermal mechanisms underlying caudal neural tube closure and spina bifida. The Zhou lab is supported by NIH R01NS132387.

Poster #4

Brendan Teiken[1], Wenying Zhang[2], Ammar Husami[2], Quinn LaFave[1], Mosab Alquraish[1], William Balistreri[1], Akihiro Asai[1], Chunyue Yin[1,3,4]

Utilizing Zebrafish to Model Liver Disease Caused by ANKS3 Deficiency

[1]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Cente, USA; [2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 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 the slowing or stalling of bile flow in the liver. Genetic disorders account for 25% of cases in children. If left untreated, cholestasis leads to pruritis or jaundice and may progress to end stage liver disease and cancer. ~30% of patients with idiopathic chronic cholestasis do not have mutations in the known genes. The lack of molecular diagnosis hampers the development of personalized treatment. Whole Genome Sequencing (WGS) was performed on a patient with neonatal cholestasis and heart defects whose previous genetic testing was negative. We used a zebrafish model to validate the potential causality of a candidate gene. We performed Cholylglycylamidofluorescein (cGamF) assays on 6-day-old fish to track bile secretion and transport, and immunostaining to examine the morphology of the bile ducts and bile canaliculi. WGS identified compound heterozygous variants in the *ANKS3* gene. *anks3* zebrafish mutants were premature lethal and a third of them displayed heterotaxy. cGamF assay indicated that the *anks3* mutant larvae had reduction of bile salts within the bile ducts. Immunostaining revealed that the mutants had winding, tortuous bile ducts, with cholangiocytes positioned closer together compared to wildtype siblings. Bile Salt Export Pump (BSEP) localized normally to presumptive bile canaliculi at 6 days post fertilization but became disorganized by 9 days, suggesting either BSEP mislocalization or bile canalicular structural defects. We discovered novel variants in the *ANKS3* gene in a patient with neonatal cholestasis, renal and heart defects. Studying the zebrafish model validated causality of Anks3 deficiency and advanced our understanding of the role of ANKS3 in liver pathology. This work is supported by NIDDK, NIH Bioimaging Facility of the Digestive Disease Research Core Center in Cincinnati, James Heubi Investigator Award, Steele Family Research Fund, HEAL Fund for Rare Liver Disease Research, Peter and Tommy Colucci Research Award for PFIC.

Poster #5

Shoshana Marcus[1], Eleonora Scalia[2], Marcus Keatinge[2], Rafael Almeida[2], Daniel Soong[2], David Lyons[2], Sarah Petersen[1]

Dynamics of Glial-Specific Damage and Remyelination in the Peripheral Nervous System

[1]Kenyon College, USA; [2]University of Edinburgh, Scotland

Schwann cells form protective coating around axons called myelin in the peripheral nervous system. When nerves are damaged, Schwann cells help repair axons in a relatively well-characterized response. However, Schwann cell recovery after glial-specific damage and demyelination remains unexplored. To study this, we built a larval zebrafish model to specifically ablate Schwann cells and induce demyelination. In this model, nitroreductase 2.0 (NTR) is knocked in under the myelin basic protein (mbp) promoter. NTR in mbp-expressing cells reacts with ronidazole (RDZ) to ablate NTR+ Schwann cells. We found that at low doses of RDZ, peripheral myelin is slowly degraded; however, overall Schwann cell numbers stay the same. This result may indicate Schwann cells are dying slowly without being replaced, or they die

rapidly and are replaced by new Schwann cells. To distinguish between these possibilities, we treated NTR+ larvae with RDZ and observed apoptosis via acridine orange. We found the proportion of acridine orange-stained Schwann cells increases in RDZ treated fish, suggesting that Schwann cells turn over during RDZ treatment. To understand whether Schwann cells remyelinate normally following glial-specific damage, we used the mbp:EGFP-CAAX transgenic strain to visual myelin sheaths before and after RDZ treatment. We found sheaths partially regenerated by 48 hours following RDZ treatment; however, regenerated sheaths were 33.8% shorter than sheaths measured before treatment. Future studies will look into Schwann cell proliferation during recovery from RDZ as well as remyelination from newly proliferated Schwann cells following complete demyelination with RDZ. Our work characterizes the effects of Schwann cell-specific damage and demyelination and contributes to a mechanistic understanding of remyelination. This work was supported by funding from Kenyon Summer Science Scholars and NIH NINDS award 1R15NS141049-01.

Poster #6

Hannah Truong[1], Bailey Weatherbee[1], Shreyasi Mukherjee[2], Aaron Zorn[1]

Multifunctional RNA-binding Transcription Factors Coordinate Cell States in Development

[1]Cincinnati Children's Hospital Medical Center, United States of America; [2]Harvard Medical School and Massachusetts General Hospital, United States of America

SOX transcription factors play essential roles in organ development, and their dysfunction contributes to disease. Recent studies identified an evolutionarily conserved RNA binding domain in SOX proteins. To investigate its developmental role, I generated domain-specific mutations in SOX2 and SOX17, factors essential for pluripotency and digestive system development, respectively. An hPSC line carrying an endogenous SOX17 RNA binding domain deletion (dARM) disrupts definitive endoderm differentiation, similar to complete SOX17 knockout. However, dARM cells also show impaired nuclear localization, since this domain contains a nuclear localization signal (NLS). To distinguish localization defects from true RNA binding requirements, I generated additional constructs carrying a C-terminal NLS. Using Gateway cloning, I introduced mutations into the RNA binding and DNA binding domains and inserted these constructs into plasmids with an inducible promoter and viral integration sites. Since SOX2 is required for pluripotency itself, and functional SOX17 is needed at the definitive endoderm stage to study downstream cell types, inducible protein degradation hPSC lines were created for both factors, stably integrating the mutant constructs to enable temporally controlled functional studies. Our findings suggest that RNA binding domain is developmentally important, particularly for SOX17-driven endoderm differentiation. I am now investigating interactions between SOX17 and candidate U1/U2 spliceosome proteins, which drive splice site and branch point selection. Preliminary data indicate protein-protein interactions between SOX17 and these factors. I am performing in situ hybridization in frog embryos to compare expression of candidates with SOX17 across tissues and developmental stages. Together, this work defines a novel mechanism by which transcription factors integrate multiple regulatory layers to ensure precise developmental gene expression. Funding: CCHMC.

Poster #7

Laura Romano, Immanuel Mamphey, Anna Pak
Investigating the effect of exposure to chemicals associated with agricultural runoff on the embryogenesis and reproductive output of freshwater snails
Denison University, United States

We are interested in the extent to which anthropogenic activities are impacting the embryogenesis of snails that inhabit freshwater ecosystems in central OH. In recent years, local bodies of water have been impacted by pollution, warmer temperatures, more severe storms, and the construction of new roads, bridges, and water treatment facilities; agricultural runoff continues to be a major issue as well. In this study, we investigated the effects of nitrate and phosphate on the embryogenesis of ramshorn snails, which can be found in local ponds and a nearby creek with water that ultimately flows into the Ohio River. Fertilizers and livestock manure are a major source of both chemicals, with the EPA recommending nitrate levels less than 5.0 mg/L and phosphate levels less than 0.1 mg/L in bodies of water such as those inhabited by the snails (noting there are different thresholds for reservoirs of drinking water). We found that embryos tolerate nitrate at levels described as “poor,” although those from a pond are delayed in hatching and exhibit an elevated heartrate. We found that embryos are sensitive to high levels of phosphate, with those from a pond failing to progress beyond the veliger larval stage, and those from the creek failing to progress beyond the hippo stage of development. Exposure to both chemicals had significant effects on reproductive output, specifically the number of eggs per clutch. Currently, we are examining the effect of nitrate and phosphate on the microbiome of embryos exposed until the onset of hatching (preliminary results to be shared). Hopefully, all of this work using local snails as a new model system in our laboratory will contribute to more informed decision-making with respect to policies that may impact freshwater ecosystems in central OH. We are supported by the Denison University Research Foundation as well as the institution itself, Denison University.

Poster #8

Peyton Morrow
Unlocking Regeneration: How Newts Develop and Restore Their Eyes
Miami University, USA

Eye tissue regeneration after injury occurs in only a limited number of vertebrates, such as zebrafish and salamanders. In this regard, the newt Pleurodeles waltl (P. waltl) can regenerate its neural retina (NR) by reprogramming the retina pigment epithelium (RPE). The RPE and NR share the same embryonic origin: the optic vesicle, suggesting that the RPE neural potential may be linked to this origin. However, eye morphogenesis has not been studied in P. waltl, and it is unclear whether the molecular and cellular mechanisms that control eye development are also essential for its eye-regeneration capabilities. Therefore, we characterized eye morphogenesis in this species using histological, immunological, RT-qPCR, and transcriptomic analyses. We showed that eye formation begins between 3 and 4 days post-fertilization (dpf) and is well developed by 12 dpf. Interestingly, our snRNA-seq analysis indicates that eye development in the newt follows a conserved program. In the adult eye, the RPE is more oxidative, while the NR is mostly glycolytic; it is unknown whether this distinctive metabolism is necessary for NR and RPE formation during development. To determine whether glycolysis or oxidative phosphorylation (OxPhos) affects eye development and/or retina regeneration, we treated embryos with well-characterized inhibitors. We found that rotenone, an inhibitor of

respiration, increased the number of pHH3-positive cells and expanded the CM in developing eyes, producing smaller eyes. These results suggest that OxPhos regulates cell proliferation and may affect NR regeneration in adult newts. Overall, this work provides a comprehensive characterization of the developing eye in P. waltl, including the role of OxPhos in regulating cell proliferation. Funding: Miami Dean Scholar to PM; KTEF Career Starter Grant to JRPE; R01EY035325 to KDRT.

Poster #9

Taylar Simmons, Dayton Gatewood, Jaclyn Olberding, Timothy Nguyen, Fan Shao, Colin Kenny, Huojun Cao, Eric Van Otterloo
Identifying redundant functions of AP-2α and AP-2ß in epidermal development.
University of Iowa, USA

Ectodermal dysplasias are rare genetic disorders marked by the abnormal development of multiple ectodermal structures. The AP-2 transcription factor family has strong links to ectodermal development, including early expression in ectoderm-derived tissues, like the neural crest and epidermis. In humans, mutations in *TFAP2A* or *TFAP2B* cause significant anomalies in these structures, most notably the craniofacial complex. While previous studies have established a role for AP-2α in embryonic epidermal development, both *Tfap2a* and *Tfap2b* are highly expressed in the epidermis. Their combined role, however, remains unknown. To investigate potential redundancy between AP-2α and AP-2ß during epithelial development and appendage formation, we are leveraging conditional mouse genetics, basic histology, RNAscope, immunofluorescence, and single-cell multiome sequencing. Initial findings reveal that the combined ectodermal loss of AP-2α and AP-2ß completely arrests the formation of ectodermal appendages, including hair follicles, mammary buds, and vibrissae. It also results in severely defective epidermal development. Ongoing work focuses on mapping high-resolution expression dynamics of *Tfap2a*/AP-2α and *Tfap2b*/AP-2ß across key stages of epidermal stratification and differentiation. Furthermore, we are identifying the shared regulatory targets of AP-2 to uncover the molecular mechanisms driving this pathology. Ultimately, this work will detail AP-2's essential role in epidermal biology, providing a broader framework to understand the pathogenesis of ectodermal dysplasias. Funding: TS, NIGMS T32GM145441; JO, NIDCR T90DE023520; HC/EVO, NIDCR R01DE033009, R01DE034668.

Poster #10

Omodasola Ola[1,2], Shelbie Wenner[1,2], Natalie Pfaltgraff[1], Esther Ushuhuda[1], Maria Mikedis[1,2]
DAZL-dependent regulation of RA signaling in progenitors during spermatogenesis
[1]Cincinnati Children's Hospital Medical center, USA; [2]University of Cincinnati, USA

During spermatogenesis, early progenitors proliferate to expand their population and then differentiate into committed progenitors in response to retinoic acid (RA). RA-dependent differentiation causes progenitors to lose their stem-like properties and commit to ultimately enter meiosis and terminally differentiate into mature spermatozoa. Deleted in Azoospermia Like (DAZL) RNA-binding protein plays a crucial regulator of early progenitors' expansion and differentiation. In Dazl global knockout and Ddx4-Cre mediated conditional knockout, tubules have fewer early progenitors, and they do not differentiate efficiently into committed progenitors. The goal of this study is to understand the molecular mechanism by which DAZL supports early progenitor expansion and differentiation into committed progenitors. We hypothesize that DAZL directly activates RA-dependent translation and indirectly activates RA-dependent transcription

to support progenitors' formation. To test our hypothesis, we developed a novel conditional mouse model where Dazl is efficiently knocked out using Neurog3-Cre. In this model, early progenitors form normally but are unable to respond to RA. We will discuss ongoing work that seeks to define how DAZL impacts the transcriptome and translatome of progenitors. This project is funded by National Institute of General Medical Sciences of the national Institute of Health. R35GM156830.

Poster #11

Paige Ramkissoon, Kimberly Cochran, Samantha Brugmann
Uncovering an Ectodermal Role of ARID1A during Craniofacial Morphogenesis
Cincinnati Children's Hospital, USA

Craniofacial anomalies (CFAs), including clefting, affect approximately 1 in 700 live births annually, and treatment of CFAs often requires several, painful, and invasive surgeries. Hence, understanding the cellular mechanisms that contribute to CFAs (e.g., apoptosis, collective migration, differentiation, and epithelial to mesenchymal transition (EMT)), is essential to address this significant biomedical burden. Coffin Siris Syndrome (CSS) is a CFA associated with mosaic loss of function of the SWI/SNF chromatin remodeling complex. While our previous work identified ARID1A, the main DNA binding component of the SWI/SNF complex, as a key modulator in the specification and differentiation of neural crest cells, we have recently found that conditional deletion of ARID1A in the surface ectoderm also resulted in CFAs including bilateral cleft lip and cleft palate with the persistence of the medial epithelial seam. Further analysis revealed the presence of oral adhesions and thickened epithelium, suggesting an unappreciated role for ARID1A in the ectoderm. To identify the transcriptional programs underlying these phenotypes, we performed bulk RNA sequencing which revealed increased expression of cell proliferation and epidermal differentiation pathways, as well as decreased expression of EMT- and cilia-related genes in samples with conditional deletion of ARID1A in the surface ectoderm. Gaining a deeper understanding of the molecular and cellular mechanisms driving the observed phenotypes may provide therapeutic avenues for the generation of soft tissues amenable for cleft repair.

Poster #12

Quentin Phillips
SOX factors modulate WNT/β-Catenin-mediated transcription during embryonic lung development
Cincinnati Children's Hospital Medical Center, USA

WNT signaling regulates numerous transcription programs throughout development, but when disrupted may cause developmental anomalies and diseases. WNTs bind FZD/LRP co-receptors to initiate an intracellular cascade to translocate b-catenin (BCAT) to the nucleus where it interacts with one of four TCF/LEF transcription factors on WNT-responsive enhancers (WRE) to activate WNT genes. 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 foregut endoderm (FG) is patterned into an esophagus or respiratory domain. SOX2 is

expressed throughout pre-patterned FG, 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-transcription. Additionally, data shows that chromatin architecture of WNT genes varies in a SOX2-dependent manner and our data demonstrated that SOX2, TCFs and BCAT co-bind 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.

Poster #13

Parna Dutta[1,2], Nathaniel King[1]
Gbx2 defines pharyngeal foregut endoderm identity and regulates pharyngeal pouch transcriptional programs
[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati, USA

Endoderm is patterned into domains with distinct organ-forming potential along the anterior-posterior axis. The anterior portion of the foregut contributes to multiple budding organs, including the thymus and parathyroid, which derive from the third and fourth pouches. Despite the immense clinical importance of these pharyngeal derivatives, the ability to direct human pluripotent stem cells (hPSCs) toward these lineages has remained significantly limited. We hypothesize this reflects the current poor understanding of molecular regulators of anterior foregut patterning. Combining single-cell RNA-seq and whole-mount immunofluorescence, we identified a complementary Otx2-Gbx2 boundary in the E8.5 mouse foregut segregating the anterior tip from more posterior pharyngeal domains, and this pattern mirrors the Otx2-Gbx2 expression established at the forebrain-hindbrain junction. Otx2 is restricted to the anterior definitive endoderm from E7.5 and it later localizes to only the rostral portion of the first pouch. In contrast, Gbx2 emerges in the more posterior foregut by the 4 somite stages and later gets restricted to pouches 2-4. Genetic lineage tracing using Gbx2-CreER confirmed its transient expression in a broad range of early endoderm and its persistent expression in the posterior pouch domains. Correspondingly, Gbx2-labelled cells contributed to large portions of both the thymus and parathyroid, but not the thyroid that forms in the region of the first pouch. In hPSC differentiation protocols, we found that induced foregut endoderm consistently adopted an anterior-biased OTX2+ identity and never expressed GBX2. Transgenic GBX2 induction at the foregut stage was sufficient to promote other pharyngeal regulators such as SIX1 and PAX1, identifying GBX2 as a potential driver of posterior pouch. This suggests that more faithfully modeling the OTX2-GBX2 patterning mechanism will be a key requirement in generating authentic pharyngeal pouch derivatives from hPSCs. CCHMC.

Poster #14

Irene Jingyun Jin, Tyler Huycke
Architectural regeneration of intestinal villi
University of Michigan, USA

Tissue architecture provides the structural basis for organ function. Yet, while many mammalian tissues can replace lost cells after injury, they often fail to regenerate the three-dimensional geometries needed to fully restore their functions. The small intestine is a unique exception, as

thousands of damaged villi can coordinately regenerate their functional structures within days following acute injury. While previous studies of intestinal repair have largely focused on stem-cell-driven epithelial restitution, we approach this problem from a different angle by asking how cells self-organize to rebuild villi following damage and why this process fails in disease contexts. Our preliminary data suggest that mechanical forces driven by myosin-mediated mesenchymal cell dynamics, extracellular fluid flow, and epithelial cell bridging coordinately contribute to architectural villus restoration. Together, these findings support a model in which damaged villus fields are rebuilt through collective tissue remodeling, rather than epithelial replacement alone or regrowth of individual villi. Defining these mechanisms may shift our understanding of intestinal repair from stem-cell-driven cell replacement toward a broader framework for rebuilding form after injury, including in organs that normally fail to regenerate architecture. Funding: This research was supported by University of Michigan Department of Molecular, Cellular, and Developmental Biology Lab Startup Funds and Kinship Foundation Searle Scholars Program.

Poster #15

Thomas Taylor[1], Matthew Riccetti[2], Jenna Green[1], Kimberly Wagner[1], Cheng-Lun Na[1], Isabella Ford[1,3], Anne-Karina Perl[1,2,4]

Reexamining Alveolarization through the Perspective of MEOX2+ Alveolar Fibroblasts

[1]Division of Neonatology and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, United States of America; [2]Division of Development Biology, Cincinnati Children's Hospital Medical Center, United States of America; [3]College of Engineering and Applied Science, University of Cincinnati, United States of America; [4]Department of Pediatrics, University of Cincinnati College of Medicine, United States of America

Alveolarization is the final stage of lung development and is defined by “secondary septation”: subdivision of large saccules into alveoli by the secondary crest myofibroblasts (SCMFs). Premature birth disrupts this process, resulting in impaired, low surface-area lungs characteristic of Bronchopulmonary Dysplasia (BPD). In addition to SCMFs, the recently identified alveolar fibroblast cell types AF1 and AF2 reside in the developing alveolus, but their functions remain unexplored. They are defined by expression of mesenchyme homeobox 2 (MEOX2), a homeodomain transcription factor unexplored in the lung but with known contribution to segmentation and cell-cycle regulation. To examine the role of alveolar fibroblasts within alveolarization, we deleted Meox2 postnatally in mice (*Meox2*$^{\Delta/\Delta}$) using *Meox2*$^{flx/flx}$ in the fibroblast-specific *Pdgfra*^{CreER}. Histology, organoids, and transcriptomics demonstrate that MEOX2 is critical to alveolar fibroblast identity and controlling differentiation within the alveolar niche. Single-cell RNA-sequencing identified that changes within alveolar fibroblasts were confined to AF1s, coinciding with their shift towards SCMF or AF2 identity. Loss of AF1-derived WNT is associated with hastened epithelial differentiation, an effect recapitulated in primary-cell organoids. Complete absence of secondary septation coincides with increased hedgehog ligand from the epithelium and decreased hedgehog-interacting protein from AF1s. To further define SCMF response to hedgehog, we treated *Meox2*$^{\Delta/\Delta}$ mice with anti-hedgehog antibody. Treatment partially rescues the SCMF defect, displaying that modulation of hedgehog is a core component of AF1 input upon alveolarization. Alveolar fibroblast dictation of secondary

septation is a novel concept, and it has strong implications for resolving SCMF dysfunction within BPD. This work was supported by NIH/NHL.

Poster #16

Andrew Fernandes, Christopher Ahn, Hee-Woong Lim
Retinoic acid signaling promotes myocardial Wnt signaling necessary for atrioventricular valve development in zebrafish
Cincinnati Children's Medical Center, USA

Perturbations of retinoic acid (RA) signaling have been associated with congenital valve defects. Although the earliest role of RA signaling in cardiovascular development is to restrict cardiomyocyte specification, the requirements for early RA signaling in valve development are poorly understood. Here, we show that RA-deficient zebrafish embryos lack atrioventricular valves (AVVs), despite having enlarged hearts with intact endocardial lining. Endocardial valve markers were lost in RA-deficient embryos. However, consistent with the enlarged hearts in RA-deficient embryos, the atrioventricular canal (AVC) cardiomyocyte markers *bmp4* and *tbx2b* were expanded. Canonical Wnt signaling has been suggested to lie upstream of *bmp4* and *tbx2b* in AVV development. Surprisingly, we found that RA-deficient embryos lack myocardial *wnt2bb* expression by 72 hpfand canonical Wnt signaling by 36 hpf, suggesting that RA deficiency creates a disconnect between signals within the AVC myocardium prior to the initiation of endocardial cushion (EC) induction. Single cell RNA sequencing of 30 hpf heart tubes revealed that RA deficiency affected patterning of atrial/AVC cardiomyocytes resulting in misexpression of genes involved in Wnt signaling and valvulogenesis. In vivo analysis confirmed a loss of *wnt2bb* expression in RA-deficient atrial/AVC cardiomyocytes by 30 hpf and ectopic expression of genes regulating valvulogenesis and AVC chamber development. Inhibition of canonical Wnt signaling from 32-44 hpf and performed CRISPR-mediated knockdown of *wnt2bb,* both were sufficient to reduce EC cell number and size. Conversely, induction of canonical Wnt signaling in RA-deficient embryos by 36 hpf was able to rescue EC marker expression. Our results suggest that early RA signaling is necessary to establish Wnt signaling in AVC cardiomyocytes, which in turn promotes endocardial cushion cell differentiation and growth. This work was funded by NIH.

Poster #17

Karla Garcia, Katie Cameron, J. Raul Pérez-Estrada, Erika Grajales-Esquivel, Katia Del Rio-Tsonis
The Two Sides of Lens Regeneration: The Dorsal-Ventral Axes
Miami University, USA

In 2021, cataract prevalence increased from 36.38% to 37.03% in the U.S alone, exacerbating personal medical expenses and negatively impacting lifestyle. Intra Ocular Lens (IOL) surgery is a traditional treatment, however, complications like capsular damage and zonule dehiscence can lead to secondary cataracts. Formulating non-invasive procedures to redress IOL complications will be facilitated by understanding lens formation and repair through the study of newt lens regeneration. Our laboratory has identified that the GRN that specifies the D-V axis during eye development is active and conserved in the eyes of adult newt, *Pleurodeles waltl* (*P. waltl*). Interestingly, the newt has plasticity in its eye tissues, where the iris pigment epithelial cells (IPECs) are able to reprogram to lens cells after lentectomy. In addition, only the dorsal iris has the ability to give rise to lens cells post-injury. Two transcription factors (TFs), TBX5 and

VAX2 are key in mediating D-V eye patterning during eye development. We hypothesize that the D-V patterning observed in P. waltl eye is dictated by the GRN that modulates the D-V axis in the developing vertebrate eye. Yet, little is known on the interactions between D-V-specific TBX5 and VAX2 and how they modulate cell plasticity. Unveiling the role that these factors play in regulating lens regeneration through D-V regulation of GRNs is important. Hybridization Chain Reaction Fluorescence In Situ Hybridization (HCR-FISH), will be used to examine spatial localization of gene expression occurring at different regeneration timepoints. Iris explants will be used to either overexpress or knockdown these TFs to dorsalize the plasticity of the ventral iris and provide insights on cell fate determination and morphogenesis in eye regeneration. This work is supported by the NIH grant R01EY035325.

Poster #18

Irina Voda, Joseph Finks, Aden Jamal, Blair Leach, Courtney Stockman, Jenna Green, Kimberly Wagner, Anne Karina Perl
Fibroblast-Derived CXCL12 Maintains Pulmonary Capillary Endothelial Identity During Postnatal Alveologenesis
Cincinnati Children Hospital Medical Center, USA

During alveologenesis, functional alveoli depend on interactions among mesenchymal, epithelial, and endothelial cells. Fibroblasts provide structural support and produce signals regulating capillary endothelial identity. CXCL12 is chemokine important for endothelial proliferation, migration and vascular development, secreted by endothelial cells and alveolar fibroblasts. While its systemic vascular role is known, its role in establishing pulmonary capillary endothelial identity during postnatal alveologenesis is poorly understood. We asked if disruption of CXCL12 signaling from Meox2+ fibroblasts alters capillary endothelial identity and alveolar development. Meox2-CreERT2; Cxcl12fl/fl mice received tamoxifen at postnatal day 1 to delete Cxcl12 in Meox2+ alveolar fibroblasts. Lung morphology and differentiation were analyzed by histology and immunofluorescence. Endothelial identity was evaluated using PLVAP, CAR4, FOXF1, ERG, and NR2F2. An in vitro fibroblast–endothelial co-culture system investigated how fibroblast signals influence endothelial differentiation. Loss of fibroblast-derived CXCL12 resulted in persistent PLVAP expression, reduced CAR4+ CAP2 endothelial cells, and emergence of NR2F2+ERG+FOXF1− endothelial population in distal lung. In controls, NR2F2+ERG+ endothelial cells retained FOXF1 expression. NR2F2+ERG+FOXF1− cells localized in distal capillary structures rather than venous vasculature, suggesting impaired capillary identity and transdifferentiation to venous endothelial cells rather than ectopic venous cell hyperplasia. Our findings suggest that disruption of CXCL12 signaling alters pulmonary capillary endothelial identity toward a venous-like phenotype, similar to that observed in lymphangioleiomyomatosis (LAM), suggesting altered endothelial specification may contribute to vascular remodeling. This work was supported by NIH/NHLBI grants R01HL167030, R01HL164418, T32HL007752-31, and F31HL162470.

Poster #19

Aidan Goodwin[1,2], Erika Grajales-Esquivel[1,2], Stacy Bendezu Sayas[1,2], Katia Del Rio-Tsonis[1,2]
Uncovering the Epigenetic Factors Driving Chicken RPE Reprogramming
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In 2024 it was estimated that, globally, 196 million people have some stage of age-related macular degeneration (AMD), a degenerative retinal disease that causes blindness as it progresses in severity; this number is expected to reach 288 million by 2040. Any research related to potential treatments for AMD, or other retinal diseases, carry extreme importance: both for increasing the quality of life of patients and decreasing the economic burden that current treatments carry. Interestingly, chromatin accessibility of retinal pigment epithelium (RPE) cells from AMD patients has been shown to be dramatically reduced in comparison to healthy RPE cells, with 91.6% of genetic loci showing a decrease in accessibility in AMD RPE as opposed to healthy ones. As such, investigating the effects epigenetic modifications have on RPE cells has direct relevance to patients and is an important area of study. DNA modifications are key elements for cell fate and specification. This is true during chick ocular development, where epigenetic modifications have been shown to be an important factor in retinal development. We have shown that DNA demethylation is integral to induce FGF2-facilitated chick RPE reprogramming at embryonic day 4 (E4), where RPE cells are still plastic, and that across all reprogrammed RPE cells the repressive histone mark H3K27me3 was decreased and the activation histone mark H3K4me3 was increased when compared to intact RPE. Exploring which genes these histone marks are associated with can be tackled by CUT&RUN, a technique that can provide accurate histone modification and protein binding site mapping with small cell counts. The data generated from CUT&RUN will be overlayed with data from recently performed ATAC-seq and scRNA-seq to provide a comprehensive picture of the epigenetic landscape of the chick RPE as it undergoes reprogramming, and how this landscape affects gene transcription. This research is supported by funding from NIH grant EY034980.

Poster #20

Lindsay Helock[1,2], Joshua Waxman[2]

***Tbx20* Balances Cardiac and Pharyngeal Progenitor Specification in Zebrafish**

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Mutations affecting the transcription factor *TBX20* are associated with numerous congenital heart defects (CHDs) in humans, including septal defects, patent foramen ovale, and valvular defects. Previous studies identified *Tbx20* expression and requirements in the myocardium and endocardium, yet the early developmental cardiovascular defects resulting from *Tbx20* loss remain incompletely understood. Here, we investigated the requirements of *tbx20* in zebrafish heart development using CRISPR/Cas9-mediated depletion and a Cre-inducible loss-of-function allele. We find that loss of *tbx20* results in hearts with dramatically reduced number of cardiomyocytes by 48 hours post-fertilization (hpf). Consistent with previous reports in zebrafish, multi-color hybridization chain reaction (HCR) *in situ* analysis revealed reduced expression of the pan-cardiac differentiation marker, *myl7*, as early as 14-somites; however, in contrast to previous findings, expression of the cardiac progenitor markers *fgf8a*, *pea3*, *tbx1*, *tbx5a*, *hand2*, and *bmp4* were unaffected. To determine if loss of *tbx20* may be leading to differentiation of adjacent cell types, we examined the *nkx2.5*:ZsYellow reporter, which is expressed in cardiac and adjacent pharyngeal mesoderm. We found that as early as 17-somites within the anterior lateral plate mesoderm, *tbx20* depleted embryos exhibited an increase of *nkx2.5*:ZsYellow+ pharyngeal cells, coupled with the loss of *nkx2.5*:ZsYellow+ cardiomyocytes. Thus, our results support that *tbx20* may be required to promote the differentiation, but not specification, of cardiomyocytes at the expense of adjacent pharyngeal progenitors.

Poster #21

Praise Joel[1], Charlotte Meyer-Gerards[2], Cally Xiao[2], Marta Grzonka[2]
Disscecting the Mitotic Survellance Pathway (MSP)
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The regulation of cell division is crucial for the propagation of multicellular organisms and for tissue regeneration. We recently contributed to the discovery of a p53-dependent mitotic surveillance pathway (MSP) that monitors cell division duration and eliminates cells that exceed a defined threshold. The MSP signaling axis of 53BP1-USP28-p53 is activated by centrosome loss-of-function or pharmacological agents that disrupt cell division. Centrosomes, composed of a pair of centrioles and pericentriolar material, organize mitotic spindles and their loss in developing mice causes prolonged mitosis, 53BP1-, USP28- and p53-dependent cell death and embryonic arrest at mid-gestation. How the time is biochemically measured during mitosis and initiates MSP activation is an open question. To dissect the mechanism of the MSP, we established mouse embryonic stem cells (mESCs) and acute pharmacological protocols to induce the MSP *in vitro*. Prolonging mitosis in mESCs triggered the formation of mitotic aggregates or foci containing the main MSP components. Time-lapse imaging of p53-eGFP and 53BP1-HcRed knock-in mESCs reporters showed that these foci start to form in the cytoplasm of mitotic cells, coalesce, and lead to p53 upregulation after release from arrest. Genetically, the assembly of mitotic foci is dependent on 53BP1 but not USP28 or p53, suggesting a scaffolding role for 53BP1. We also identified RIF1, a known partner of 53BP1 in the DNA damage response during interphase, as a novel player in the MSP. RIF1 localizes to the mitotic foci, and its knockout in mESCs transforms the foci into elongated thread-like structures and blunts p53 stabilization. Collectively, our findings define a 53BP1-RIF1 mitotic scaffold that initiates and propagates the MSP, establishing it as a novel cell cycle checkpoint.
Funding Sources (Deutsche Forschungsgemeinschaft (German Research Foundation), Department of Cell & Developmental Biology, University of Michigan Medical School).

Poster #22

Shili Wu, Fangli Weng, Josefina Perez, Laurence Lemaire
Mapping Neural Cell Identities in the Central Nervous System of *Ciona intestinalis*
Saint Louis University, USA

During the metamorphosis of *Ciona*, an invertebrate chordate, its larval central nervous system (CNS) undergoes extensive remodeling. Most differentiated neurons are eliminated while other populations, acting as neural progenitors, are retained and contribute to the adult CNS. Identifying these progenitors and their potential will highlight *Ciona* CNS remodeling and, given its evolutionary relationship with vertebrates, the evolution and regeneration of the vertebrate nervous system. To uncover their identity, we mapped distinct larval neural cell types identified by single-cell transcriptomics and investigated their embryonic lineage and post-metamorphic developmental outcomes. Cell type-specific differentially expressed genes (DEGs) were used to generate fluorescent reporters to characterize cell identities, and in situ hybridization chain reaction (HCR) was used to confirm the gene expression patterns. Our results indicated that we identified a putative neural progenitor population in the region controlling tail movement, called the motor ganglion, as the expression of its specific reporter gene was retained in the juvenile CNS after metamorphosis. In contrast, a *Foxd+* cell population in the same motor control region corresponds to ascending motor ganglion (AMG) neurons, as indicated by their inhibitory neuronal transcriptomic signature and ascending axonal projections. Their shared

characteristics with vertebrate spinal V1 interneurons suggest a potential evolutionary relationship. Lineage tracing also identified *Foxd+* AMG neurons as originating from the posterior neural plate border, corresponding to the *Ciona* b-lineage. Further work will map the fate of the putative neural progenitors in the juvenile CNS and evaluate the evolutionary relationships of the identified cell types with vertebrate cell types. This work is funded by the Office of the Vice President for Research and the Provost Office of Saint Louis University (RI award 01978 and grant 001654).

Poster #23

Jacob Weaver, Anil Upreti, Brad Wagner, Jared Tangeman, Michael Robinson
FOXE3 Promotes Lens Development via Active Chromatin Maintenance and Distinct Repression of Neural Gene Programs
Miami University, USA

FOXE3, a transcription factor essential for eye development, is expressed in the lens placode and persists in the lens epithelium throughout life. Foxe3 mutations cause severe ocular anomalies including aphakia, Peters anomaly, and cataracts, but *FOXE3* transcriptional targets and regulatory mechanisms remain poorly understood. To identify FOXE3 targets, we utilized *Foxe3* knockout (KO) mice carrying a CRISPR/Cas9-mediated mCherry knock-in that abolishes FOXE3 protein. RNA-seq was performed on wild-type and KO lenses at E12.5, E15.5, and P0.5. To map direct binding sites, CUT&RUN profiling for FOXE3 was performed at P0.5 using FOXE3-specific antibodies relative to an IgG control. To evaluate epigenetic regulation, CUT&RUN for H3K4me3, H3K27me3, and H3K27ac was conducted in E15.5 control and KO lenses. Differential expression analysis revealed 268 consistently downregulated genes and 529 progressively upregulated genes in *Foxe3* KO lenses. FOXE3 binding peaks were enriched at regulatory regions of 87 downregulated genes and 118 upregulated genes. Direct targets positively regulated by FOXE3 included key lens development genes (*Pitx3, Gja3, Pdgfra, Bfsp1, Sox1*). Direct targets negatively regulated by FOXE3 were enriched for neural development (*Rorb, Rax, Sox11*). In Foxe3 KO lenses at E15.5, 21% of positively regulated targets showed reduced H3K4me3 and 11% show increased H3K27me3, with a subset (*Sox1, Gja3, Dmrta2*) also showing moderately reduced H3K27ac. In contrast, negatively regulated targets exhibited no consistent alterations in these histone marks. Together, these findings support a model in which FOXE3 maintains an active chromatin state at lens-development genes while repressing neural programs through a distinct mechanism. This research was funded R21 EY033471, R01 EY036378 and Sigma Xi G20230315-5759.

Poster #24

Josefina Perez[1], Shili Wu[1], Fangli Weng[1], Wei Lin[1], Michael Levine[2], Laurence Lemaire[1]
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 for vertebrate nervous system formation. This process starts with the invagination of the neural plate. Its borders then converge to close the tube, which propagates like a zipper. Afterward, cell intercalation and proliferation extend the tube. This process involves thousands of cells in vertebrates. The invertebrate chordate *Ciona* closes its neural tube with fewer than 20 neural cells, allowing single-cell resolution tracking and facilitating the study of its molecular mechanisms. In *Ciona*, the transcription factor Lmx1 is expressed in the dorsal-most cells of the developing tube before its closure. In

vertebrates, Lmx1 paralogs are involved in neural tube patterning, and no morphogenetic functions have been uncovered. Here, we found that in the absence of Lmx1, neural tube closure is delayed but recovered at later stages. Additionally, overexpression of a repressive *Lmx1* variant prevents proper dorsal neural cell intercalation, impeding zipper progression. This intercalation defect is absent in embryos where *Lmx1* was knocked down, suggesting that Lmx1normally represses early expression of genes involved in later morphogenetic steps. Lmx1, acting as a gatekeeper, might facilitate their coordination. Furthermore, Pax3/7, ZicL, and Smad transcription factors regulate *Lmx1* expression. Their orthologs are present at the vertebrate neural plate border, suggesting that *Lmx1* regulation is conserved across chordates. It raises the possibility of an unrecognized role for Lmx1 during vertebrate neural tube morphogenesis. This initial phase of this work was supported by a grant (NS076542) from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health. The Office of the Vice President for Research and the Provost Office of Saint Louis University (RI award 01978 and grant 001654) currently funds the project.

Poster #25

Gopal Kushawah

Spatio-temporally regulated maternal RNA decay factor essential for zebrafish development

Stowers Institute for Medical Research, USA

Maternal mRNA decay is essential for the Maternal-to-Zygotic Transition (MZT). While the temporal dynamics of maternal mRNA decay are well-characterized, spatial mechanisms remain underexplored. To resolve the spatio-temporal dynamics of maternal RNA decay, we integrated SLAM-seq with single-cell RNA-seq data to map the spatial dynamics of maternal RNAs during early zebrafish development. This analysis identified Cth1 as the first maternally encoded RNA-decay factor in zebrafish shown to undergo spatiotemporal regulation outside the germline, through conserved cis-elements in its 3′ untranslated region (3′UTR). 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. 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. These findings establish Cth1 as a key component of the maternal program and provide the first direct evidence that spatio-temporally regulated mRNA decay, outside the germline, contributes to early vertebrate development. Fundings: NIH-R01 GM136849, NIH-R21 OD034161 and NIH-R35 GM161459.

Poster #26

Courtney Stockman[1], Irina Voda[1,2], Blair Leach[1,3], Joseph Finks[1], Aden Jamal[1,4], Kimberly Wagner[1], Jenna Green[1], Anne-Karina Perl[1,5]

A Specialized Fibroblast Niche Instructs Alveolar Capillary Endothelial Identity Through Localized CXCL12 Signaling

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Formation of the alveolar gas-exchange surface requires coordinated differentiation of epithelial, endothelial, and mesenchymal cells into an exceptionally thin air-blood barrier composed of a single alveolar epithelial cell and a single capillary endothelial cell. This architecture is established during secondary septation, when specialized fibroblasts drive septal formation and coordinate maturation of the surrounding tissue. Although fibroblasts are increasingly recognized as specialized stromal niches that coordinate tissue development, it remains unknown whether they directly instruct endothelial cell fate during alveolar vascular development. To determine whether fibroblast-derived CXCL12 instructs alveolar capillary endothelial identity, we conditionally deleted *Cxcl12* in Meox2? fibroblasts during postnatal lung development. Loss of fibroblast-derived CXCL12 disrupted secondary septation, impaired CAP1-to-CAP2 differentiation, depleted mature CAR4? CAP2 endothelial cells, and redirected endothelial differentiation toward a venous-like fate. These changes occurred despite CXCL12 expression from other cellular sources in the developing lung. Reduced ERK1/2 and p38 MAPK signaling throughout the developing capillary endothelium accompanied these defects, suggesting that localized CXCL12 signaling promotes acquisition and maintenance of mature capillary identity. Together, these findings demonstrate that endothelial cells require spatially localized CXCL12 signaling, rather than overall tissue ligand abundance, to establish alveolar capillary identity. They identify fibroblast-derived CXCL12 as an instructive developmental niche signal that links mesenchymal patterning to endothelial cell fate during alveolar vascular development. This study was supported by NIH/NHLBI grants R01 HL167030 (AKP), R01 HL164418 (AKP), and T32 HL007752 (CAS). We thank the CCHMC Transgenic Animal and Genome Editing Core for the design and generation of the Meox2CreERT2 mouse.

Poster #27

Bitan Saha, Amulya Adavalli, Joshua Waxman

Cardiac contractions establish endocardial heterogeneity needed for cardiac jelly homeostasis and chamber-specific growth

Cincinnati Children's Hospital Medical Center, USA

Defects in endocardial development are associated with chamber-specific congenital malformations, however the influence of endocardial cell heterogeneity on vertebrate cardiac chamber development and congenital heart defects remains poorly understood. Here, using zebrafish and human induced pluripotent stem cells (hiPSCs), we show that atrial endocardial cells are instructive for differential deposition of cardiac jelly, chamber-specific growth, and morphology. A knock-in transgenic reporter and single cell RNA-seq show that zebrafish *nr2f1a* is uniquely expressed in the atrial endocardium by cardiac looping. Single cell RNA-seq also

revealed unique molecular signatures and functional diversity between *nr2f1a*$^{+}$ atrial endocardial cells and *nr2f1a*$^{-}$ ventricular endocardial cells. In contrast to previously examined cardiovascular contexts, within the endocardium *nr2f1a* and cardiac contraction-initiated Notch signaling cell-autonomously define atrial and ventricular endocardial populations in a mutually antagonistic manner. Furthermore, the delineation of atrial and ventricular endocardial populations is necessary for proper chamber-specific development as *nr2f1a* mutant zebrafish exhibit hypertrabeculation-like growth within the ventricles. We also find that repression of Notch signaling is sufficient to induce NR2F2^{+} atrial endocardial identity in hiPSC-derived endocardial cells. Knockdown of the hyaluronidase *hyal2b*, which is repressed in *nr2f1a*$^{+}$ atrial endocardial cells and expanded in *nr2f1a* mutant hearts, was found to be sufficient to restore the proper cardiac jelly deposition that is absent in *nr2f1a* zebrafish mutants. Thus, these results illustrate that mutual antagonism downstream of biomechanical forces define atrial and ventricular endocardial populations in the developing heart, which influence chamber specific growth and morphology, and whose disruption may underlie etiology of congenital heart defects. This work was funded by the American Heart Association.

Poster #28

Yam Prasad Aryal[1], Yilun Huang[1], Yu Lan[1,2,3], Rulang Jiang[1,2,3]

***Sf3b2* heterozygous mice exhibit defects in vertebral patterning and strain-background dependent ocular and craniofacial abnormalities**

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Craniofacial microsomia (CFM), also known as oculo-auriculo-vertebral spectrum, is characterized by mandibular hypoplasia with variable ocular, auricular, and vertebral anomalies. Heterozygous loss-of-function variants in *SF3B2*, a core component of the U2 spliceosome, are a major genetic cause of CFM, but the developmental mechanisms underlying *SF3B2*-associated spliceosomopathy remain poorly understood. To investigate these mechanisms, we generated conditional *Sf3b2* mutant mice. We show that constitutive heterozygous deletion of *Sf3b2* caused severe developmental defects in a strain-dependent manner. In the C57BL/6J background, fewer than 25% of *Sf3b2*$^{+/-}$ embryos survived to birth, with more than 50% embryonic lethality by E12.5. Surviving mutants exhibited developmental delay, reduced body size, microphthalmia (>70%), and craniofacial defects (~30%). In contrast, *Sf3b2*$^{+/-}$ mice on a mixed CD1 genetic background were born at Mendelian ratios and did not have overt ocular and craniofacial abnormalities. Regardless of genetic background, *Sf3b2* heterozygous mice consistently exhibited vertebral homeotic transformations including C7 to T1, T1 to T2, S4 to S3, and loss of L6. These vertebral defects correlated with dysregulated *Hox* gene expression similar as previously reported in *Sf3b1*$^{+/-}$ and *Sf3b4*$^{+/-}$ mouse embryos, indicating that the SF3B complex plays a key role in patterning of the anterior-posterior axis through regulation of Hox gene expression. This work was supported by Division of Developmental Biology at Cincinnati Children's Hospital Medical Center.

Poster #29

Nirpesh Adhikari[1], Yu Lan[1,2,3], Rulang Jiang[1,2,3]

Twist1 acts upstream and as an obligatory partner of Alx1 in specifying frontonasal identity of the neural crest-derived craniofacial mesenchyme

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Dominant-negative mutations in *TWIST1* cause a severe frontonasal dysplasia syndrome named as Saethre-Chotzen syndrome (SCS), characterized by craniosynostosis and facial dysostosis with hypoplastic maxilla and mandible, whereas biallelic disruptions of the *ALX1* gene cause the autosomal recessive frontonasal dysplasia syndrome FND3, characterized by severe midfacial hypoplasia, bilateral cleft lip, and extreme microphthalmia. Genetic studies in animal models have shown Twist1 function is required for the activation of a number of ectomesenchyme genes including *Alx1* in the embryonic cranial neural crest cells (CNCCs). Recent studies have shown that Alx1 plays a crucial role in the molecular determination of frontonasal identity of the CNCC-derived craniofacial mesenchyme. We have shown that transgenic overexpression of *Alx1* throughout the CNCCs resulted in formation of a duplicated premaxilla at the expense of the maxillary structures accompanied by significant downregulation of expression of the *Dlx1* and *Dlx2* genes. However, we found that *Twist1* heterozygosity prevented the transgenic Alx*1*-induced maxillary-to-premaxillary transformation. Furthermore, spatiotemporally induced specific inactivation of *Twist1* in the *Alx1*-expressing CNCCs severely disrupted frontonasal development. Together, these results reveal stage-dependent Twist1 functions in CNCC fate specification and in regulating the regional identities of the craniofacial structures. This work was supported by the National Institutes of Health National Institute of Dental and Craniofacial Research (NIH/NIDCR) grant R01DE029417.

Poster #30

Ekasit Sonpho[1], Vidyanand Sasidharan[1], Charles A.S. Banks[1], Eric J. Ross[1], Carlos Guerrero-Hernández[1], Frederick G. Mann Jr.[1], Carolyn Brewster[1], Neelanshi Jain[1], Beatrice Broglia[1], Sean A. McKinney[1], Kexi Yi[1], Zulin Yu[1], Alejandro Sánchez Alvarado[1,2]

Geranylgeranylation is Broadly Required for Vesicular Trafficking and Tissue Regeneration in Planarians

[1]Stowers Institute for Medical Research, USA; [2]Howard Hughes Medical Institute, USA

Tissue regeneration relies on coordinated cell biology across lineages, yet how irreversible lipid post-translational modifications contribute to this coordination remains poorly understood. Protein prenylation anchors small GTPases and lamins to membranes, but its *in vivo* roles during whole-organism regeneration are unclear. Here, in the planarian *Schmidtea mediterranea*, we show that RNAi of geranylgeranyl pyrophosphate synthase (*ggpps*), and convergent RNAi of the downstream geranylgeranyl transferase *pggt-b*, impair tissue homeostasis, blastema growth, organogenesis, stem cell migration, and epidermal differentiation. Single-cell transcriptomics across three independent atlases shows that prenylation pathway enzymes are broadly expressed across cell types including stem cells. Disrupted geranylgeranylation reduces anti-prenyl immunoreactivity and remodels Rab GTPase distribution, manifesting as altered large extracellular vesicle composition with selective Rab GTPase accumulation as cargo. These findings establish that broad-spectrum protein

geranylgeranylation supports coordinated tissue regeneration, with Rab-dependent vesicular trafficking as a major affected cellular process. This project has been funded by the Stowers Institute for Medical Research and the Howard Hughes Medical Institute.

Poster #31

Anna Statsenko[1], Amartya Mitra[2], Lucy Lucy Bailei[1], Itunu Adewoye[1], Elke Buschbeck[2], Mark Charlton-Perkins[1]
Vitreous chamber expansion drives refractive development and emmetropization
[1]Miami University, USA; [2]University of Cincinnati, United States

Emmetropization is the developmental process that coordinates ocular growth and optical power for focused vision. In humans, the eye is usually hyperopic early on and gradually reaches emmetropia as it grows. Disruptions in this process lead to refractive disorders like myopia, but the mechanisms behind normal refractive development are not well understood. Zebrafish serve as an accessible vertebrate model to study how individual ocular compartments grow and contribute to emmetropia. We combined micro-ophthalmoscopy and optical coherence tomography to study refractive development and ocular growth in zebrafish. Micro ophthalmoscopy provided noninvasive assessment of retinal focal plane position, while optical coherence tomography quantified axial length and ocular compartment dimensions. Similar to humans, zebrafish were strongly hyperopic during early development and gradually approached emmetropia as the eye grew. The most rapid refractive transition occurred between 2 weeks and 1-month post-fertilization and was strongly associated with body size. Analysis of individual eye compartments revealed that expansion of the vitreous chamber was the primary structural change associated with refractive development. In contrast, the lens, retina, and other ocular compartments grew more uniformly over time. These results indicate that refractive maturation is not simply the consequence of proportional growth across the entire eye. Instead, it depends largely on selective expansion of the vitreous chamber, which increases axial length and moves the retina toward the focal plane. Together, these findings demonstrate that zebrafish and humans share a similar developmental progression from early hyperopia toward emmetropia. Our results outline the normal emmetropization process and highlight vitreous chamber expansion as a key factor in refractive development. This framework will help us understand how genetic and environmental factors disrupt ocular growth and lead to myopia.

Poster #32

Bau-Lin Huang[1], Sean Davis[2], Eiki Koyama[3], Maurizio Pacifici[3], Susan Mackem[1]
A pivotal Wnt antagonist role in promoting digit joint (interzone) specification by constraining Wnt activity
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Unlike long bone joint formation, which occurs within already condensed cartilage, digit joint and phalanx formation is a sequential and repetitive process in digits. In long bones, Wnts have long been considered to direct joint formation. In digits, depending on Bmp response, phalanx forming region (PFR, chondroprogenitors) cells in distal digit tips can adopt a chondrocyte (high Bmp response) or joint fate (low Bmp response). It is unclear how Wnts may interact with Bmps to modulate digit phalanx-joint formation. We find that in digit progenitors, Wnts cooperate with Bmps to promote chondrogenic rather than interzone fate by inhibiting Gsk3beta to stabilize pSmad1/5. In this study, we tested the role of Wnt pathway by analyzing the effect of

constitutive betaCatenin activation (bCatCA) on the impaired digit joint induction in 5'Hoxd-/- digits. Unexpectedly, only bCatCA expressed selectively in interdigit mesenchyme, rather than in PFR cells, was able to restore 5'Hoxd-/- digit joint formation indirectly and cell non-autonomously. RNA profiling revealed that interdigit bCatCA induced Wnt antagonists that act to lower digit tip pSmad1/5 levels and restore interzone specification in 5'Hoxd-/- digits. Genetic removal of the bCatCA-induced downstream target Dkk2 in 5'Hoxd-/- interdigits prevented joint rescue by bCatCA and, conversely, Wnt ligand overexpression in digit tip inhibited joint progenitor specification in wildtype digit tips. Furthermore, in cultured limb buds, small molecule Gsk3beta inhibitors increased pSmad1/5 level and resulted in higher Bmp activity in digit tips. We propose that, before the digit mesenchymal progenitors transit into the PFR, Wnt antagonists are required for Gsk3beta stabilization to prevent excess, precocious pSmad1/5 level in progenitors. The balance between Wnt and Wnt antagonists maintains digit progenitor plasticity to adopt phalanx or interzone fates in response to varying Bmps. Funding: NIH Intramural Program.

Poster #33

Han Liu[1], Jingyue Xu[1], Eri Iwasawa[1], Yueh-Chiang Hu[1,2], Yu Lan[1,2], Jason Tchieu[1,2], Hee Woong Lim[1], Rulang Jiang[1,2]

Deletion at the *SIX2* locus causes frontonasal dysplasia through chromatin domain fusion and altered enhancer-gene interactions

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Several studies have identified heterozygous deletion of the *SIX2* gene as a cause of an autosomal dominant frontonasal dysplasia (FND) syndrome and suggested *SIX2* haploinsufficiency as a disease mechanism. *SIX2* encodes a member of the SIX family DNA-binding transcription factors and is physically directly adjacent to *SIX3*, with the chromatin region containing a topologically associating domain (TAD) boundary located between these two genes. We generated human pluripotent stem cell (hPSC) lines carrying *SIX2* deletions mimicking the genomic changes in the FND patients and found that such deletions disrupted the TAD boundary and significantly increased interactions of the *SIX3* gene with *SIX2* distal enhancers. We generated conditional mutant mice with inducible deletion of *Six2* mimicking the FND-associated *SIX2* deletions and found that the *Six2-TADb*$^{del/+}$ mice are born with severe midline frontonasal clefting, whereas *Six2*$^{+/-}$ mice do not have such defects. Disruption of craniofacial development in the *Six2-TADb*$^{del/+}$ embryos correlated with ectopic *Six3* expression in the developing frontonasal mesenchyme. Furthermore, transgenic misexpression of *Six3* in the embryonic frontonasal mesenchyme is sufficient to disrupt midfacial morphogenesis. Moreover, mice heterozygous for a deletion containing both *Six2* and *Six3* genes do not exhibit FND. These results indicate that *SIX2*-related FND is caused by TAD fusion-induced activation of *SIX3* expression and that medical interpretation of phenotypic impacts from copy number variations needs to consider effects of local chromatin architecture. Our novel mouse and hPSC models provide valuable tools for understanding chromatin-level gene regulation. This work was supported NIH/NIDCR grants R21DE032877 and R01DE032558.

Poster #34

Le Xu

Mesothelial Plasticity Specifies a Pleural Immune Circuit that Orchestrates Lung Regeneration

Cincinnati Children's Hospital Medical Center, USA

Background: A key feature of lung regeneration after pneumonectomy (PNX) is its spatial bias toward the organ periphery, where dysplastic and/or fibrotic tissues are also primarily built up in many degenerative lung diseases. The cellular and molecular players that dictate these divergent outcomes remain poorly understood. Here we aimed to investigate the cellularmechanismsand outcomes ofthe context-dependent reprogramming of mesothelial cells under physiological or pathological perturbations. **Methods:** We performed scRNA-seq and scATAC-seq on mouse lungs at day 7 post-PNX versus sham control, and integrated these with in-house generated scRNA-seq from a mouse orthotopic lung allograft model of tissue degeneration. Lineage tracing and conditional inactivation were performed as major genetic approaches. **Results:**Mesothelial cells are found drastically expanded after PNX with pleura thickened from a single layer to patches of 3–5 cell layers. scRNA-seq resolved heterogeneoussubgroups of expanded mesothelial cells that arranged along a differentiation trajectory validated by *Wt1?????* lineage tracing. In the CLAD allograft lung, mesothelial cells are similarly expanded but stalled in the ECM-high state, failed to generate the inflammatory subpopulation which highly express chemokines recruiting pro-regenerative monocytes. scATAC-seq identified TWIST1 as a transcription driver of mesothelial activation; Mesothelium-specific *Twist1* deletion blocked mesothelial expansion and immune cell recruitment, which in turn led to reduced lung regeneration. **Conclusion:**Our findings here establish a diagram in which context-dependent mesothelial cell reprogrammingdictates peripheral remodeling of tissue regeneration or degeneration in the lung. This work was supported by CCHMC startup funds and NIH/NHLBI Pathway to Independence K99/R00HL171830 (to L.X.).

Saturday, September 26

6:30-9:30 pm **Poster Session 2 (Fountian Square Room)**

6:30-8:00 **Odd numbers** Posters 35-71

8:00-9:30 **Even numbers** Posters 36-72

Poster #35

Paloma Jolly
Investigating the effects of elevated retinoic acid on vascular development in zebrafish
Miami University, USA

As zebrafish develop from newly fertilized eggs to larvae, their vascular system is at the forefront of that growth. Due to cell specialization, blood vessels grow and sprout to create a network throughout the organism that allows for circulation. Cells that will become the blood vessels in zebrafish are assigned as precursor cells, or angioblasts, and move to the designated area in the embryo to begin forming the main vessels in the trunk: the dorsal aorta and posterior cardinal vein, by vasculogenesis. After the main vessels are formed by vasculogenesis, vessel sprouts develop off of the network through angiogenesis. During this early development, the zebrafish embryos need specific quantities of chemical signals to help support growth and development of organ systems. One such chemical is retinoic acid (RA), which is used to determine how angioblasts and other specialized cells develop into blood vessels. Both insufficient and excess amounts of RA can cause significant issues with vessel development as seen in mouse and chicken embryos. Previous studies determined the impact that excess RA has on zebrafish vasculature, specifically in the initial formation of the dorsal aorta and posterior cardinal vein. Expanding on this, this project also explored the impact that varying concentrations of RA had on zebrafish angiogenesis. Following the RA treatments, I utilized the confocal microscope to take images of live and immunostained embryos for further analysis. I specifically examined the dorsal aorta diameter in the zebrafish trunk, the number of ISV sprouts in the tail, the diameter of the caudal vein plexus, and the amount of abnormal ISV sprouts in the tail. Based on current analysis, the dorsal aorta diameter and the number of ISV sprouts were unaffected. From observing differences in abnormal sprout morphology in relation to the increased RA doses, we are currently exploring other ways to quantitatively analyze these differences. Funded by Miami University.

Poster #36

Amalia Hunt, Joseph Lee, Kunqi Shi, Chanda Maambo, Karen Hicks
Identifying Genes Involved in Seasonal Reproduction of *Physcomitrium patens* using QLTseq
Kenyon College, USA

Many organisms align their reproductive cycles with environmental cues. This study aims to apply quantitative genetics to discover genes involved in the seasonally regulated reproduction of moss, using the model moss *Physcomitrium patens*. Previous work in the Hicks lab has revealed that the mechanism that converts environmental cues to the initiation of the reproductive cycle is largely distinct from the previous characterized mechanism in flowering plants. Specifically, we aim to harness the natural genetic differences between strains of *P.*

patens as an avenue to identify and characterize new genes involved in the species' seasonal reproduction. Strains that vary in their reproductive timing in response to cooler temperatures were crossed, and reproductive timing was measured in the recombinant progeny. Using QTLseq analysis, several genomic regions were identified as differing significantly between early reproductive progeny and late reproducing progeny in response to cool temperatures, and 1-7 candidate genes within each region were selected based on predicted impact of variants on protein structure/function. These genes are currently being knocked out in parental strains using CRISPR-Cas9 and their phenotypic effects will be assessed. This work was funded by NSF 2523491 and the Kenyon College Summer Science Scholars Program.

Poster #37

Taylor Roy[1,2], Sophia Hsin-Jung Li[1], Maple Adkins-Threats[1], Xufeng Xue[1], Jim Wells[1,2], Aaron Zorn[1,2]

Modeling human fetal gastrointestinal tract in a microfluidic device

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

The primitive gut tube is the embryonic precursor which undergoes regional identity to form the gastrointestinal tract. Spatial patterning of the gut tube first occurs along the anterior-posterior (A-P) axis which gives rise to three main regions including the foregut, midgut, and hindgut. Along these regions, patterning occurs along the dorsal-ventral (D-V) axis through signaling pathways which establish organ determinization and organization. Reproducing this regional patterning in vitro is challenging, which limits the ability to study this developmental process in a physiologically relevant human 3D model. Our developed microfluidic "gut tube chip" allows for precise spatial control and signaling gradients while enabling live imaging of dynamic processes. By seeding human pluripotent stem cells (hPSC) into the gut tube chip, we can investigate these gradient mechanisms that occur during human gastrointestinal development. Through optimization experiments, our preliminary data reveals that microfluidic devices provide a platform for spatially controlled signaling and tissue organization as a 3D model. Overall, this system presents as a unique model for investigating developmental mechanisms and gastrointestinal disease. Funding: CCHMC Cure Award to Aaron Zorn.

Poster #38

Jonathan Yii

Tandem Knockdown of *Celsr2* and *Celsr3* in *Xenopus tropicalis* on the Genetic Pathogenesis of Congenital Hydrocephalus

Department of Pediatrics, Yale University School of Medicine, United States

Congenital hydrocephalus (CH), the abnormal accumulation of cerebrospinal fluid (CSF) in the cerebral ventricles, affects ~1.5/1000 births and is typically treated by surgical intervention. In *Mus musculus*, combined loss of the core planar cell polarity (PCP) genes *Celsr2* and *Celsr3* disrupts ependymal ciliogenesis and planar polarity, resulting in lethal hydrocephalus; *Celsr2* is expressed in ependyma but not the choroid plexus, supporting a cilia-mediated mechanism for ventriculomegaly. Therefore, we investigated whether disruption of this pathway produces a conserved ventricular phenotype in *Xenopus tropicalis*, where the ventricular system is optically accessible *in vivo*. Dual CRISPR/Cas9 RNP complexes targeting exon 1 of *celsr2* and *celsr3* were co-injected into one-cell-stage embryos, and ventricular morphology was assessed at stage 45 using optical coherence tomography (OCT). Approximately 25.4% of the injected

tadpoles (n=122) versus 5.3% of uninjected controls (n=245) exhibited hydrocephalus-associated phenotypes, including ventriculomegaly and aqueductal stenosis ($p<0.0001$). We genotyped 10 uninjected controls, 10 phenotypically normal injected, and 20 abnormal injected tadpoles by Sanger/Inference of CRISPR Edits. Injected tadpoles yielded indel scores from 63–96% and knockout scores from 57–92% in both genes. Editing was comparable across phenotypic groups, consistent with F0 mosaicism: whole-animal genotyping does not resolve editing within the ependyma itself. These preliminary findings indicate that simultaneous disruption of *celsr2* and *celsr3* produces hydrocephalus-like ventricular abnormalities in *X. tropicalis* and support its use as a rapid vertebrate model for investigating PCP-associated CH. Future work will directly examine ependymal ciliary organization, planar polarity, and CSF flow via OCT particle tracking and cilia imaging to determine if the cellular mechanisms described in mice are conserved in *Xenopus*.

Poster #39

Alexandra Kelly[1], Eleonora Scalia[2], Marcus Keatinge[2], Rafael G. Almeida[2], Daniel Y. H. Soong[2], David A. Lyons[2], Sarah C. Petersen[1]

Debris Clearance and Repair Following Glial-Specific Damage in the Peripheral Nervous System

[1]Kenyon College, USA; [2]University of Edinburgh, United Kingdom

Peripheral myelin is produced by Schwann cells to enable rapid electrical signaling and trophic support for axons. When peripheral nerves are injured, mature Schwann cells transdifferentiate to a general repair state, demyelinating injured axons, clearing debris via autophagy, and supporting axon regrowth. Myelin debris is cleared in part through Schwann cell autophagy, which promotes axon regrowth and new myelin. However, it is unclear how debris is cleared with glial-specific damage in the absence of autophagic Schwann cells. To study this, we built a larval zebrafish model in which nitroreductase 2.0 (NTR) is tagged with mCherry and expressed under the control of the *myelin basic protein promoter* (*mbp*). NTR+ Schwann cells are ablated with the drug ronidazole (RDZ), causing peripheral demyelination and, to our surprise, swellings along peripheral axons. While mCherry+ debris was observed following demyelination, this is rapidly cleared within 2 days. We therefore questioned whether phagocytic macrophages clear Schwann cell debris along peripheral nerves in response to glial-specific demyelination. We found that with RDZ treatment, macrophages accumulated peripheral nerves and were laden with NTR-mCherry debris. When co-treated with RDZ and the macrophage inhibitor Ki202227, fewer macrophages accumulated and the amount of mCherry debris within macrophages was reduced. Finally, we questioned whether debris clearance and macrophages have a role in resolution of axon swellings. We have found that macrophages, but not regenerated Schwann cells, are necessary for the resolution of swellings. We are currently testing for a synergistic effect when both Schwann cells and macrophages are inhibited. Altogether, we have found that macrophages are required for debris clearance during glial-specific damage and are necessary for recovery of axon swellings. This work was supported by NIH R15 NS141049 and Kenyon College Summer Science Scholars Fellowships.

Poster #40

Callan McKernan[1,2], Shelbie Wenner[2,3], Omodasola Ola[2], Natalie Pfaltzgraff[2], Maria Mikedis[2,4]
Induced Expression of Meiotic Transcription Factor MEIOSIN is Not Sufficient to Broadly Promote Premature Meiotic Entry
[1]University of Michigan, College of Literature, Science, and The Arts, Ann Arbor, MI, United States; [2]Reproductive Sciences Center, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; [3]Medical Scientist Training Program, University of Cincinnati College of Medicine, Cincinnati, OH, United States; [4]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States

Meiosis is a specialized cellular division that produces haploid 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 required during meiotic prophase I results in cell death or chromosomal missegregation and can lead to infertility. MEIOSIN and STRA8 proteins form a transcription factor complex required to initiate meiosis and upregulate meiotic gene expression. The loss of either protein results in failure to initiate meiosis. In mouse spermatogenesis, mitotic spermatogonia transiently express STRA8, but not MEIOSIN, but do not enter meiosis. Six days later, they co-express STRA8 with MEIOSIN and proceed to enter meiosis. The aim of this study is to evaluate whether MEIOSIN expression in STRA8-positive mitotic spermatogonia is sufficient to induce premature entry into meiosis. We have generated a mouse model carrying a Cre-inducible Meiosin transgene. Recombination mediated by Ngn3-Cre results in constitutive Meiosin expression (ind.Meiosin) in STRA8-positive mitotic spermatogonia. Based on immunostaining and histology, 5% of spermatogonia expressing ind.Meiosin enter meiosis early. Consistent with these results, RNA sequencing analysis of ind.Meiosin and control testes demonstrate that ind.Meiosin does not broadly upregulate meiotic gene expression. We conclude that MEIOSIN is not sufficient to broadly induce premature meiotic entry. Additional factors, such as post-transcriptional regulator MEIOC, likely act alongside the MEIOSIN-STRA8 transcription factor to drive meiotic entry. This work is supported by CCHMC Trustee Award (MMM) and NIGMS Award R35GM156830 (MMM).

Poster #41

Isabella Ford, Thomas Taylor, Matthew Riccetti, Jenna Green, Kimberly Wagner, Cheng-Lun Na, Anne-Karina Perl
Meox2 Controls Myofibroblast Function Through Indirect Regulation of Hedgehog
Cincinnati Children's Hospital Medical Center, USA

Infants born prematurely undergo dysregulated alveolarization in the lungs, leading to impaired generation of alveoli, known as Bronchopulmonary Dysplasia (BPD). The distal region of the lungs contains various fibroblast populations that are necessary for successful alveolarization and often hindered within BPD. During this second phase of alveolarization, myofibroblasts form secondary crests when large saccules divide into alveoli and undergo programmed cell death, thinning the septal walls. Our lab has identified Meox2 as a transcription factor important for alveolar fibroblast function and specification. To explore the role of Meox2, we used $Meox2^{flx/flx}$ $Pdgfra^{CreER}$ mice to delete Meox2 in fibroblasts with postnatal recombination. Using histochemical and immunofluorescences stains, we were able to identify increased myofibroblast differentiation when Meox2 was deleted on PN1 and a milder phenotype when

deletion occurred PN7. Suggesting a critical role of Meox2 during the early postnatal period. Recent research has highlighted the importance of the hedgehog signaling pathway upon myofibroblast differentiation in the lung. Meox2-deleted lungs showed increase sonic hedgehog signaling. To explore whether $Meox2^{+}$ cells control myofibroblast fate through hedgehog regulation, we treated mice with an anti-sonic hedgehog antibody (5E1) at P3 following P1 Meox2 recombination. While 5E1 treatment of wild-type mice impairs secondary septation, hedgehog-high $Meox2^{flx/flx}$ mice treated with 5E1 appear to exhibit secondary septation more similar to control mice. By linking Meox2-dependent secondary septation to hedgehog, we were able to define an indirect role for alveolar fibroblasts for control of myofibroblasts. This study is among the first that link hedgehog to myofibroblast dysfunction in alveolar simplification and maybe BPD. This work was supported by NIH/NHLBI grants R01HL167030, R01HL164418, T32HL007752-31, and F31HL162470.

Poster #42

Shannon Barr, Abdul Rafay Ali Jan, Lily Vondrell

Root Cap-Specific Gene Editing Using a Tissue-Specific Promoter with Cas9 as a Tool for Dissecting Root Gravitropic Signaling

Ohio Wesleyan University, USA

Roots of land plants are adapted for anchorage and the uptake of water and mineral ions from the soil. They accomplish these roles by sensing and responding to a variety of environmental cues such as light direction, moisture gradients, and ion concentration. Earth's gravity vector also serves as a constant directional stimulus, resulting in an overall root system having vertical primary roots and non-vertical lateral roots. Gravity sensing in roots is localised to a population of cells in the root cap that contain starch-filled plastids that sediment, or sink, to the lowest points in the cell. The importance of these organelles for gravity sensing is illustrated by the strong reduction in gravity responses when starch biosynthesis is disrupted through mutation, as is the case in the pgm1 mutant. However, the loss of starch synthesis capability throughout the plant leads to pleiotropic effects, such as reduced growth rates and altered circadian rhythms, complicating interpretation of gravitropic phenotypes in starchless mutants. To overcome this limitation, we sought to edit the PGM1 gene in a restricted region of the root containing the root cap using CRISPR guide RNAs specific for the PGM1 gene and driving the expression of the Cas9 nuclease with the SOMBRERO promoter, expressed only in the central and peripheral regions of the root cap. Heritable non-mosaic lines with Cas9 driven by EC1.2 egg cell specific promoter were done in parallel to provide a whole-plant starchless control. Edited lines will be tested with assays for their gravitropic-response, initiation time, and growth rate using ROTATO, a rig of rotation, used to image four-day old seedlings under IR light in dark. These tissue-specific mutants offer a greater physiologically controlled system for investigating starch-independent mechanisms. This work was supported by NASA (grant #80NSSC21K0585) and the Ohio Wesleyan Summer Scholarship and Research Program.

Poster #43

Olivia Boyea[1], Kendall Martin[2], Hee-Woong Lim[2], Joshua Waxman[2], Jennifer Schumacher[1]
Expression of novel genes during cardiovascular development in zebrafish
[1]Miami University, USA; [2]Cincinnati Children's Hospital Medical Center, USA

Congenital heart defects (CHDs) affect 1% of births, often resulting from dysregulated cardiac development and genetic mutations. We used the transparent zebrafish (*Danio rerio*) model to analyze orthologous cardiovascular genes that may be associated with CHDs. To accomplish this, we employed co-expression analysis to identify novel regulatory genes, and then analyzed where and when these genes are expressed during early cardiogenesis stages. *pabpc4* and *usp28* were identified as candidates due to co-expression with the ventricular marker *vmhc*. Pabpc4 is involved in post-transcriptional regulation of gene expression and Usp28 enables cysteine-type deubiquitinase activity. We hypothesized *pabpc4* and *usp28* will be expressed in cardiac muscle during early stages of heart development. Sequences were identified via ZFIN, and primers were designed using Primer3 and ApE. Following PCR amplification of 96 hpf cDNA and subsequent gel purification, purified DNA templates were used to synthesize antisense RNA probes. Gene expression patterns were analyzed using traditional in situ hybridization (ISH), imaged via Zeiss V8 microscopy, and analyzed qualitatively based on observed staining patterns. *pabpc4* was found to be expressed in the fins at 48 hpf, in cells on the surface of the yolk at 72 hpf, in the cardiac ventricle and the swim bladder at 96 hpf. Expression was found in the somites, craniofacial muscles and in a distinct small spot on the lateral head, hypothesized to be a gland, at all stages. Initial PCR amplification of *usp28* was unsuccessful. Future research will include investigating the staining patterns of *pabpc4* at higher resolution, and reattempting the *usp28* PCR. Altogether our results provide new insights into novel genes that may regulate cardiovascular development. Funded by Miami University Start-Up funds.

Poster #44

Kahlan Kahmann[1,2], Madison Ringer[1,2], Yanzhen Zhang[1,2], Matt Kofron[1,2], Cristina Cebrian[1,2]
Hyaluronic Acid Deposition in the Nephrogenic Zone is Dispensable for Nephron Progenitor Cell Viability
[1]Cincinnati Children's Hospital, United States; [2]University of Cincinnati, United States

Hyaluronan Synthase 2 (HAS2) is an enzyme that polymerizes hyaluronan (aka hyaluronic acid), at the plasma membrane. This hyaluronic acid is then deposited outside the cell, contributing to the extracellular matrix, supporting tissue structural integrity, and modulating cell adhesion, migration, and differentiation. Therefore, *Has2* is a gene critical for the production of extracellular matrix, influencing many aspects of embryonic development. While the role of HAS2 is well understood in both heart and bone development, with complete knock out models leading to embryonic lethality, its role in the development of the kidney is not so well understood. Throughout kidney organogenesis, *Has2* is highly expressed by nephron progenitor cells. Given its relevance in other organs, and the progenitor-specific expression of *Has2* in the embryonic kidney, we hypothesized that HAS2, via its role in hyaluronic acid deposition in the nephrogenic zone, modulates progenitor cell behavior. We have used a mouse model of conditional deletion of *Has2* in nephron progenitor cells and their progeny. At birth, the kidneys of these mice lack hyaluronic acid binding protein in the nephrogenic zone, indicating that *Has2* is ablated efficiently in the progenitors and that these cells are the main source of hyaluronic acid in the nephrogenic zone. However, no severe phenotype is observed in these mice; kidney

size is not affected, and size of the nephrogenic niches is comparable to that of littermate controls. Interestingly, we observe a reduction in macrophage infiltration in the nephrogenic zone. In conclusion, our data indicates that compromising hyaluronic acid deposition in the nephrogenic zone does not severely affect the viability of nephron progenitor cells. Funding from: NIDDK DK132052

Poster #45

Ethan Kendall, Nour Sayed, Aryesh Acharjee, Jacob Weaver
Expression pattern of *FOXE3* during early stage chick development
Miami University, USA

FOXE3 is a transcription factor expressed throughout lens development in mammals, fish and amphibians. Within the lens of these vertebrate classes, *FOXE3* expression initiates in the lens placode and remains expressed in the lens epithelium through adulthood. In addition to the lens, *FOXE3* expression has been documented in the developing forebrain/midbrain boundary (PMID: 10652278, 10890982) and in pharyngeal arch neural crest cells (PMID: 26854927) in mice and in the prechordal plate of zebrafish (PMID: 18327772). *FOXE3* expression has not been characterized in any known avian species. We conducted HCR-FISH in chicken stages HH9-HH12 to explore *FOXE3* expression. While *FOXE3* was strongly detected in the lens, we also observed *FOXE3* expressing cells in the early embryo near the neural tube. To determine if *FOXE3* is expressed in chick neural crest cells, we multiplexed HCR probes for both *FOXE3* and *PAX7*, a transcription factor expressed in a subset of neural crest cells. While we observed no co-localization of *FOXE3* and *PAX7*, we did observe *FOXE3* expression in the axial mesoderm (notochord) at HH12. We failed to see any *FOXE3* expression in the brain at HH9-HH12 in the chick. This work was supported by a grant R21EY036999 from the NEI.

Poster #46

Guimba, James Eschtruth, Luke Hanson, Stepannil Kalasava, Lilly Richardson, Alexanna Taylor, Gary M. Lange
Developmental impacts of environmentally relevant exposure to alternative bisphenols in the juvenile through pubertal period in rats (Rattus norvegicus)
Department of Biology, Saginaw Valley State University, United States

Bisphenol-S and/or Bisphenol-F are chemical agents found in many plastics used in everyday life. Both bisphenols are now common replacements for the original, widely used bisphenol, Bisphenol-A. This change in types of bisphenols used in the manufacture of plastics has occurred in the last several years because Bisphenol-A has become recognized as a chemical of concern by the general public. Unfortunately, while Bisphenol-S and Bisphenol-F are technically NOT Bisphenol-A, both have similar chemical structures, and both are endocrine disruptors of concern due to their impact on neuroendocrine relevant receptors on cells. However, we do not have as detailed an understanding on how Bisphenol-S and/or Bisphenol-F may exert their disrupting effects. All bisphenols can and do leach from plastics and are inadvertently ingested by organisms. Research about the physiological and developmental impacts of Bisphenol-S and/or Bisphenol-F ingestion are limited, but evidence suggests both exhibit estrogenic properties and alter the neuroendocrine system in ways that can shape development and physiology. We theorize that juvenile exposure to Bisphenol-S or Bisphenol-F may specifically influence behavior by reshaping organization and physiology during juvenile development through the completion of puberty, resulting in potential changes affecting various

behaviors. In our research, exposure to either Bisphenol-S or Bisphenol- F occurred post weaning at environmentally relevant levels. At maturity, neuromuscular development was assessed. Behaviorally, measures related to stress responses and reproductive behavior were measured. These data are compared with respect to developmental outcomes. Work was funded in part by the Field-Spicer Fellowship in Research awarded to the anchor author.

Poster #47

Shantanu Karandikar, Laura Rolfs, Taylor Brittain, Jennifer Gutzman
Role of Non-Muscle Myosin IIA in Kidney Development and *MYH9*-Related Disease.
University of Wisconsin-Milwaukee, USA

MYH9-related disease (*MYH9*-RD) is a rare multisystemic disorder characterized by hematologic, visual, auditory and variable renal manifestations resulting from mutations in the *MYH9* gene. *MYH9* encodes the non-muscle myosin IIA (NMIIA) heavy chain which is a cytoskeletal motor protein essential for regulating cellular processes such as cell division, migration, and signaling. Despite the established roles for NMIIA, the mechanisms by which *MYH9* mutations cause tissue-specific pathologies remain unclear. To identify the role of *MYH9* during development, we established and characterized zebrafish models carrying loss-of-function mutations in *myh9a* and *myh9b*, two zebrafish orthologs encoding NMIIA heavy chain. It was determined that *myh9b* is the most critical NMIIA-encoding gene, as *myh9b* mutants develop pericardial edema and exhibit a partially penetrant lethal phenotype. Since pericardial edema can be indicative of abnormal kidney development, and patients with *MYH9*-RD often develop renal failure, we examined *myh9a* and *myh9b* mutants for potential defects in renal structure and function. Using a dextran injection assay, we demonstrated that *myh9b* is required for maintaining the glomerular filtration barrier integrity. TEM analysis revealed that both *myh9a* and *myh9b* contribute to maintain normal podocyte foot process morphology, while *myh9b* is specifically required for maintaining normal slit diaphragm architecture and glomerular basement membrane thickness. Notably, microinjections with human *MYH9* mRNA partially rescued pericardial edema and impaired glomerular filtration in *myh9b* mutants, confirming functional conservation between species. Together, these findings establish *myh9* zebrafish mutants as a genetically tractable *in vivo* model for organ development and for exploring therapeutic interventions for *MYH9*-RD. Funding source: Eunice Kennedy Shriver National Institute of Child Health and Human Development/NIH R03HD094034 and the UWM Research Growth Initiative.

Poster #48

Hariharan Karthikeyan[1], Nicole A. Edwards[1], Lu Han[1], Karyn L. Robert[2], Steven A. Vokes[3], Sumeda Nandadasa[2], Aaron M. Zorn[1]
Mechanisms of Hedgehog/GLI-dependent regulation of Tracheoesophageal morphogenesis
[1]Division of Developmental Biology, Cincinnati Children's Hospital and Medical Center, Cincinnati, OH 45220, USA; [2]Department of Pediatrics, UMass Chan Medical School, Worcester, MA, USA; [3]Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712, USA

Hedgehog signaling through Gli transcription factors is required for tracheoesophageal morphogenesis, and disruption of this process results in birth defects ranging from esophageal atresia/tracheoesophageal fistula to laryngotracheoesophageal clefts. Despite its importance,

the mechanisms by which GLI transcription factors influence mesenchymal cell behavior during tracheoesophageal morphogenesis remain poorly defined. We hypothesize that Hedgehog-dependent GLI signaling coordinates extracellular matrix remodeling, cell adhesion, and mesenchymal cell migration within the splanchnic mesenchyme to drive tissue separation. A combination of single-nucleus multiome analysis of Gli2/3 allelic series with Gli3 genomic binding data from microdissected embryonic mouse foregut has identified multiple GLI-regulated genes, including the extracellular matrix remodeling enzyme ADAMTS20. Consistent with a functional role in morphogenesis, loss-of-function ADAMTS mutants exhibit tracheoesophageal clefts, suggesting that GLI-dependent ECM remodeling contributes to proper tissue separation. Ongoing experiments use live imaging of mouse foregut slice cultures to examine how altered GLI dosage affects mesenchymal cell behavior, including cell migration, adhesion dynamics, and tissue organization. Together, these studies will reveal how Hedgehog-GLI signaling integrates transcriptional regulation with extracellular matrix remodeling and adhesion-dependent cellular behaviors to control tracheoesophageal morphogenesis. This work is funded by NICHD P01 HD093363.

Poster #49

Sachin Wagh, Kristina Ly
Temporal Dynamics of Basement Membrane Remodeling During Stria Vascularis Development
Creighton University, USA

The stria vascularis is a highly specialized cochlear epithelium essential for generating the endocochlear potential required for hearing. Its proper assembly depends on the coordinated development of three distinct cell types: marginal cells, intermediate cells, and basal cells. Marginal cells, derived from the otic epithelium, establish epithelial polarity and ion transport machinery, while intermediate cells, derived from the neural crest, are required for electrochemical gradients. Unlike most epithelial tissues, the stria vascularis contains a unique capillary network that penetrates the epithelial layers and lies in close association with marginal and intermediate cells. Development of this functional epithelium requires remodeling of the basement membrane. However, the signals that allow the neural cells and capillary network to enter the epithelial layers and locally remodel the basement membrane are still unknown. In this study, we investigated the role of marginal cell differentiation on the neural crest-derived cells and capillary ingression. Cochlear tissues collected at E14.5, E16.5, E18.5, P0, and P3 were analyzed using immunofluorescence and histological approaches. We found that basement membrane remodeling starts at E18.5 to allow capillary network entry into the stria vascularis. Interestingly, some neural crest-derived cells entered the epithelial layer before detectable basement membrane remodeling, suggesting that their ingression may occur independently of extensive basement membrane remodeling. In our mutant deficient in marginal cell differentiation, the basement membrane remodeling was absent at P0 and P3, as assessed by fibronectin and laminin expression. Also, GLUT1 immunostaining revealed an abnormal capillary network within the developing stria vascularis. Overall, the findings of this study will provide insights on the role of basement membrane remodeling in stria vascularis development. This project is funded by startup to JR.

Poster #50

Benjamin Akande[1], Sylvie Earlewine[1], Grace Leonard[1], Zoe Zwick[1], Jonathon T Hill[2], John Postlethwait[3], Saulius Sumanas[4], Jennifer A Schumacher[1]
Solving an enigma: dissecting TGF-β activity during cardiac outflow tract formation in the zebrafish heart.
[1]Miami University, USA; [2]Brigham Young University, USA; [3]University of Oregon, USA; [4]University of South Florida, USA

Formation of the cardiac outflow tract (OFT), which gives rise to the aorta and pulmonary artery, is driven by interactions between the second heart field (SHF) and neural crest cells (NCC). Aberrations of these interactions have been implicated in OFT defects, which account for ~1/3 of congenital heart defect (CHD) cases. Despite advances in identifying drivers of OFT formation, the cellular and molecular etiology of OFT defects remains poorly understood. Here, we define a genetic and signaling framework governing early OFT patterning in a novel zebrafish mutant called *pendulum* (*pen*). Morphological assessment reveals that *pen* mutant larvae exhibit severe cardiopharyngeal defects, including a malformed aortic valve, absent or reduced OFT smooth muscle, a diminished ventral aorta, and craniofacial cartilage abnormalities. Bulk transcriptomic analysis of *pen* larvae reveals hyperactivation of TGF-β signaling in *pen* mutants, confirmed by increased pSmad2/3 in the OFT domain. Paradoxically, baseline TGF-β signaling activity is crucial for promoting smooth muscle cell differentiation, yet *pen* mutants show excess TGF-β activity along with loss of OFT smooth muscle. As such, we hypothesize that hyperactivation pushes signaling beyond its productive range. Notably, pharmacological inhibition of TGF-β signaling partially rescues the OFT smooth muscle in *pen* mutants, supporting tight regulation of TGF-β in proper OFT formation. Genetic mapping identified a nonsense mutation in *wipf3*, a regulator of actin cytoskeleton activity, in *pen* mutants, implicating an impaired contractile machinery as a possible contributing factor. Ongoing work aims to validate the genetic interaction between *wipf3* and TGF-β signaling in coordinating SHF-NCC during early OFT development. Together, these findings identify a signaling threshold that governs cardiac OFT formation, offering new mechanistic insights that may underlie CHD. Funded by AHA Predoctoral Fellowship.

Poster #51

Benjamin Akande[1], Sylvie Earlewine[1], Grace Leonard[1], Zoe Zwick[1], Jonathon T Hill[2], John Postlethwait[3], Saulius Sumanas[4], Jennifer A Schumacher[1]
Solving an enigma: dissecting TGF-β activity during cardiac outflow tract formation in the zebrafish heart.
[1]Miami University, USA; [2]Brigham Young University, USA; [3]University of Oregon, USA; [4]University of South Florida, USA

Formation of the cardiac outflow tract (OFT), which gives rise to the aorta and pulmonary artery, is driven by interactions between the second heart field (SHF) and neural crest cells (NCC). Aberrations of these interactions have been implicated in OFT defects, which account for ~1/3 of congenital heart defect (CHD) cases. Despite advances in identifying drivers of OFT formation, the cellular and molecular etiology of OFT defects remains poorly understood. Here, we define a genetic and signaling framework governing early OFT patterning in a novel zebrafish mutant called *pendulum* (*pen*). Morphological assessment reveals that *pen* mutant larvae exhibit severe cardiopharyngeal defects, including a malformed aortic valve, absent or reduced OFT smooth muscle, a diminished ventral aorta, and craniofacial cartilage

abnormalities. Bulk transcriptomic analysis of *pen* larvae reveals hyperactivation of TGF-β signaling in *pen* mutants, confirmed by increased pSmad2/3 in the OFT domain. Paradoxically, baseline TGF-β signaling activity is crucial for promoting smooth muscle cell differentiation, yet *pen* mutants show excess TGF-β activity along with loss of OFT smooth muscle. As such, we hypothesize that hyperactivation pushes signaling beyond its productive range. Notably, pharmacological inhibition of TGF-β signaling partially rescues the OFT smooth muscle in *pen* mutants, supporting tight regulation of TGF-β in proper OFT formation. Genetic mapping identified a nonsense mutation in *wipf3*, a regulator of actin cytoskeleton activity, in *pen* mutants, implicating an impaired contractile machinery as a possible contributing factor. Ongoing work aims to validate the genetic interaction between *wipf3* and TGF-β signaling in coordinating SHF-NCC during early OFT development. Together, these findings identify a signaling threshold that governs cardiac OFT formation, offering new mechanistic insights that may underlie CHD. Funded by AHA Predoctoral Fellowship.

Poster #52

Benjamin Callaway, Caiden Duskey, Diego Pareja, Isabella Klapwijk, Mia Angulo, Ronald Henkel, Aidan Kraan, Sarah Alhilu, Mark Charlton-Perkins

A CRISPR-based Zebrafish Model of NF1 that Provides Novel Insights into CNS Tumors

Miami University of Ohio, USA

Neurofibromatosis type 1 (NF1) is a tumor predisposition syndrome that affects both the peripheral and central nervous systems. While existing mouse models have provided important insights into peripheral nerve tumor development, they incompletely model central nervous system manifestations such as optic pathway gliomas. Zebrafish offer several experimental advantages, including rapid development, low cost, and optical transparency for in vivo imaging. Because early lethality in germline NF1 mutants has limited investigation of tumor initiation, we hypothesized that a glial-specific, conditional NF1 repression model in zebrafish would enable improved study of CNS tumor development. We generated transcriptional repression by replacing the nuclease domain of Cas9 with a zebrafish Krüppel-associated box (KRAB) repressor domain. Guide RNAs targeting both zebrafish NF1 paralogs were expressed under a U6 promoter and co-injected with the CRISPR interference construct. A GFAP enhancer drives NF1 repression in glial lineages of both the CNS and PNS. Tumor development was assessed by gross morphology, histological staining, and confocal microscopy. NF1-repressed embryos displayed phenotypes consistent with NF1 pathology. Glial tumors were observed throughout the body, with evidence of Schwann cell hyperplasia and hypercellularity in paraspinal ganglia. Optic nerve thickening was consistently present, and optic pathway gliomas were identified in both pre- and post-chiasmatic regions. These lesions were hypercellular, enriched for GFAP-positive glia, and associated with ocular protrusion reminiscent of the human condition. We have developed a rapid and scalable zebrafish model of NF1 that recapitulates key CNS features of the disease. This system provides a powerful platform for studying early tumor initiation and progression, particularly in the optic pathway. This work is supported by NIH/NINDS 1R01NS133200-01A1.

Poster #53

Itunu Adewoye[1], Anna Statsenko[1], Amartya Mitra[2], Elke Buschbeck[2], Mark Charlton-Perkins[1]
Identifying Altered Refractive Development in a Zebrafish Model of Myopia
[1]Miami University, USA; [2]University of Cincinnati, USA

Myopia is a common refractive disorder in which excessive ocular growth causes images to focus in front of the retina, resulting in blurred distance vision. The increasing prevalence of myopia and the risk of vision-threatening complications associated with severe disease highlight the need to better understand the developmental mechanisms that regulate ocular growth and refractive state. Zebrafish provide a useful vertebrate model for investigating these processes because their eyes undergo coordinated changes in size, ocular dimensions, and refractive state during postembryonic development. We combined micro-ophthalmoscopy with optical coherence tomography (OCT) to characterize refractive development and ocular growth in juvenile zebrafish. During normal development, we identified a marked change in refractive state between two weeks and one-month post-fertilization that was accompanied by changes in ocular dimensions, including the vitreous chamber. Using these measurements, we have also identified a zebrafish mutant exhibiting altered refractive development consistent with a myopic phenotype. Ongoing characterization is examining the relationship between refractive error, ocular size, and individual ocular compartments to determine the anatomical basis and developmental progression of this phenotype. Together, these studies establish a framework for linking changes in ocular growth to refractive state in vivo and for identifying genetic mechanisms that contribute to the development of myopia.

Poster #54

Yanzhen Zhang
Defining the Role of ETV4/5 in Nephron–Collecting Duct Connectivity and Cystic Kidney Development
Cincinnati Children's Medical Center, United States of America

Mammalian kidney development relies on reciprocal inductive interactions between the ureteric bud and metanephric mesenchyme, ultimately resulting in the fusion of developing nephrons with the collecting duct system. This fusion creates a continuous channel, ensuring that glomerular filtrate can be properly cleared from the nephron into the collecting system. We have shown that removing ETV4 and ETV5 from nephron progenitor cell (NPCs) using the Six2-TGC mice disrupts NPC maintenance and nephron-to-collecting duct connectivity while also present a proximal tubule-specific cystic phenotype. Since the Six2-TGC model has been shown to affect NPCs, here we utilized the Six2TGCΔSix3 (Six2TGCΔ3) mouse model to ablate Etv4/5 from NPCs and derivatives. We have performed conventional histological analysis (H&E and PAS), immunofluorescence on thin sections and whole kidney imaging of mutant and control littermates at P0. Unexpectedly, the nephrogenic zone in mutant kidneys appears normal and further quantification of the volume of the nephrogenic niches revealed no size differences between mutants and littermate controls. However, the cystic phenotype and disruption in the connection between the nephron and the collecting duct remained, suggesting that Etv4/5 are required for the fusion of nephrons to collecting ducts. Altogether, these data indicate that niche maintenance is not primarily affected by the loss of Etv4/5 in this model. Furthermore, the cystic and the failed connection phenotypes are not caused by defects in NPC maintenance but by specific roles of Etv4/5 in connection/cyst development. The question remains whether the lack of connection causes the cystic phenotype. We are currently using prenatal Dextran injections

and whole kidney imaging to investigate if the cystic structures correspond to blind nephrons. Further studies will be needed to determine the relevance of blind nephrons in cystic kidney disease. Supported by NIH grant R01DK132052.

Poster #55

Clare Austin, Thomas Gallagher, Sharon Amacher
Understanding segmentation clock oscillatory gene regulation and function
The Ohio State University, USA

The segmentation clock controls vertebrate somite formation. In zebrafish, segmentation clock genes oscillate with 30-minute periodicity in the e presomitic mesoderm (PSM), the region that gives rise to somites. Maintenance of segmentation clock oscillations requires rapid synthesis and decay of oscillatory components. The adaptor protein Pnrc2 promotes rapid decay of half the known zebrafish segmentation clock oscillatory gene transcripts, including *rhov,* whose role in segmentation is unknown. Transcripts of the *rhov* gene, which encodes a highly active atypical Rho family GTPase, are among the most highly upregulated transcripts in *pnrc2* mutants. *rhov* transcripts were shown to oscillate in the zebrafish PSM (Krol et al, 2011); we show that *rhov* transcripts are specifically expressed in PSM cells and are overexpressed in *pnrc2* mutants. Rho family GTPases are known to regulate cell polarity, cell adhesion, and cytoskeletal rearrangements, and some function during mammalian segmentation (Nakaya et al., 2004). To understand the role of *rhov* during zebrafish development, we are characterizing crispant and mutant phenotypes. The mechanism by which Pnrc2 promotes rapid RNA decay of oscillatory gene transcripts is unknown. In human cells, Pnrc2 does not appear to directly bind RNA but does interact with transcript decay-associated and P-body-associated proteins (Cho et al, 2009). We are endogenously tagging Pnrc2 with an ALFA epitope, a short peptide with high affinity for an ALFA-specific nanobody (Boswell et al, 2023). After recovering a functional tagged allele, we will use ALFA nanobody variants fused to fluorophores, halo tags, and degrons to analyze tissue-specific and subcellular Pnrc2 localization, identify Pnrc2-interacting proteins, and characterize phenotypes induced by rapid, acute loss of Pnrc2 function, respectively. This work will help uncover the molecular regulation of the segmentation clock and likely other ultradian oscillators. Funding NIH R35 (SLA).

Poster #56

Kristina Sattler[1,2,3], Brian Paleo[1,2,3], Mackenzie Roth[4], Kara Bradley[4], Megan Winkler[4], Wakako Yoshioka[5], Ichizo Nishino[5], Christoph Lepper[1,2,3], Kelly Crowe[6]
Assessment of Lectin Staining Biomarkers for GNE Myopathy Gene Therapy
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GNE myopathy (GNEM) is a rare, autosomal recessive disorder caused by mutations in the UDP-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase (*GNE*) gene, which encodes an enzyme required for sialic acid (SA) biosynthesis. Reduced SA production is associated with progressive skeletal muscle wasting; however, robust molecular biomarkers of SA deficiency and therapeutic restoration remain lacking. As gene therapy for

GNEM continues to advance, there is a critical need for biomarkers that enable early, objective assessment of therapeutic response that may precede detectable clinical improvements. Here, we systematically compare a panel of glycan-binding lectins to identify sensitive and robust biomarkers of SA deficiency and restoration in GNEM. This panel of glycan-binding lectins was evaluated for differential binding under altered sialylation using fluorescent staining and flow cytometry in a *GNE*-deficient cell model, followed by fluorescent staining of skeletal muscle biopsies from GNEM and non-GNEM patients. Subterminal glycan-binding lectins SBA, PNA, and VVA showed greater sensitivity to hyposialylation than terminal glycan-binding lectins. By flow cytometry, SBA and PNA increased 90.38- and 50.03-fold, respectively, in *GNE*-deficient cells and decreased 96.06% and 91.83% following *GNE* restoration. In GNEM muscle, PNA showed the greatest increase (5.3-fold) and most consistent discrimination from non-GNEM muscle. Overall, PNA demonstrated the most robust and consistent performance across models, supporting its promise as a biomarker to reflect restoration of sialylation that would occur in skeletal muscle after GNEM gene therapy. Further validation in clinical samples will establish its utility as a pharmacodynamic biomarker. This work was supported by the Neuromuscular Disease Foundation (NDF).

Poster #57

Dayton Gatewood[1], Jaclyn Olberding[1], Taylar Simmons[1], Timothy Nguyen[1,2], Fan Shao[1], James Thomas[1], Erliang Zeng[1], Brad Amendt[1], Huojun Cao[1], Colin Kenny[1], Eric Van Otterloo[1]
Reinforcement of the frontonasal neural crest identity by TFAP2 transcription factors
[1]University of Iowa, USA; [2]Cincinnati Children's Hospital, USA

The development of the vertebrate face is a highly precise process dependent on the proper deployment of cranial neural crest cells. When this process is disrupted, it results in debilitating congenital craniofacial defects. The transcription factor (TF) family TFAP2 is fundamentally required for normal craniofacial morphogenesis, with paralogs AP2α and AP2ß being specifically associated head and face development. Mutations in these transcription factors lead to severe conditions, including cleft lip and/or palate, branchio-oculo-facial syndrome, and Char syndrome. Previous studies demonstrate that both individual and combined loss of *Tfap2a* and *Tfap2b* result in primary and secondary palate clefting, as well as distinct midface defects. However, the specific genetic interactions and protein co-factors associated with these AP2 paralogs remain unresolved. To address this gap, this study investigates the cellular, genomic, and molecular intersections of TFAP2 paralogs during cranial neural crest development. The project is structured around three specific aims. First, we will identify the interacting co-factors of AP2 in cranial neural crest cells. This will be achieved by generating compound conditional knockouts utilizing a Wnt1-Cre driver, followed by comprehensive analysis through gross morphology, skeletal staining, and co-immunoprecipitation assays. Second, we will characterize the direct regulatory targets of AP2 within the cranial neural crest using an *in vivo* enhancer-specific reporter allele for Alx-1 and fluorescent imaging, complemented by *in vitro* functional testing of AP2-regulated enhancers. Finally, we will determine the cellular consequences associated with the neural crest-specific loss of TFAP2 by conducting targeted proliferation and cell death assays. Together, these aims will define the regulatory networks and critical interacting factors of AP2 during craniofacial development. This is funded through grant R01DE034668 from the NIDCR.

Poster #58

Ashley Cunha[1,2], Margaret Hanlon[1], Hee Woong Lim[1,2], Joshua Waxman[1,2]
Cephalic epicardial-derived Igf2b signaling is required for zebrafish outflow tract growth
[1]Cincinnati Children's Hospital Medical Center, USA; [2]University of Cincinnati College of Medicine, USA

30% of congenital heart defect cases have malformations of the cardiac outflow tract (OFT), which include persistent truncus arteriosus, double outlet right ventricle, and Tetralogy of Fallot. Complex interactions between several different cardiac cell types are required for normal OFT development, yet our understanding of the etiology of OFT defects remains incomplete. Here, we used zebrafish embryos to examine the requirement of cephalic epicardial cells (EpCs) in OFT development. The cephalic EpCs are a distinct epicardial population derived from the anterior pericardium that covers the smooth muscle of the bulbus arteriosus (BA) on the distal OFT. Almost nothing is understood about the transcriptional signatures and functional requirements of vertebrate cephalic EpCs, as studies have almost exclusively focused on proepicardial organ-derived EpCs that initially cover the ventricle. Using a zebrafish single cell transcriptomic dataset from 96 hours post-fertilization embryos, our data support that cephalic EpCs are a unique fibroblast/smooth muscle cell-like epicardial subpopulation. CellChat analysis between the cephalic EpCs and BA smooth muscle cells and subsequent hybridization chain reaction (HCR) *in situ* support Igf2b and the Itga5;Itgb3b integrin pair as a candidate ligand-receptor pair expressed in the cephalic EpCs and underlying smooth muscle of the BA, respectively. CRISPR-mediated knockdown of *igf2b* resulted in hearts with smaller BAs due to reduced cellularity. Thus, our data support that cephalic EpCs have a unique epicardial identity similar to fibroblast/smooth muscle cells and a previously unrecognized requirement promoting OFT tract growth. This project is funded by the NIH.

Poster #59

Nicholas Russell[1], Sarah Trovillion[1], Esther Won[2], Nicole Edwards[2], Daniel Swarr[1,3], William Zacharias[1,3], Debora Sinner[1,3]
BAF-Dependent Chromatin Remodeling Coordinates Patterning Signals and Matrisome Programs during Respiratory Tract Development
[1]Cincinnati Children's Hospital Medical Center, Department of Neonatology and Pulmonary Biology, United States of America; [2]University of Michigan Medical School, Cell and Developmental Biology, United States of America; [3]University of Cincinnati College of Medicine, Department of Pediatrics, United States of America

Congenital foregut and airway malformations cause neonatal morbidity, yet the chromatin mechanisms coordinating epithelial identity, mesenchymal patterning, and airway morphogenesis remain unclear. Canonical BAF (cBAF) chromatin-remodeling complexes regulate developmental gene programs, and our prior work implicated Arid1a in respiratory epithelial differentiation. Here, we define compartment-specific requirements for ARID1A/ARID1B-containing cBAF complexes during tracheoesophageal separation, airway maturation, cartilage formation, and lung branching. Using ShhCre, epithelial deletion of Arid1a/Arid1b blocked tracheoesophageal separation and caused severe pulmonary hypoplasia, with reduced Nkx2-1, Wnt7b, and Cldn18, abnormal Trp63 and Sema3A localization, disrupted SHH/BMP4 domains, impaired basement-membrane programs, and non-cell-autonomous tracheal smooth muscle defects. In contrast, mesenchymal deletion with Tbx4rtTA;TetOCre induced at E9.5 preserved tracheoesophageal separation but disrupted Sox9-positive cartilage

condensation, extracellular matrix programs, and lung branching. Pharmacologic BRG1 inhibition in mouse trachea-lung explants impaired epithelial branching, abolished SOX9-positive mesenchymal condensations, depleted Wnt, BMP, FGF, myogenic, and matrisome programs, and reduced distal epithelial Sox9 while preserving Nkx2-1. In Xenopus, BRG1 inhibition disrupted foregut separation and altered E-cadherin organization, demonstrating conserved BAF-dependent foregut morphogenesis across vertebrates. Together, these findings identify cBAF complexes as chromatin-level integrators of epithelial-mesenchymal signaling and link altered BAF dosage to tracheoesophageal separation defects, cartilage malformation, impaired branching, and pulmonary hypoplasia, providing a clinically relevant framework for BAFopathy-associated airway and lung phenotypes, including Coffin-Siris syndrome. Partially supported by RO1HL R01HL156860 and the Schmidlapp Program to DS.

Poster #60

Caroline Eichhorn[1], Sarah Trovillion[1], Nicholas Russell[1], Anne-Karina Perl[1,2], Zhiyuan (ZY) Chen[2,3], Hee-Woong Lim[2,4], Debora Sinner[1,2]

Investigating ARID1a/ARID1b-mediated pathways in lung development and disease

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Bronchopulmonary dysplasia (BPD), the most common morbidity of very preterm birth, is characterized by arrested alveolar development and lifelong respiratory risk. Although abnormal mesenchymal differentiation contributes to impaired alveologenesis, the chromatin-mediated mechanisms that define and maintain pulmonary fibroblast identity remain unclear. We previously found that respiratory mesenchymal deletion of the canonical BAF (cBAF) subunits ARID1A and ARID1B, as well as pharmacological inhibition of cBAF in vitro, causes lung hypoplasia, loss of alveolar fibroblast markers, perinatal lethality, and reduced expression of *Tcf21*, a transcription factor required for a subset of alveolar fibroblasts. We hypothesized that cBAF preserves alveolar fibroblast identity through TCF21-dependent transcriptional programs. Using a pulmonary mesenchyme-targeted *Meox2-CreERT2* mouse model, we deleted *Arid1a/Arid1b* in alveolar fibroblast precursors at the onset of alveologenesis. Mutant lungs showed increased mean linear intercept and reduced surface area, consistent with impaired septation and alveolar simplification. Sorted mutant fibroblasts had reduced *Tcf21* transcripts, as confirmed by RNAscope. In IMR-90 lung fibroblasts, CUT&RUN for ARID1A and BRG1 (the ATPase subunit of the complex), combined with RNA-sequencing after pharmacologic cBAF inhibition, showed that *TCF21* expression is cBAF-sensitive. Integration with published TCF21 perturbation datasets revealed co-localization of TCF21-binding sites with cBAF peaks and enrichment of TCF21 targets among cBAF-regulated genes. These findings identify a mesenchymal cBAF–TCF21 regulatory axis required for alveolar fibroblast differentiation and normal alveolar development. Linking chromatin remodeling to arrested alveolarization may reveal molecular targets to restore lung development in infants at risk for BPD. Partially supported by R01HL156860 and the Schmidlapp Program to DS, and by T35HL113229-15 to CE.

Poster #61

Matthew Janik[1], Monica Blatnik[1,2], Thomas Gallagher[1], Sharon Amacher[1]
Investigating Btg/Tob1 proteins in post-transcriptional regulation of oscillatory gene transcripts
[1]The Ohio State University, USA; [2]Oberlin College, USA

In vertebrates, somitogenesis is controlled by the segmentation clock, a molecular oscillator that regulates periodic gene expression in the unsegmented presomitic mesoderm. Segmentation clock periodicity is maintained by core oscillatory genes encoding Hes/Her transcriptional repressors that act in an autoregulatory negative feedback loop. In zebrafish, *pnrc2* is required for rapid decay of *her1* transcript and additional oscillatory gene transcripts. Using RNA-Seq, our lab has identified over 1,700 transcripts that are overexpressed in *pnrc2* mutant embryos. Strikingly, *pnrc2* mutants lack overt embryonic phenotypes, leading us to hypothesize that additional mechanisms protect *pnrc2* mutants from transcript over-translation. Consistent with this hypothesis, our lab has previously shown that many accumulated transcripts, which have shortened poly(A) tails, are disengaged from ribosomes and thus likely translationally repressed in *pnrc2* mutants. Notably, several transcripts encoding members of the Btg/Tob1 family of CCR4-NOT cofactors, which promote deadenylation and repress translation, are overexpressed in *pnrc2* mutants. Preliminary polysome profiling data indicates that *btg1*, *btg2*, and *tob1b* transcripts are engaged with ribosomes in *pnrc2* mutants, suggesting that those genes are overexpressed at both mRNA and protein levels in *pnrc2* mutants. Multiplexed knockdown of *btg1*, *btg2*, and *tob1b* using a highly efficient F0 CRISPR strategy causes abnormal segment formation in *pnrc2* mutant embryos at gRNA doses that have no effect in wild-type embryos, lending support to our hypothesis that *pnrc2* mutants employ Btg/Tob1 proteins as compensatory machinery to buffer against transcript accumulation. Furthermore, double knockdown of *tob1a* and *tob1b*, but not *btg1* and *btg2*, leads to *her1* misexpression in wild-type embryos, suggesting a potential role for *tob1* gene function in segmentation clock dynamics. This work is supported by the NIH (R35 and T32).

Poster #62

Bolu Osifalujo, Hariharan Karthikeyan, Aaron Zorn
Characterizing the Cell Biology of Tracheoesophageal Morphogenesis
Cincinnati Children's Medical Hospital Center, USA

During early embryonic development, the trachea and esophagus arise from a common foregut tube that must undergo precise tissue remodeling and patterning to establish two distinct organs. Proper tracheoesophageal separation requires coordinated interactions between the foregut endoderm and surrounding mesenchyme and is regulated by conserved signaling pathways, including Hedgehog (HH) signaling. HH signaling activates GLI transcription factors, which regulate gene expression and cellular patterning within the developing foregut. Disruption of HH/GLI activity results in severe tracheoesophageal defects, including abnormal tissue organization and impaired separation, yet the cellular mechanisms through which HH signaling controls this morphogenesis remain poorly understood. We hypothesize that GLI-mediated HH signaling regulates cellular organization and patterning during tracheoesophageal morphogenesis. To investigate this, we examined the spatial expression of candidate mesenchymal patterning markers and HH pathway components across the developing foregut. RNA in situ hybridization and immunostaining was performed on thick vibratome sections which preserves tissue architecture while enabling assessment of molecular expression across the

length of tracheoesophageal separation. This approach provides a spatial framework for defining cell populations, tissue organization, and molecular patterning during morphogenesis. Ultimately, these analyses will help determine how spatially coordinated cellular organization contributes to tracheoesophageal separation and provide insight into the cellular basis of HH/GLI-dependent foregut development. This work is funded by NICHD P01 HD093363.

Poster #63

Archana Singh[1], Kendall E Martin[1,2], Margaret A Hanlon [1], Joshua S Waxman[1,3]
Nr2f1a and Tbx5a cooperatively promote atrial cardiomyocyte differentiation in zebrafish
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Mutations in genes that affect the proper establishment and maintenance of cardiomyocyte (CM) populations are associated with congenital heart defects. While Nr2f and Tbx5 transcription factors (TFs) have respectively been implicated in atrial CM differentiation, the functional relationship of these factors in cardiac development *in vivo* is not well understood. Here, we find that while zebrafish *tbx5a* mutants do not have defects in atrial differentiation, depletion of *tbx5a* in *nr2f1a* mutants exacerbates atrial CMs differentiation defects found in *nr2f1a* mutants alone. Specifically, there is a dramatic expansion of ventricular markers coupled with a loss of pacemaker cardiomyocytes in *tbx5a*;*nr2f1a* deficient embryos by 72 hours post-fertilization (hpf) compared to *nr2f1a* mutants alone. Temporal analysis of atrial and ventricular markers beginning at 30 hpf showed that in control and *tbx5a* mutants, the atrial CMs are co-labeled with ventricular markers. The atria then resolve to have atrial CM specific gene expression. The transition is reduced in the smaller atria of *nr2f1a* mutants. However, in *tbx5a*;*nr2f1a* deficient embryos ventricular CM marker expression in the venous pole is never lost, supporting an early cooperative role in promoting atrial differentiation. Despite the expansion in overlap of atrial and ventricular markers, there was not an expansion of atrioventricular valve expression in *nr2f1a* and *tbx5a* mutants, indicating that their cooperative requirement is specific to atrial and pacemaker CM fate. Together, our findings show a previously unrecognized, early cooperative requirement between Nr2f1a and Tbx5a that is needed for atrial CM differentiation within the venous pole of the vertebrate heart, informing us of regulatory networks that are relevant to congenital heart defects. This work is supported by fundings from National Institutes of Health.

Poster #64

Mohammad Fazle Azim[1], Kristen Edgeworth[1,2], Stuart Hilton[1], Alanta Burdrys[1], Erika Conant[1], Viktoriya Coneva[1], Karen A. Hicks[1]
Investigating the functional conservation of EARLY FLOWERING 3 in land plants
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Seasonal environmental cues such as photoperiod regulate reproductive timing in land plants. The molecular mechanisms controlling reproduction have been extensively studied in flowering plants like Arabidopsis, but little is known about the degree of conservation across all land plants. Since flowering plants like Arabidopsis diverged from non-vascular plants such as

Physcomitrium patens 450 million years ago, using both allows us to study the conservation of the regulation of seasonal reproduction. EARLY FLOWERING 3 (ELF3) plays a key role in photoperiodic induction of flowering in Arabidopsis, rice, and soybean. It is a core component of the circadian clock and is involved in physiological processes that are strongly regulated by the environment. We identified four *P. patens* ELF3 homologs with conserved amino acid sequences in both the N- and C-terminal regions, which are important for regulating key proteins in Arabidopsis. We found that the expression of three homologs is regulated by photoperiod and the circadian clock. Using CRISPR-Cas9, we generated elf3abc triple-knockout lines and found that reproductive development did not occur in the absence of ELF3 under inductive conditions with a short photoperiod. To test whether PpELF3 is functionally conserved, we expressed *PpELF3* in Arabidopsis *elf3-1* mutants with elongated hypocotyls. Constitutive expression of *PpELF3* rescued their elongated hypocotyl phenotype in *elf3-1* mutants, but not to the wild-type level, suggesting partial functional conservation of ELF3. We will also test flowering time and circadian function in these complementation lines to better understand the functional conservation. This work was funded by NSF 2523491, NSF 1656091 and Kenyon College.

Poster #65

Yizi Zhang[1], Xinyuan Li[1], Hailong Lyu[2], Courtney Stockman[1], Kathleen Cook[1], Xiaoyue Chang[1], Xufeng Xue[2], William Zacharias[1], Anne-Karina Perl[1], Le Xu[1]

Mesothelial plasticity driven by mechanical force orchestrates lung regeneration

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During breathing, the lung is constantly exposed to rhythmic mechanical alternations, yet the biological readout of these mechanical signals is poorly understood. Following pneumonectomy (PNX), a surgical mouse model that triggers elevated mechanical tension, the remaining lung lobes undergo compensatory regrowth with de novo regeneration of alveoli, the gas-exchange units. Mesothelial cells lie on the outer surface of the lung where mechanical tension remains highest at baseline and after PNX. Whether these cells can respond to mechanical cues, and how physical signals are transduced into biological consequences that could promote alveolar regeneration remain largely unknown. In this study, we sought to investigate the plasticity of mesothelial cells under mechanical stretch and their role in regulating the cell behaviors of alveolar type 2 (AT2) cells, the stem cells for alveologenesis. Here, we established a "lung-bath" method to efficiently isolate pure primary mesothelial cells from intact mouse lungs within a short time window. Preliminary results suggest that primary mesothelial cells are responsive to mechanical stretch with increased nuclear YAP/TAZ levels compared with unstretched controls. Furthermore, PNX-activated mesothelial cells, but not their quiescent counterparts, promoted AT2 organoids formation and growth. In ongoing studies, we focus on interrogating mechanotransduction pathways in mesothelial cell activation under elevated mechanical tension *in vitro* (stretch device) and *in vivo* (PNX). The downstream mechanisms by which activated mesothelial cells regulate AT2 growth will be investigated in our organoid and genetic mouse models. Taken together, our studies here uncover a role for mesothelial mechanobiology in regulating AT2 stemness during lung regeneration. This work was supported by the Xu R00 grant (GR400875-001).

Poster #66

David Paulding[1], Genevieve Kendall[1,2]
Core mechanisms of infantile rhabdomyosarcoma across diverse fusion oncoproteins
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Infantile rhabdomyosarcoma (RMS) is a rare pediatric sarcoma most often seen in children under two years of age. Like other RMS subtypes, it is characterized by the appearance of skeletal muscle-like tumor cells and the expression of skeletal muscle developmental proteins. Infantile RMS is genetically driven by fusion oncogenes. The recognition of this infantile subtype and its molecular drivers are recent and no patient-derived models exist. Among the most common fusion oncogenes are *VGLL2::NCOA2*, *VGLL2::CITED2*, *TEAD1::NCOA2*, and *SRF::NCOA2*. We have published an in vivo zebrafish and mouse allograft models for the *VGLL2::NCOA2* fusion oncogene which replicated the histology and transcriptomics of patient tumors. In the infantile RMS fusion oncogene family, the 5' fusion partners each function in skeletal muscle development, suggesting a common transcriptional mechanism for each of these fusion oncoproteins. We therefore hypothesize that infantile RMS tumorigenesis is driven through the binding and regulation of a common set of skeletal muscle genomic targets by the fusion oncogenes. We will test this with zebrafish and human myoblast models in a cross-species approach. We have adapted our published embryonic zebrafish mRNA injection model and have shown consistent, reproducible expression of FLAG-tagged fusion oncoproteins at the six hour old early-gastrulation stage and quantified their survival. We are performing bulk RNAseq to identify regulated targets and will characterize shared genomic occupancy with CUT&RUN experiments. We will complement these experiments in transduced, fusion-expressing human myoblasts and overexpress VGLL2, TEAD1, SRF, NCOA2, and CITED2 full-length and fragment constructs to dissect the tumorigenic elements of the fusions. This work will provide a platform for identifying mechanistic similarities and differences between the fusion oncogenes driving infantile RMS. Project funded by NIH R01 1R01CA272872 to GK and T32 T32CA269052 to DP

Poster #67

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Schwann Cell Derived ErbB Signaling Influences Anterior Lateral Line Development
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The zebrafish lateral line is a mechanosensory system specific to aquatic vertebrates enabling water flow detection. The system consists of sensory organs called neuromasts innervated by the lateral line nerves, organized into separate anterior and posterior lateral lines (LLs). Within the cranium, anterior LLs develop directly adjacent to multipotent neural crest cells (NCCs). Previous work from the Prince lab has shown complete NCC ablation leads to anterior LL development failure. Our studies aim to delineate the specific roles NCC derivatives have on anterior LL development. NCC-derived Schwann cells (SCs) have been shown to regulate intercalary neuromast formation in the posterior LL, therefore we tested the hypothesis that a similar mechanism acts in the anterior. The nerve and SCs comigrate by means of ErbB signaling, creating the inhibitory niche that suppresses precocious intercalary neuromast formation in the posterior (Lush & Piotrowski, 2014). We tested the role of ErbB signaling in the anterior LL using the pharmacological inhibitor AG1478. Treated specimens showed precocious

development of intercalary neuromast SO3 within the supraorbital branch of the anterior LL. SO3 normally forms at 4 days post fertilization, but in the absence of ErbB signaling we observed SO3 was already forming, or in many cases fully present, by 3 days post fertilization. Also, we observed a lack of SCs surrounding the anterior LL nerve, suggesting ErbB signaling is also required for their normal comigration. Upon transient AG1478 treatment, we noticed INCs are returned to quiescence in the relative absence of SCs. This may suggest a previously undescribed role for ErbB signaling within INCs, diverging from the posterior mechanism. Future directions will focus on the presence of ErbB receptors and how ErbB signaling influences Wnt and Fgf signaling within INCs of the anterior LL system. This work is funded by the NSF IntBIO Award.

Poster #68

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Netrin-1 expression and function in craniofacial development

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Cleft palate is a common major birth defect for which the cause is often unknown. Whereas most children born with cleft palate receive surgical palatal repair during early childhood, about 25% of the patients with repaired palate experience palatal functional deficit known as velopharyngeal dysfunction (VPD), with significant impairment of speech and/or hearing. It has been suggested that defect or disruption of palatal innervation is a possible reason for VPD in at least some of those patients, but little is known about the molecular regulation of palatal innervation. Recent human GWAS studies have shown a strong association of the *Netrin-1* (*NTN1*) locus with cleft lip and palate (Leslie et., 2016; Tao et al., 2023). NTN1 is known to regulate axon guidance, but its expression and function in palate development is not well understood. RNAseq analysis confirmed *Ntn1* and its receptors' expression in neural crest derived palatal mesenchyme cells in mouse embryos. RNAscope analyses show *Ntn1* expression in the palatal epithelium and subregions of the palatal mesenchyme, in addition to the brain, optic cup, optic stalk, and tongue in E13.5 and E15 mouse embryos. Preliminary analysis of *Ntn1-/-* mutant mouse embryos confirmed critical Ntn1 function in multiple developmental processes but its function in palate development and palatal innervation requires further investigation. This work is supported by NIH R01 DE033890 to RJ and YL and NIH R01 DE035481 to YL

Poster #69

Cole Milas, Paul Huber, Betsy Schock

Using HCR to assess Cell Differentiation in Neural Crest-Induced Explants

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Blastula stem cells can be isolated from *Xenopus* embryos and reprogrammed to different cell fates though administration of small molecules or growth factors. This is a powerful system for assessing gene function, cell type specification, and terminal differentiation of vertebrate stem cells. Recently, a small molecule methodology has been developed that results in the induction of neural crest (NC) cells from *Xenopus* blastula stem cells, herein referred to as NC-induced explants. When NC-induced explants are grafted back into an embryo, these cells migrate and differentiate into multiple cell types (chondrocytes, melanocytes, cranial nerves).

While these findings suggest that NC-induced explants are competent to differentiate into multiple NC derivatives when placed in an inductive environment, it remains unclear whether they possess the intrinsic ability to differentiate in an *ex vivo* environment. To address this question, we analyzed transcriptome data from NC-induced explants cultured *ex vivo*, and found that these cells express markers for NC derived cell types, but also express markers for neural plate border (NPB) derivatives (placodes, hatching gland, cement gland). Using *in situ* hybridization chain reaction (HCR), we confirmed the presence of these NPB derivatives and found that the chondrocytes, otic placode, and cement glands could organize into structures and patterns that mimic those observed in whole embryos. Overall, our work suggests that NC-induced explants cultured *ex vivo* display hallmark properties of organoids, as they are capable of differentiation and self-organization independent of an embryonic environment. Funding for this project was provided to BS from NIDCR (R00DE031825).

Poster #70

Laura Romano, Thao Trang Nguyen

Investigating the effect of temperature on the embryogenesis and reproductive output of freshwater snails

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We are interested in the extent to which anthropogenic activities are impacting the embryogenesis of snails that inhabit freshwater ecosystems in central OH. In recent years, local bodies of water have been impacted by pollution, warmer temperatures, more severe storms, and the construction of new roads, bridges, and water treatment facilities; agricultural runoff continues to be a major issue as well. In this study, we specifically investigated the effects of temperature on the embryogenesis of two species of freshwater snails that inhabit the same local pond. Predictably, there was a correlation between temperature and the time it took to reach key developmental milestones. Ramshorn snails were more vulnerable than bladder snails, with only bladder snails successfully hatching at 10ºC and 30ºC; neither species hatched at 40ºC. Both species also exhibited a significant increase in heartrate as temperature increased. When we investigated the impact of temperature on reproductive output, we found significant differences in the length of time until eggs were laid as well as in the number of eggs per clutch. Collectively, our data indicate that both species are vulnerable to temperatures beyond their typical range, but particularly the ramshorn snail. This study highlights how the effects of temperature may differ between even closely-related and/or sympatric species such that a comparative approach is necessary to avoid a generalized prediction with regard to the effects of climate change. Hopefully, all of this work using local snails as a new model system in our laboratory will contribute to more informed decision-making with respect to policies that may impact freshwater ecosystems in central OH. We are supported by the Denison University Research Foundation as well as the institution itself, Denison University.

Poster #71

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MEOX2+ Fibroblast Progenitors Respond to Neonatal Hyperoxia During Alveolar Development

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Bronchopulmonary dysplasia (BPD) is a chronic lung disease affecting premature infants born during the saccular phase of lung development. Requirement of respiratory support leads to alveolar simplification and impaired secondary septation driven by disruption of pulmonary fibroblast populations. We demonstrated that neonatal hyperoxia alters the ratio of myofibroblasts and lipofibroblasts in a mouse model of BPD, and identified Meox2 as a transcription factor expressed in progenitors, however their fate following injury and their role in restoring fibroblast subtype balance remain unknown. Using a Meox2-CreER;mTmG lineage trace model, pups were exposed to 90% O2 from PN1-PN7 then returned to room air until PN10. Immunofluorescence was used to quantify GFP+ lineage traced cells, MEOX2+ cells, myofibroblast, and lipofibroblast populations. The Meox2-lineage tagged GFP+ population significantly increased in the hyperoxia+recovery group vs controls (20.39% vs 16.01%), while MEOX2+ cells increased during room air recovery without reaching significance (16.26% vs 22.30%); Myofibroblast abundance returned to control levels, suggesting secondary crest myofibroblast numbers normalize after injury, whereas lipofibroblasts remained elevated in recovery animals (34.81% vs 23.49%). The recovering MEOX2+ population and the expansion of GFP+ cells suggest that MEOX2+ progenitors are responding to injury, and the persistent lipofibroblast elevation indicates fibroblast subtype balance has not fully normalized despite myofibroblast recovery. Three days of room air recovery fails to restore MEOX2+ progenitor fate following neonatal hyperoxia, demonstrating that fibroblast dysregulation persists beyond the initial injury and may contribute to sustained alveolar dysfunction in BPD. This study was supported by NIH/NHLBI grants R01 HL167030 (AKP), R01 HL164418 (AKP). We thank the CCHMC Transgenic Animal and Genome Editing Core for the design and generation of the Meox2CreERT2 mouse.

Poster #72

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Differentiation of hPSCs into tracheal-esophageal mesenchyme *in vitro* as a model to study human disease and development

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The trachea and esophagus are adjacent organs essential for respiration and digestion, both arising from a common embryonic foregut. Current in vitro models remain limited, particularly for studying mesenchymal lineages. This study aimed to optimize protocols for differentiating human pluripotent stem cell (hPSC)-derived esophageal and respiratory mesenchyme into

smooth muscle and cartilage by modulating key developmental signaling pathways. TGFβ-1 robustly promoted smooth muscle differentiation in both mesenchymal populations, as indicated by upregulation of smooth muscle markers. Neither TGFβ-1 treatment nor modulation of WNT, BMP, or Hedgehog pathways, nor extended 3D aggregate culture, induced chondrogenic differentiation. Positive control experiments verified that chondrogenesis requires appropriate progenitors and conditions, underscoring the importance of lineage competency. We also applied this system to model Down syndrome (DS) using isogenic Trisomy 21 (Tri21) and disomic control (Di21) hPSC lines, which confer elevated risk for tracheoesophageal birth defects. Preliminary findings indicate that Tri21-derived esophageal and respiratory mesenchyme exhibited more rapid transcriptomic progression and diminished Hedgehog signaling responsiveness, accompanied by downregulation of FOXF1, a Hedgehog-dependent transcription factor, pointing to impaired Hedgehog-FOXF1 signaling as a candidate contributor to DS tracheoesophageal defects. Collectively, our findings identify TGFβ-1 as a key regulator of smooth muscle differentiation and highlight the requirement for additional inductive cues for chondrogenesis. This platform provides a framework for modeling tracheoesophageal development and its dysregulation in congenital disorders such as Down syndrome. This work was supported by the Zorn Endowment (S-GR901116-001; EN45175 / Perinatal Pulmonary Development).