Thursday, November 5th

1:20-2:40 PM: Selected talks session 1

1:20-1:40 PM: Chris Petell (Strahl lab, Postdoc)
Title: The BAH domains of PBRM1 are readers of unacetylated H3 and H4 histone tails
Abstract: Epigenetic regulation of gene expression is mediated in part through the specific recognition of histone tails and modification states by chromatin "reader" domains embedded in epigenetic effector proteins or their complexes. While many families of reader modules have been investigated, one family that remains poorly understood is the bromo-adjacent homology (BAH) domain. A pre-eminent member of this family is PBRM1 (BAF180), which contains two C-terminal BAH domains in tandem whose roles are yet to be elucidated. PBRM1 is a member of the PBAF nucleosome remodeling complex and is a key regulator of gene expression as mutations found in various diseases, especially renal cancers, are related to PBRM1, and subsequently PBAF, function. We therefore sought to determine the binding preferences of the BAH domains of PBRM1 and how they affect its biological role. Initial structural modeling of the two BAH domains revealed the presence of regions likely to interact with histones. Binding assays further confirmed that both BAH domains broadly interact with histone tails, with a preference for H3 and H4 peptides that are unacetylated. Additional mutagenesis of residues predicted to drive BAH-histone interactions confirmed the specificity of the BAH domains' interaction with histones. Current studies are aimed at examining the importance of one or both BAH domains, in conjunction with its adjacent bromodomains that bind to H3 acetylation, for chromatin engagement and in gene regulation.

1:40-2:00 PM: Hui-Chia Yu-Kemp (Peifer lab, Postdoc)
Title: Nonmuscle Myosin II Drives Actomyosin Bundling at Nascent Adherens Junctions
Abstract: E-cadherin-mediated adherens junctions (AJs) is essential for maintaining tissue integrity. Disruption of E-Cadherin-based AJs often happens during carcinoma progression. The molecular mechanisms regulating the formation and dynamics of the AJs are still not fully understood. The actomyosin cytoskeleton is one component shown to regulate E-Cadherin accumulations at AJs, and thus modulates cell shape and border tension. It is unclear how the cells assemble the contractile actomyosin cytoskeleton while AJ forms. We addressed this question by combining cultured ZO-1/ZO-2 KD MDCK cells, which form a highly organized actomyosin cytoskeleton at ZAs, and super-resolution microscopy. We also applied imaging analysis software to unveil the process of actomyosin structure formation as junctions reforms in 3D. We tested two mechanisms driving the formation of the junctional cytoskeleton: 1) Actin polymerization, or 2) Reorganization of F-actin via myosin. At steady state, the cells assemble tightly bundled F-actin decorated by a sarcomeric myosin array at AJs. When we triggered junction reformation via calcium-switch experiments, we observed that myosin minifilaments form dramatically expanded arrays starting at very young junctions, while F-actin is not yet well-organized. These myosin arrays extend many microns from the cell borders in the x-y dimension yet are >1μm long in the Z dimension. The myosin arrays appear tightly associated with the apical plasma membrane, apical to the forming E-Cadherin AJs. As junctions mature, the breadth of the myosin array shortens, F-actin becomes bundled, E-Cadherin enriches at the apical membrane and cells become polarized. We were surprised to find that actin polymerization machines (Arp2/3, Formin, and Ena/VASP) appear dispensable for this process. In contrast, myosin function is essential. ROCK inhibition disrupts myosin localization to cell borders, while junctional myosin loses its sarcomeric organization when the myosin ATPase is inhibited. Both conditions lead to less-bundled junctional F-actin, although E-Cadherin can still concentrate at cell-cell contacts. Our results reveal a major role for myosin in regulating the establishment of the

supramolecular cytoskeleton at the ZA and suggest there maybe two independent pathways to generate F-actin and E-Cadherin pools at E-Cadherin-based junctions.

2:00-2:20 PM: Phil Lange (Damania lab, Postdoc)
Title: Adenosinergic signaling as a novel target for viral lymphomas
Abstract: Epstein-Barr virus (EBV) is among the most prevalent human pathogens, causing lifelong infections in >90% of the adult population. Importantly, EBV is an oncogenic virus that is associated with numerous B cell lymphomas, lymphoproliferative disorders, and other malignancies. While current therapies have acceptable outcomes for some EBV+ lymphomas, others exhibit extremely poor prognosis despite aggressive chemotherapy. Thus, novel therapeutic targets are needed for the treatment of EBV-positive lymphomas. The adenosine signaling axis represents one of the most promising and targetable immunomodulatory pathways. Extracellular ATP and adenosine drive inflammatory and immunosuppressive signaling, respectively. For instance, ATP signaling via P2 receptors on lymphocytes and myeloid cells is critical for robust activation and inflammatory responses. On the other hand, adenosinergic signaling counteracts inflammatory stimuli, promotes tolerance, and induces immunosuppressive pathways. Ectonucleotidases regulate the extracellular purine concentrations by sequentially converting ATP to adenosine, thereby driving immunosuppressive adenosinergic signaling and opposing immune cell activation. Here I demonstrate robust ectonucleotidase expression and activity in EBV transformed primary B cells. Further, we observed similarly high ectonucleotidase expression in EBV infected tissues using a humanized mouse model of aggressive EBV lymphomagenesis. Notably, inhibiting ectonucleotidase activity in this model resulted in increased inflammatory cytokine expression, decreased viral burden and gene expression, and prolonged survival. Thus, adenosinergic signaling represents a potential target for the treatment of EBV+ malignancies.

2:20-2:40 PM: Leiah Carey (Campbell lab, Postdoc)
Title: Oncogenic RAS Q61 mutants as novel targets for drug discovery efforts
Abstract: The three RAS genes (HRAS, KRAS and NRAS) encode for GTPases that regulate cellular growth via cycling between inactive and active states. When this regulation is compromised, aberrant RAS signaling manifests in numerous types of cancer. RAS is the most frequently mutated oncogene in cancer (~30%), with 99% clustered at 3 mutational hotspots (G12, G13, and Q61). It is becoming clear that all RAS mutations are not created equal and their distinctive differences may harbor attractive therapeutic targets. Recently anti-Ras G12C inhibitor drugs have reached phase 2 clinical trials, however these drugs specifically target only 1 oncogenic RAS mutant. Inhibitors to other oncogenic RAS mutants are needed. Biochemical and biological studies conducted on a subset of KRAS and NRAS Q61 mutants in collaboration with the Der lab, show distinct differences from G12/G13 oncogenic mutants. Intriguingly, we also observe residue- and isoform-specific differences within the Q61 codon. These findings correlate with observations that distinct RAS mutations impart differences in their signaling and tumorigenic properties. Understanding these differences will have important biological and clinical implications. Molecular dynamic (MD) and NMR studies on oncogenic RAS Q61 mutants suggest a subset adopt distinct structural ensembles that promote accessibility of druggable pockets. We hypothesize that select oncogenic RAS Q61 mutants may adopt conformations that are vulnerable to structure-based drug discovery efforts, and will present preliminary biochemical, NMR and MD results for a subset of RAS mutations (KRAS Q61R/Q61H; NRAS Q61R) that support differences in structure and ligand binding pockets. Our novel combinatory experimental and computational structure-based analysis to identify unique druggable binding pockets of mutated RAS Q61 GTPases may pave new directions for treatment of RAS oncogenic-driven cancers.

4:00-4:30 PM: Poster Session 1

Christopher Abdullah (Duronio lab, Postdoc)
Title: Characterization of a predicted H3.3K36 trimethylation reader in Drosophila
Abstract: Mutations in the replication-independent histone H3.3 (K36M and G34R) have been identified as drivers of human cancers. Both of these mutations have also been shown to disrupt H3.3 post-translational modifications (PTMs) on H3.3K36. Much of the current research looked at the effects of the writers and erasers that deposit these PTMs and how this dysregulation may be driving cancer progression. However, little research has tried to assess the effects of these mutations on histone reader proteins. ZMYND11 has been identified in humans as an H3.3-specific K36 trimethylation (K36me3) reader that regulates pre-mRNA processing and RNA Pol II elongation. Interestingly, data has also suggested that ZMYND11 functions as a tumor suppressor in mouse xenograft models. We hypothesize that the reader's functions are perturbed in cancers that harbor K36M or G34R mutations. Our lab uses *Drosophila melanogaster* to study the structure and function of chromatin. Bioinformatic analyses have identified an uncharacterized gene, CG8569, predicted to be the ortholog in *Drosophila*. Few reagents currently exist in *Drosophila* that can be used to study this gene. Here we discuss the current generation of tools to study this gene including generating CRISPR alleles of a tagged and null allele of the gene in flies, RNAi studies, generation of antibodies for the CG8569 protein.

Cole Edwards (Der lab, Graduate student)
Title: YAP1 overexpression drives resistance to the KRASG12C specific inhibitor MRTX1257 in KRASG12C-mutant cancers
Abstract: Mutations in the KRAS oncogene are among the most frequent driver events in human cancers. With decades of failed efforts, KRAS has been considered 'undruggable'. However, recent discoveries have led to inhibitors that can directly bind and inactivate one KRAS mutant, with a glycine-12 to cysteine-12 substitution (G12C), and early clinical evaluation show promising responses in $KRAS^{G12C}$ mutant lung cancers. However, as with essentially all targeted therapies, acquired mechanisms of drug resistance are likely to limit the long-term effectiveness of G12C inhibition (G12Ci). To identify mechanisms of resistance to G12Ci, we determined if overexpression of the HIPPO pathway component, YAP1, can drive resistance to the MRTX1257 G12Ci. MRTX1257 is a $KRAS^{G12C}$ mutant-selective covalent inhibitor that demonstrates >1000-fold selectivity over WT KRAS. In a panel of $KRAS^{G12C}$ cell lines, we determined that MRTX1257 potently inhibited KRAS signaling, blocking ERK phosphorylation (IC_{50} ~ 1 nM) and cellular proliferation. Activation of the YAP1 transcriptional co-regulator has been shown to overcome KRAS addiction in KRAS-mutant cancers. In concordance with these observations, we found high YAP1 protein expression significantly correlated with resistance to MRTX1257 in a 3D *in vitro* viability assay. We determined that overexpression of wild-type (WT) YAP1 or constitutively active $YAP1^{S127A}$ (S127A) drove resistance to MRTX1257 in a panel of $KRAS^{G12C}$ -mutant cancer cell lines. YAP1 overexpression also drove resistance to inhibition of ERK MAPK inhibitors, but not other conventional cytotoxic or molecularly targeted chemotherapeutics. Ongoing studies involve the evaluation of targeting YAP1 signaling in combination with G12Ci.

Kanishk Jain (Strahl lab, Postdoc)
Title: Acetylation-mediated histone tail accessibility governs the read-write mechanism of H3K4
Abstract: The highly positively charged nature of histone tails along with the PTMs that decorate them govern their equilibrium between free/accessible states and collapsed/DNA-bound states in nucleosomes. This dynamic structural feature of histone tails affects how reader proteins

recognize and bind to particular PTMs. Specifically, acetylation of the H3 N-terminal tail has been linked to increased recognition by the PHD finger from BPTF to H3K4me3. To test whether these structural dynamics go beyond protein-protein recognition and influence histone writers, we have tested the effect of downstream H3 N-terminal tail acetylation on the catalytic activity of the MLL1 complex. Our results demonstrate that mononucleosome acetylation on H3 K9, 14, and 18 significantly increases MLL1 activity and binding affinity for the H3K4 residue as a substrate. Furthermore, mass spectrometric analysis of MCF-7 cells treated with butyrate, an HDAC inhibitor, revealed a significant positive correlation between rising levels of histone acetylation and H3K4 methylation. Together, these observations show that the dynamic structural environments around nucleosomes broadly impact how protein complexes engage with, modify, and eventually regulate chromatin structure and gene expression.

Clint Stalnecker (Der lab, Postdoc)
Title: A multi-omic approach to overcoming resistance to the direct inhibition of mutant KRASG12C
Abstract: The recent development of small molecule inhibitors selective for one specific KRAS mutant, KRASG12C, with four currently under clinical evaluation, has begun to challenge the perception that KRAS is 'undruggable'. However, as essentially all targeted therapies are limited by de novo and acquired cancer cell resistance, combination treatment strategies will be needed for G12C inhibitors to maximize the extent and duration of anti-tumor efficacy. We focused our studies on the potent and selective KRASG12C inhibitor MRTX1257, an analog of the clinical candidate MRTX849. In a panel of KRASG12C mutant cell lines, we observed a robust rebound in reactivation of the KRAS downstream effector ERK and subsequent resistance to KRASG12C inhibition. To determine strategies to overcome resistance to direct KRASG12C targeting, we performed a high-throughput drug sensitivity and resistance screen (DSRT) that contained a diverse set of >500 clinically actionable drugs. We measured both cellular viability and death endpoints to identify combinations that would be cytostatic and/or cytotoxic, with a preference for cytotoxic combinations. Furthermore, we used a multi-omic approach to profile changes in the kinome (using multiplexed kinase inhibitor bead pull-down coupled with mass spectrometry, MIB-MS), phosphoproteome (LC-MS/MS), and transcriptome (RNAseq) for pathway activation mapping. Using these complementary data sets, we identified potential strategies that were both shared and unique among KRASG12C-mutant lung, colorectal, and pancreatic cancer cell lines. We identified combinations that have been previously reported with KRASG12C inhibitors, namely RTKs and components of the PI3K-AKT-mTOR pathway. Additionally, we also report novel combinations and provide mechanistic rationale for their activity.

Joseph Szulczewski (Hahn lab, Postdoc)
Title: Optogenetic control of vinculin-actin interactions to probe durotaxis
Abstract: During metastasis, cancer cells directionally migrate towards stiffer matrices in a process known as durotaxis. Cells sense the stiffness of their environment through protein complexes known as focal adhesions, which link the actin cytoskeleton to the extracellular matrix. This linkage is mediated by vinculin recruitment to focal adhesions, where the vinculin head domain binds to Talin and the tail domain binds to F-actin. It is unclear how vinculin force transduction is translated into signals that control directional migration. Traditional assays such as global knockdown or overexpression provide little information regarding the integration of signals from different force environments across the cell. We therefore hope to modulate force transduction locally, by creating a vinculin analog with actin binding controlled by light. The analog will be based on LOV2, a protein domain that undergoes major conformational changes when irradiated. Based on our previous studies of light-mediated allosteric control (Dagliyan et al. Science, 2016) we predicted that LOV2 could be inserted in a specific surface loop of vinculin, where its conformational changes would allosterically affect the actin binding site. A vinculin tail

domain labelled with mCherry fluorescent protein strongly co-localized with both actin and focal adhesions. When LOV2 was incorporated into loop2 of this domain, co-localization was disrupted in the dark but reversibly restored by irradiation. In contrast, insertion of LOV2 into a different loop (loop1) produced an adduct with good colocalization in the dark, but disrupted upon irradiation. These results suggest that LOV2 insertion can be used to control the actin interactions of full-length vinculin. We will locally activate or inhibit vinculin-mediated intracellular force transduction in polarized cancer cells to understand the stretch induced signaling that controls directed migration in durotaxis.

4:30-5:00 PM: Poster Session 2

Lindsey Buckingham (Muss and Bae-Jump labs, Clinical Fellow)
Title: The interplay of obesity and frailty in treatment tolerance and outcomes for elderly patients with endometrial cancer
Abstract: Background: Endometrial cancer (EC) is the 4th most common cancer in US women. Median age at diagnosis is 61 years old, and 70% of EC deaths occur in women aged 65 years and older. Obesity is a well-known risk factor for developing and dying from EC. Additionally, a relationship between decreased muscle density and increased myosteatosis has been reported, suggesting a relationship between intra-abdominal fat and sarcopenia. In the elderly population, obesity rates increase along with sarcopenia rates, and both obesity and sarcopenia have been correlated with increasing frailty in women with cancer. We hypothesize that decreased muscle density correlates with higher levels of frailty in elderly EC patients. Furthermore, we suspect frail patients will have greater toxicity and worse quality of life during EC-directed therapy. Because EC usually occurs in obese, older women, this population is an apt target for investigation of the interplay of obesity, sarcopenia, and frailty.
Methods: Target accrual for this study is 100 patients with new diagnoses of Type I or Type II EC. All patients will undergo baseline functional and cognitive testing, and will answer quality of life surveys. We will follow patients prospectively through surgery and adjuvant therapies and catalogue post-operative complications, chemotherapy toxicities, and progression free and overall survivals. Quality of life will be measured at several timepoints throughout treatment.
Aims: We aim to assess the association of bioimpedance analyses of sarcopenia with CT measurements of skeletal muscle density in EC patients. We will use this data to compare body composition, treatment tolerance and response, quality of life, and surgical outcomes in EC populations that are <60 years of age to those 60 years and older.

Daniel Cortes (Maddox and Gladfelter labs, Postdoc)
Title: Contractile ring component density dynamics influences contraction kinetics
Abstract: Cytokinesis, the physical separation of one cell to two daughters, is required for proper cell proliferation. Proper completion of cytokinesis requires the formation of an actomyosin contractile ring, which is assembled around the cell in the cortex immediately below the plasma membrane. As it matures, the contractile ring restructures from a band to a tight chord, suggesting significant changes in contractile ring component organization during cytokinesis. Previous work has demonstrated the overall amount of several ring components change throughout cytokinesis, supporting ring restructuring. While these measurements show the global changes that occur in cytokinesis however, less is known about changes on the mesoscopic and molecular scales. In this work we set out to further bridge the gap between the theoretical models of cytokinetic ring components and quantitative cell biology. To this end, we used custom PDMS microfluidic devices to position C. elegans zygotes such that the contractile ring forms in plane with a high N.A objective light sheet. This setup allowed us to accurately measure ring component intensity over the length of cytokinesis. We then incorporated these data into a new agent-based model of cytokinesis where we simulate the complex load-dependent binding dynamics of myosin filaments built in Cytosim. In our model, actin treadmilling and overall shortening provide for a dynamic actin meshwork. Using this system, we generate contractile rings on the scale of C. elegans 1-cell contractile rings, that exhibit protein density dynamics like those measured in our in vivo data and show a model for how these rings may contract and how changing component organization may affect contraction dynamics.

Danielle File (Perou lab, Postdoc)
Title: Expression of Immune-related Gene Signatures in Untreated Metastatic Breast Cancer Tumors

Abstract: Background: Metastatic breast cancer (MBC) remains incurable. There is insufficient characterization of the molecular alterations that allow tumor cells to gain the ability to metastasize, contributing to inadequate metastasis prevention and treatment. It is known that breast primary and metastatic tumors have differential expression of immune-related gene signatures, however whether this occurs as a result of the metastatic process itself or secondary to treatment exposure remains unknown. The current literature regarding the biology driving metastatic disease comes from analysis of tumors obtained from patients who previously received cancer-directed treatments, thus capturing both genomic drivers of metastasis and alterations that occurred in response to selective pressures exerted by treatment, which cannot be readily distinguished.
Methods: Using clinical data and whole transcriptome sequencing results from two previously enrolled studies, we will compare gene expression patterns from tumors obtained from patients with de novo MBC (untreated) to those from patients with recurrent MBC (treated). Supervised analysis using Significance Analysis of Microarrays will be performed on a) primary breast tumors from de novo versus recurrent MBC, b) metastatic tumors from de novo versus recurrent MBC, and c) paired primary breast versus metastatic tumors from de novo MBC. Each analysis will assess the expression of immune-related individual genes and published gene signatures. Rationale: By eliminating concurrent detection of therapeutic resistance genes there will be a unique opportunity to statistically search for gene expression patterns specific to metastases. By also comparing gene expression from patients with de novo versus recurrent MBC, we will identify genes affected by treatment exposure, which can be further explored in studies of mechanisms of therapeutic resistance. Additionally, this comparison will allow for identification of unique biologic features of de novo MBC, which may inform novel treatment approaches. Conclusion: Despite the potential to identify true genomic drivers of metastasis inherent to the study of de novo MBC, samples from patients with de novo MBC have rarely been included in genomic studies. This project will characterize immune-related gene expression patterns of untreated metastases.

Wenxia He (Marzluff lab, Postdoc)
Title: Use of CRISPR technology to investigate the mechanism of histone mRNA degradation
Abstract: Histone mRNAs differ from all other cellular mRNAs in that they do not end in a polyA tail, but end instead in a stemloop. Histone mRNAs are tightly cell-cycle regulated and most of the regulation in mammalian cells is postranscriptional. An important regulatory step is rapid changes in the rate of histone mRNA degradation to balance histone mRNA levels with the rate of DNA replication. The stem-loop at the 3' end of histone mRNA binds SLBP and is the critical cis-element that controls histone mRNA half-life. The SLBP-stem-loop RNA complex is involved in all steps of histone mRNA metabolism. SLBP-mediated replication-dependent histone mRNAs decay depends on translation and UPF1 recruitment to the 3' UTR of histone mRNA when DNA replication is inhibited. UPF1 recruitment results in activation of 3'hExo and TUTase 7 to initiate histone mRNA degradation. Using crispr/cas9, we deleted exon2 (aa 14-50) of SLBP, on the N-terminal and found the rate of degradation of histone mRNA became slower. Dr.Sutapa Chakrabarti's lab (Free University of Berlin), have found that this region of SLBP directly binds Upf1, providing a mechanism to recruit Upf1 to histone mRNA. We postulate that Upf1 binds to the terminating ribosome and also to the N-terminal region of SLBP. This results in activation of 3'hExo and TUT7 to initiate histone mRNA degradation. We have knocked out both TUT7 and 3'hExo and will also determine the regions in these proteins required for histone mRNA degradation.

Christopher Jensen (Tuchman and Muss labs, Postdoc)
Title: Geriatric-assessment-guided interventions to address functional deficits in older adults undergoing treatment for multiple myeloma: feasibility and preliminary efficacy

Abstract: Background: Multiple myeloma (MM) is largely a disease of older adults, with a median age at diagnosis of 69. There is substantial heterogeneity in the aging population, which may not be captured by chronologic age alone. One approach to capturing this variation is the geriatric assessment (GA). Among patients with MM, deficits identified via GA predict treatment tolerance and survival. Despite this prognostic significance, clinic-based interventions targeting these deficits have not been thoroughly evaluated. Methods: We will test a clinic-based program to address GA-identified deficits in adults 65 and older treated for MM via a single-arm prospective trial. Patients will complete a baseline GA, including validated measures of physical function, psychosocial function, cognition, nutrition, hearing and vision. Patients with deficits will be referred to evidence-based interventions. Patients will complete a follow-up GA 3 months from baseline. Patient satisfaction with the intervention(s) will be assessed via questionnaire. Aims: 1) Assess the feasibility of the program by measuring adherence to, and satisfaction with, recommended interventions. 2) Assess preliminary by exploring changes in GA domains and symptom measures before and 3 months after intervention, with particular attention to physical function and social support / isolation. Anticipated results: Our hypothesis is that brief assessments of deficits and timely referral can be completed during a clinic visit, and that older patients with MM will engage in recommended interventions. We further hypothesize that these interventions will result in significant improvement in relevant functional domains among patients referred for interventions.

Rebecca Steiner (Matera lab, Postdoc)
Title: Elucidating the impact of SMA-causing mutations on the SMN complex and surrounding proteome.
Abstract: Small nuclear ribonucleoprotein (snRNP) assembly, essential for proper spliceosome biogenesis, has been implicated in many diseases including acute myeloid leukemia and myelodysplastic syndrome. Splicing defects have been shown to occur early-on in hematopoiesis, indicating the potential for snRNP assembly errors to be disease-initiating. A critical protein involved in snRNP assembly is Survival Motor Neuron (*SMN1*), which, in complex with Gemin proteins and Sm proteins, chaperones formation of the core snRNP particles. A homozygous deletion or mutation of *SMN1* causes a neurodegenerative disease, spinal muscular atrophy (SMA). SMA is characterized by the loss of motor neurons, leading to muscle weakness, presenting as a spectrum of severities. In severe SMA patients, there is a global splicing defect, which is thought to contribute to disease progression. Using *Drosophila* as a model organism, our lab has developed an allelic series of transgenic flies expressing SMA-causing missense mutations in *SMN*. *SMN1* mutations recapitulate the full range of SMA severities; however, how these mutations impact the SMN complex and global proteome is unknown. Through immunoprecipitation and mass spectrometric analysis, we have identified distinct proteins that interact with the SMN complex. Additionally, we have analyzed the global proteome of domain-specific SMN mutations, revealing unique processes being altered. Combined, these datasets provide mechanistic insight into the function of the SMN complex in the proteome.

Jackson Trotman (Calabrese lab, Postdoc)
Title: Elements at the 5' end of Xist harbor SPEN-independent transcriptional antiterminator activity
Abstract: The *Xist* lncRNA requires Repeat A, a conserved RNA element located in its 5' end, to induce gene silencing during X-chromosome inactivation. Intriguingly, Repeat A is also required for production of *Xist*. While silencing by Repeat A requires the protein SPEN, how Repeat A promotes *Xist* production remains unclear. We report that in mouse embryonic stem cells, expression of a transgene comprising the first two kilobases of *Xist* (*Xist* -2kb) causes transcriptional readthrough of downstream polyadenylation sequences. Readthrough required Repeat A and the ~750 nucleotides downstream, did not require SPEN, and was attenuated by splicing. Despite associating with SPEN and chromatin, *Xist*-2kb did not robustly silence

transcription, whereas a 5.5-kb *Xist* transgene robustly silenced transcription and read through its polyadenylation sequence. Longer, spliced *Xist* transgenes also induced robust silencing yet terminated efficiently. Thus, in contexts examined here, *Xist* requires sequence elements beyond its first two kilobases to robustly silence transcription, and the 5' end of *Xist* harbors SPEN-independent transcriptional antiterminator activity that can repress proximal cleavage and polyadenylation. In endogenous contexts, this antiterminator activity may help produce full-length *Xist* RNA while rendering the *Xist* locus resistant to silencing by the same repressive complexes that the lncRNA recruits to other genes.

Andrew Waters (Der lab, Postdoc)
Title: Targeting p130Cas- and Microtubule-dependent MYC Regulation Sensitizes Pancreatic Cancer to ERK MAPK Inhibition
Abstract: To identify therapeutic targets for KRAS-mutant pancreatic cancer, we conducted a druggable genome siRNA screen and determined that suppression of *BCAR1* sensitizes pancreatic cancer cells to ERK inhibition. Integrative analysis of genome-scale CRISPRCas9 screens also identified *BCAR1* as a top synthetic lethal interactor with mutant KRAS. *BCAR1*encodes the SRC substrate p130Cas. We determined that SRC inhibitor-mediated suppression of p130Cas phosphorylation impairs MYC transcription through a DOCK1-RAC1-beta-catenin dependent mechanism. Additionally, genetic suppression of *TUBB3*, encoding the beta III-tubulin subunit of microtubules, or pharmacological inhibition of microtubule function, decreased levels of MYC protein and potently sensitized pancreatic cancer cells to ERK inhibition. Accordingly, the combination of a dual SRC/tubulin inhibitor with an ERK inhibitor cooperated to reduce MYC protein and to synergistically suppress the growth of KRAS-mutant pancreatic cancer. Thus, we demonstrate that mechanistically diverse combinations with ERK inhibition suppress MYC to impair pancreatic cancer proliferation.

Friday, November 6th

8:00-9:40 AM: Selected talks session 2

8:00-8:20 AM: Katherine Barnett (Ting lab, Postdoc)
Title: Inflammasome Activation by SARS-CoV-2
Abstract: Since its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 35 million people worldwide causing significant morbidity and mortality with over 210,000 deaths in the United States alone. Individuals with coronavirus disease 2019 (Covid-19) typically have mild symptoms, while others progress to acute respiratory distress syndrome (ARDS), a condition fueled by a dysregulated immune response known as cytokine storm. Patients with Covid-19 have elevated serum levels of several different proinflammatory cytokines, including one of the drivers of cytokine storm, IL-1β. A major source of IL-1β production is through activation of the inflammasome, an innate immune signal transduction complex that acts through multiple sensors to trigger IL-1β release and pyroptotic cell death. In addition to heightened IL-1β, patients with severe Covid-19 have elevated levels of lactate dehydrogenase (LDH), a molecule released upon cell membrane rupture and a hallmark of pyroptotic cell death. Together, these data suggest that SARS-CoV-2 activates the inflammasome and that this process is likely exacerbated in patients with severe disease. In this study, we investigate the prevalence of inflammasome biomarkers in severe Covid-19 patients and the activation of the inflammasome by SARS-CoV-2 infection in well-differentiated primary human airway epithelia. Through this work, we seek to understand the activation of inflammation by a highly immune-evasive virus and evaluate strategies for dampening inflammation in severe Covid-19 patients.

8:20-8:40 AM: Evan Dewey (Sekelsky lab, Postdoc)
Title: Deciphering Mitotic Crossover and Bloom Syndrome Helicase Mechanisms in Cancer
Abstract: Genome stability is critical to homeostasis and cancer prevention. Bloom Syndrome Helicase (human: *BLM*) preserves genome stability through DNA repair, with *BLM* loss causing numerous cancer types. BLM cancers are thought to be associated with increased mitotic crossovers (mitCOs) from homology-directed repair of double strand break DNA that lead to loss of heterozygosity and genome instability. Despite potentially carcinogenic effects, precise mitCO mechanisms remain elusive. This is due to difficulty in precisely interpreting mitCO products, as key heteroduplex DNA, consisting of DNA from both repaired and template homologous chromosomes, is masked by mismatch repair (MMR). Using *Drosophila*, I will accurately define mitCO mechanisms using a unique and complete MMR knockout (both canonical and short-patch) paired with CRISPR/Cas9 and with *BLM* knockout. This will define mechanisms of so-called "spontaneous" and Cas9-mediated mitCOs. While initial *BLM* studies focused on its loss in cancer, cancer genome catalogs instead show some cancers with *BLM* copy gain and overexpression, suggesting different function. In addition to homology directed repair, BLM repairs DNA replication forks, and I hypothesize both activities could promote cancer transformation, growth, and/or stability via overexpression. *BLM* overexpression in normal *Drosophila* epithelia indicates that excess Blm does not appear to function in epithelial transformation, pointing instead to a role in cancer growth and stability. I will assess this with BLM inhibition of *BLM* overexpressed patient-derived tumor cells and *BLM* overexpression coupled to tumor suppressor knockdown and oncogenic overexpression in *Drosophila* epithelia. By combining *Drosophila* and patient-derived tumor cells, I will examine how mitCOs lead to genome instability, provide insight into mitCO mechanisms for use in genome editing, and define how BLM overexpression aids cancer transformation, growth, and stability.

8:40-9:00 AM: Emily Harrison (Pecot lab, Postdoc)
Title: A circle RNA regulatory axis promotes lung squamous metastasis via CDR1-mediated regulation of Golgi trafficking
Abstract: Lung squamous carcinoma (LUSC) is a highly metastatic disease with a poor prognosis. Using an integrated screening approach, we found that miR-671-5p reduces LUSC metastasis by inhibiting a circular RNA (circRNA), CDR1as. Although the putative function of circRNA is through miRNA sponging, we found that miR-671-5p more potently silenced an axis of CDR1as and its antisense transcript, cerebellar degeneration related protein 1 (CDR1). Silencing of CDR1as or CDR1 significantly inhibited LUSC metastases and CDR1 was sufficient to promote migration and metastases. CDR1, which directly interacted with adaptor protein 1 (AP1) complex subunits and COPI proteins, no longer promoted migration upon blockade of Golgi trafficking. Therapeutic inhibition of the CDR1as/CDR1 axis with miR-671-5p mimics reduced metastasis in vivo. This report demonstrates a novel role for CDR1 in promoting metastasis and Golgi trafficking. These findings reveal a miRNA/circRNA axis that regulates LUSC metastases through a previously unstudied protein, CDR1.

9:00-9:20 AM: Tara Walhart (Weissman lab, Postdoc)
Title: Targeting SMARCB1 in Chordoma Development
Abstract: Chordoma is a rare aggressive primary malignancy of the axial skeleton, originating from notochordal tissue. There are three histological subtypes conventional, poorly-differentiated and dedifferentiated. While conventional possess mutations in common oncogenes and tumor suppressor genes, poorly-differentiated chordomas arise from inactivation of SMARCB1 (SNF5). SMARCB1 is a key subunit in the SWI/SNF chromatin-remodeling complex. Currently, there are no approved therapies for chordomas, especially the highly aggressive pediatric SMARCB1^{neg} poorly-differentiated chordomas (PC). To identify mechanisms by which SMARCB1

loss drives PC developments, we examined the effects of SMARCB1 re-expression in the SMARCB1^{neg} chordoma cell lines, Ch22 and UM-Chor5. Using an inducible SMARCB1 vector (pIND20-SNF5), we developed stable Ch22 and UM-Chor5 cells with inducible SMARCB1 expression. 24h and 48h after induction of SMARCB1, we observed an increased protein level of SMARCB1. SMARCB1^{neg} chordoma cell lines lacked expression CDKN2A expression wasn't detected. Our data establish SMARCB1 re-expression in SMARCB1^{neg} chordomas alters the repertoire of SWI/SNF complexes, perhaps restoring those associated with cellular differentiation. Our finding supports a model where SMARCB1 inactivation blocks the conversion of growth-promoting SWI/SNF complexes to differentiation-inducing ones. Therefore, restoration of SMARCB1 in SMARCB1^{neg} chordomas cells creates a unique opportunity to identify therapeutic vulnerabilities.

9:20-9:40 AM: Jennifer Klomp (Der lab, Postdoc)
Title: CHK1 protects oncogenic KRAS-expressing cells from DNA damage and is a target for pancreatic cancer treatment
Abstract: KRAS is a well-validated driver of the growth of pancreatic ductal adenocarcinoma (PDAC), largely through the ERK MAPK cascade. To identify genes essential for this growth, we performed a CRISPR/Cas9 loss-of-function screen targeting the "druggable genome" in a panel of KRAS-mutant PDAC cell lines. The serine/threonine kinase *CHEK1*/CHK1 was a hit involved in the three major biological processes identified: cell cycle, DNA damage repair (DDR), and macromolecule metabolic processes. A separate, extended screen using this library identified DDR genes including an upstream CHK1 activator, ATR, as sensitizers of PDAC cells to the autophagy inhibitor chloroquine (CQ). *CHEK1* was also identified in a "druggable genome" siRNA library screen as a modulator of PDAC sensitivity to the ERK-selective inhibitor SCH772984 (ERKi). To better understand how CHK1 inhibition enhances sensitivity to ERKi and CQ, we used the clinical candidate CHK1 inhibitor, prexasertib (CHK1i). CHK1i alone potently blocked the growth of KRAS-mutant PDAC cell lines and patient-derived organoids (PDOs), and increased apoptosis at doses correlated with CHK1i-induced S-phase arrest. CHK1i + ERKi and CHK1i + CQ both synergistically blocked PDAC cell growth. CHK1i + ERKi + CQ further inhibited proliferation and enhanced apoptosis in PDAC cell lines and PDOs. We found that CHK1i alone activated ERK and AMPK, and increased autophagic flux, providing a mechanistic basis for increased efficacy of the combinations of CHK1i with ERKi and/or CQ. To better understand how activation of ERK promotes PDAC survival in the presence of CHK1i, we performed a CRISPR/Cas9 loss-of-function screen using a library targeting 1,200 known/putative ERK substrates. We identified loss of *RIF1*, a key component of non-homologous end-joining (NHEJ) repair, as a sensitizer of PDAC cells to CHK1i-mediated growth suppression and apoptosis. In summary, we demonstrate that targeting the DDR pathway enhances the efficacy of ERK MAPK and/or autophagy inhibitors to treat KRAS-mutant PDAC.

11:00-11:30 AM: Poster Session 3

Nathaniel Burkholder (Strahl lab, Postdoc)
Title: Deciphering the histone interactions and reader functions of ASH1L and the PHD-BRD reader domain family in biology and cancer
Abstract: Mutation or dysregulation of the proteins that read, write, and/or erase histone post-translational modifications are thought to underlie ~50% of all human cancers. One such family of proteins that are frequently altered in cancer is those containing paired histone reading plant homeodomains (PHD) and bromodomains (BRD). ASH1L is a histone H3 lysine-36 (H3K36) dimethylase which is altered in ~7% of cancers and contains a poorly understood triple histone reader PHD-BRD-BAH domain module. Although ASH1L and other PHD-BRD proteins contain commonly studied reader domains and have been linked to cancer, how they regulate normal and

cancer development is still not well understood. Thus, there is a critical need to systematically characterize the histone reading interactions and mechanistic processes for ASH1L and the rest of the PHD-BRD family, especially with respect to how these domains in combination can form multivalent interactions with nucleosomes. To address how ASH1L and PHD-BRD readers function, I will employ a suite of innovative histone-binding assays, structural methodologies, and cellular-based analyses. Specifically, I will use modernized histone peptide and nucleosome screening assays, X-ray crystallography and Cryo-EM in parallel, and a knockdown/complementation system of ASH1L in normal and breast cancer cell lines. I hypothesize that ASH1L targets specific acetylated and methylated histone tails via its BRD-PHD-BAH module to regulate homeotic gene expression, but that aberrant expression of ASH1L promotes breast cancer through increased expression of pro-growth and survival genes. Through this examination, this work aims to further decipher the "Histone Code" and provide new insights into cancer biology.

Jonathan DeLiberty (Bryant and Der labs, Graduate student)
Title: Identification and characterization of novel targets for autophagy inhibition in pancreatic ductal adenocarcinoma
Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with a 5-year survival rate of 10%. There are multiple current directions for new PDAC treatments, one of which is perturbing the altered metabolic demands associated with tumorigenicity. One such metabolic process is autophagy, or "self-eating", which is a lysosomal-mediated process whereby cells degrade and recycle damaged organelles and macromolecules in order to sustain growth. Autophagy can be divided into four distinct phases: initiation, nucleation, maturation, and degradation. Our lab recently demonstrated that PDAC cells become more dependent on autophagy following inhibition of the RAF-MEK-ERK mitogen-activated protein kinase cascade. This dependency can be therapeutically targeted by combining autophagy inhibitors such as hydroxychloroquine (HCQ) with ERK and MEK inhibitors. Currently, HCQ is the only clinically used autophagy inhibitor; however, it is limited by low potency and lack of specificity. In order to better understand how to improve HCQ treatment, we performed a CRISPR/Cas9-mediated genetic loss-of-function screen in the presence of a related analog, chloroquine (CQ), to identify combination drug treatments that enhance anti-proliferative activity. Interestingly, a number of top hits were genes that encode proteins upstream in the autophagic pathway. To validate these results, I have begun treating PDAC cells with anti-autophagy combinations, using inhibitors against specific nodes of the autophagy pathway including ULK (initiation), VPS34 (nucleation), and lysosomal acidification (degradation). Preliminary data suggests that vertical inhibition of the autophagy pathway results in a further reduction of autophagic flux relative to inhibition of any single node and can also synergistically kill PDAC cells. Ongoing studies are aimed at understanding compensation to ULK monotherapy and elucidating the mechanism underlying the synergy observed with anti-autophagy inhibitor combinations.

Craig Goodwin (Der lab, Postdoc)
Title: Combination therapies with CDK4/6 inhibitors to treat KRAS-mutant pancreatic cancer
Abstract: Pancreatic ductal adenocarcinoma (PDAC) patients have a dismal five-year survival rate in the advanced metastatic setting; therefore, the development of targeted therapies is a significant unmet clinical need. The two most frequent genetic events in PDAC (mutational activation of KRAS and loss of the tumor suppressor *CDKN2A*) converge on activation of the kinases CDK4 and CDK6, which promote G1 cell cycle progression. We found that CDK4/6 inhibitors (CDK4/6i), which are pharmacologic mimics of $p16^{INK4A}$ function, elicited single-agent activity in a subset of KRAS-mutant PDAC cell lines. However, applying Reverse Phase Protein Array (RPPA)-based pathway activation mapping analyses, we observed widespread CDK4/6i-induced compensatory signaling activity/expression changes leading to increased ERK-MAPK

signaling. Concurrent treatment with the ERK1/2 inhibitor (ERKi) SCH772984 reversed this phenotype, synergistically reduced cell growth, and increased both apoptosis and G1 arrest in PDX cell lines and organoid models by a well-defined mechanism. Next, we used a CRISPR/Cas9 druggable genome library loss-of-function screen to identify genes that modulated sensitivity to CDK4/6i. We identified a functionally diverse array of genes that enhanced growth suppression in combination with CDK4/6i, centered around distinct signaling nodes including cell cycle regulation and mitosis, PI3K-AKT-mTOR signaling, SRC family kinase signaling, cell metabolism and biosynthesis, and DNA damage and repair pathways, suggesting ways to overcome *de novo* or acquired CDK4/6 inhibitor resistance in the clinic. Identified synergistic combinations were validated using siRNA and small-molecule inhibitor-based approaches. Our observations suggest that CDK4/6 inhibitors alone, or in novel combinations, may benefit PDAC patients clinically.

Priya Hibshman (Der lab, Graduate student)
Title: Defining the role of MYC in KRAS-driven pancreatic cancer
Abstract: The RAF-MEK-ERK signaling network is the key effector pathway driving KRAS-dependent growth of pancreatic ductal adenocarcinoma (PDAC). We recently demonstrated that ERK is a therapeutic target in PDAC, and that the MYC transcription factor and oncoprotein is a key ERK substrate. PDAC sensitivity to ERK inhibition (ERKi) correlated with loss of MYC. Either KRAS depletion or ERKi resulted in loss of MYC, and MYC suppression alone inhibited PDAC tumorigenic growth. We have initiated a comprehensive evaluation of the specific contributions of MYC to KRAS-dependent PDAC growth. First, RNA-Seq demonstrated that KRAS depletion or ERKi globally suppressed the MYC transcriptome, supporting a significant block in MYC function upon loss of KRAS-ERK signaling. Second, acute *KRAS* suppression or ERKi in both human and mouse PDAC caused striking alterations in cell morphology, with significant cell enlargement and flattening, and enhanced actin stress fiber organization. These changes were largely phenocopied upon *MYC* suppression. Third, applying reverse phase protein array (RPPA) pathway activation mapping to *KRAS* or *MYC* siRNA-treated PDAC cell lines, we observed alterations in both shared and distinct signaling networks. Loss of either *KRAS* or *MYC* induced compensatory upregulation of KRAS effector signaling, suppressed mitosis, and induced G1 arrest, whereas only *KRAS* depletion activated pro-apoptotic proteins. Additionally, *KRAS* suppression increased E-cadherin whereas MYC suppression reduced it, suggesting opposing consequences on epithelial-to-mesenchymal transition (EMT). Our studies show that KRAS-dependent PDAC growth is mediated through both MYC-dependent and -independent processes. Our ongoing studies involve further evaluation of MYC in KRAS-dependent cellular processes and the use of pharmacologic inhibitors of MYC to further assess MYC as a therapeutic target in KRAS-mutant PDAC.

Jordan Koehn (Weeks lab, Postdoc)
Title: Transcriptome-wide discovery of RNA-ligand target sites
Abstract: Currently, only 0.05% of the human genome has been drugged with fewer than 700 proteins targeted. Similar to proteins, certain RNA molecules can fold back on themselves to form complex structures that can contain pockets or clefts with sufficient structural sophistication to allow specific and high-affinity binding by small molecule ligands. Small molecule binding to messenger RNAs can modulate protein gene products by upregulating or downregulating protein translation efficiency or by altering mRNA abundance or stability. Recent studies have revealed that diverse motifs within a transcriptome can be highly structured and may represent a substantial untapped opportunity for discovering therapeutically useful RNA-ligand interactions. Therefore, a robust method for discovering ligands and mapping RNA-ligand interactions across the transcriptome would have high therapeutic value, especially in cancer biology. Our goal is to develop a technology for transcriptome-wide identification of RNA-ligand binding sites and discovery of novel small molecule ligands that bind RNA with high-affinity and selectivity. Our strategy will employ an innovative fragment-based ligand discovery approach coupled to a concise and direct read-out of RNA-ligand binding sites by high-throughput sequencing. Once validated, we hope our technology will enable rapid identification of therapeutically relevant RNA motifs with high-information-content structures able to bind function-modulating small molecule ligands.

Susanna Stroik (Ramsden lab, Postdoc)
Title: Identifying POLQ's Role in Replicative Repair and Rescue
Abstract: Genomic instability is a hallmark of cancer known to singlehandedly drive oncogenesis and engineer resilient cancer cells by driving mutation. Notably, the standard of care for many cancers is treatment with DNA damaging agents such as chemotherapeutics and radiation therapy with the goal of damaging cancer cells beyond repair. Thus, understanding the mechanisms in which DNA damage is repaired under these conditions is of the upmost

importance for both cancer prevention and its treatment. Amongst DNA damage, the most severe threat to the genome is DNA double strand breaks (DSBs). DSBs have 3 major repair mechanisms which can be deployed in response to their accumulation – homologous recombination (HR), classical non-homologous end-joining, and Theta-mediated end-joining (TMEJ). The latter is upregulated in many cancers, specifically those in which HR genes are mutated (BRCA1, BRCA2, etc.). However, little is known about the role this pathway plays in repairing breaks which occur during DNA replication, a cell cycle stage which many cancer therapeutics induce DNA damage. My project focuses on the role the central protein in TMEJ, Polymerase Theta (POLQ), plays during genome duplication and the mutagenic signature of such a role. POLQ relies on the use of microhomologies at break sites to initiate repair and thus has a distinct, measurable repair signature. In my preliminary work, I have established that POLQ deficiency renders cells sensitive to Camptothecin (CPT) treatment, a topoisomerase I poison which induces S-phase specific DSBs. Further, POLQ deficient cells display a prolonged replication stress response after acute dosage of CPT, implying POLQ contributes to repair of replicative DSBs. Using single molecule DNA combing, I've established that POLQ plays a role in fork protection, recovery, and restart after the induction of replicative breaks. Further, this role in replication recovery is exacerbated under conditions where alternative rescue pathways are absent. Ongoing work is focused on the engineering of an inducible broken replication fork reporter system, with which I will measure the kinetics and repair signatures of individual replicative DSBs. In summary, I've identified a new function for POLQ during DNA replication and seek to identify how this function contributes to cancer genome evolution.

11:30-12:00 PM: Poster Session 4

Mitchell Butler (Bear lab, Postdoc)
Title: Investigating the spatiotemporal regulation of protrusive actin networks during cell migration
Abstract: Cell migration is essential for many important physiological processes, including embryonic development, wound healing, and the immune response. In addition, the signaling pathways and mechanisms governing cell motility are often aberrantly utilized to facilitate cancer metastases. Cell migration can be directionally biased by extracellular guidance cues, a process known as haptotaxis, and these cues are sensed and responded to through integrin receptor complexes. Haptotaxis requires the highly conserved Arp2/3 complex and its critical role in the polymerization of branched actin networks within cellular protrusions. Using cell lines derived from an Arpc2 conditional knockout mouse, we seek to explore novel aspects of how actin networks in cellular protrusions are regulated by diverse extracellular substrates. CRISPR-mediated labeling of the essential Arpc2 subunit of the Arp2/3 complex allows us to observe branched actin dynamics and affords optogenetic control of the endogenous Arp2/3 complex. High-resolution time-lapse imaging reveals striking differences in the organization and behavior of Arp2/3 depending on the substrate, and optogenetic manipulations provide additional insights into how Arp2/3 dynamics and actin network organization are regulated in a substrate-dependent manner. Micropatterning reveals that Arp2/3 is enriched in protrusions as they extend across patches of fibronectin, and that close proximity to regions of fibronectin engagement is not sufficient for this enrichment. Perturbation of factors required for haptotaxis such as nascent adhesions, Rho GTPase and RhoGEF activity can additionally alter Arp2/3 and protrusion dynamics in a substrate-dependent manner. Steps are now being taken to characterize how polymerases of linear actin arrays or bundles such as Formins and Ena/VASP proteins are also regulated by extracellular cues and haptotactic signaling. Ultimately, these efforts are geared towards developing a deeper and more comprehensive understanding of fundamental cell behaviors by examining how the motility machinery is assembled and organized within cells in response to their environment.

Nate Diehl (Der lab, Graduate student)
Title: Life without KRAS: profiling the KRAS-dependent kinome to identify novel therapeutic vulnerabilities in pancreatic cancer
Abstract: Oncogenic KRAS mediates signaling activities through a network of kinases (the kinome). To broadly characterize the KRAS-dependent kinome in pancreatic ductal adenocarcinoma (PDAC), we applied the Multiplexed Inhibitor Bead/Mass Spectroscopy (MIB/MS) chemical proteomics strategy to monitor kinome-wide changes of >200 kinases following 72 hours of siRNA-mediated knockdown of KRAS. We hypothesized that upregulated kinases may act as compensatory activities to overcome loss of KRAS function, whereas downregulated kinases may contribute to KRAS driver function. KRAS suppression-induced upregulated kinases included JAK1, DDR1, ERK1 and SRC family members YES1 and SRC. Reverse phase protein array (RPPA) pathway activation analysis confirmed activation of STAT transcription factors, reactivation of ERK1/2 signaling, and increased SRC signaling. Pharmacologic inhibition of SRC and DDR1, but not JAK, suppressed PDAC growth, supporting SRC and DDR1 as compensatory activities. Downregulated kinases included those involved in both the DNA damage response and cell cycle regulation, such as WEE1, AURKA, CHK1, CDK1, and PLK1. Western blotting verified loss of PLK1, WEE1, CHK1, and CDK1 as concurrent with loss of KRAS protein. To assess whether inhibition of these downregulated kinases conferred sensitivity to the ERK inhibitor SCH772984 (ERKi), we used the clinical candidate WEE1 inhibitor adavosertib (WEE1i). The combination of WEE1i and ERKi synergistically suppressed proliferation of PDAC cell lines and patient-derived organoids, and increased apoptosis. Together, our data suggest that concurrent inhibition of the DNA damage response pathway and the ERK MAPK cascade may be an effective treatment for KRAS-mutant pancreatic cancer.

Brigid Grabert (Gilkey lab, Postdoc)
Title: Implementation of QI coaching versus physician communication training for improving HPV vaccination in primary care: A randomized implementation trial
Abstract: Health departments (HDs) are at the forefront of efforts to improve HPV vaccine uptake in the US. Most notably, HD staff routinely conduct in-person quality improvement (QI) coaching to help primary care clinics improve their vaccine delivery systems. Some HDs also engage outside experts to conduct remote communication training to help vaccine prescribers recommend HPV vaccine more effectively. To guide future HD programming, we sought to understand the implementation strengths and challenges of each intervention. In a cluster randomized trial, we allocated 855 primary care clinics in 3 US states to receive QI coaching, communication training, or both interventions combined. In each arm, we assessed adoption (or the % of clinics receiving the intervention out of those invited), reach (median number of total staff and prescriber participants per clinic), and delivery cost. More clinics adopted QI coaching than communication training or the combined intervention (63% vs 16% and 12%, both $p < .05$). In contrast, communication training and the combined intervention reached more total staff per clinic than QI coaching (median= 5 and 5 vs 2, both $p < .05$), including more prescribers per clinic (2 and 2 vs 0 both $p < .05$). QI coaching cost $439 per clinic, including costs incurred from follow up ($129/clinic), session preparation ($73/clinic), and travel ($69/clinic). Communication training cost $1,287 per clinic, with most cost incurred from clinic recruitment ($653/clinic). QI coaching was lower cost and had higher adoption, but communication training achieved higher reach, including to highly influential vaccine prescribers. Thus, communication training is a promising intervention, although HDs will need to take care to overcome recruitment challenges.

Jessica Islam (Basch lab, Postdoc)
Title: Mental Health of Cancer Survivors during the COVID-19 Pandemic in the United States: An analysis of the COVID-19 Impact Survey

Abstract: The COVID-19 pandemic has affected the mental health of adults, including cancer survivors, in the United States (U.S.). Our objective was to evaluate mental health symptoms and estimate determinants of mental health symptoms among cancer survivors during the COVID-19 pandemic in the U.S. We used nationally-representative data of 10,760 U.S. adults from the COVID-19 Impact Survey. We defined cancer survivors as a self-reported diagnosis of cancer (n=854, 7.6%). We estimated the association of mental health symptoms among cancer survivors using multinomial logistic regression and calculated adjusted odds ratios (aOR) with 95% confidence intervals (95% CI). We estimated determinants of experiencing at least one mental health symptom 3-7 times in the last seven days among cancer survivors using Poisson regression models to estimate adjusted prevalence ratios (aPR) and 95% CI. Cancer survivors were more likely to report feeling nervous, anxious or on edge (aOR:1.42, 95% CI: 1,07-1.90), depressed (aOR:1.69, 95% CI:1.28-2.24), lonely (aOR:1.47, 95% CI:1.09-1.98), and hopeless (aOR:1.55, 95% CI:1.14-2.10) 3-7 days per week in the last seven days when compared to adults without cancer. Among cancer survivors, adults aged 30-44 years (aPR: 1.87, 95% CI:1.18-2.95), females (aPR:1.55, 95% CI:1.12-2.13), without a high school degree (aPR: 1.79, 95% CI; 1.05-3.04), and adults with limited social interaction (aPR:1.40, 95% CI:1.01-1.95) were more likely to experience at least one mental health symptom 3-7 times/day in the past 7 days. Cancer survivors are experiencing mental health symptoms during the COVID-19 pandemic, particularly young adults, adults without a high school degree, females, and survivors with limited social support.

Aleksandra Skrajna (McGinty lab, Postdoc)

Title: Deciphering the nucleosome interactome

Abstract: In all eukaryotic cells, nucleosomes provide basic organization of genomic DNA and serve as signaling hubs for genome-templated processes. These functions are tuned by chromatin enzymes that introduce chemical modifications to nucleosomes and control their positioning and occupancy. Altered DNA methylation and histone modifications are recognized epigenetic defects in cancer, developmental and neurodegenerative diseases, yet our understanding of how the chromatin enzymes recognize their physiologic target, the nucleosome, is limited. High and medium resolution structures of chromatin enzymes bound to nucleosomes show multivalent interactions including histones and DNA. Nearly all reported interactions also include the anchoring of an arginine into a patch of acidic residues on the nucleosome surface. We performed a comprehensive, unbiased nucleosome interactome proteomic screen to assess the role of the acidic patch in nucleosome binding proteome-wide and to identify novel nucleosome binding hot-spots. This was accomplished through nucleosome affinity pulldowns from mouse embryonic stem cell nuclear lysates, using a reconstituted library of nucleosomes bearing mutations that collectively disrupt all exposed histone surfaces. To quantitively compare binding differences across the library we utilized isobaric tandem mass tags (TMT) and multidimensional mass spectrometry. Remarkably, we found that over 50% of nucleosome binding proteins identified in our screen require the acidic patch for binding and that 18% and 8% of proteins also require one or two additional nucleosome disk surfaces. Perhaps more surprisingly, almost half of the nucleosome disk surface contributes only minimally to nucleosome binding nuclear proteome-wide. Overall, we paired about 300 proteins representing diverse nuclear functions including DNA methylation (DNMT1 and 3, Tet1), histone modification (HAT complexes, kinases), nucleosome remodeling (SWI/SNF), DNA repair (MRN complex), DNA replication (ORC complex), RNA processing, and cell cycle progression (APC/C) with specific nucleosome surface requirements. We selected previously unknown nucleosome binder – APC/C– and showed that it directly interacts with the nucleosomes and that this interaction is acidic patch-dependent. Altogether our results illuminate the pervasiveness of acidic patch binding, establish universal patterns of nucleosome recognition and provide a reliable resource that details the nucleosome interactome.

Siyuan Su (Liu lab, Postdoctoral Fellow)

Title: SPOP and OTUD7A control EWS-FLI1 protein stability to govern Ewing sarcoma growth

Abstract: The EWS-FLI1 fusion oncoprotein occurs in the majority of Ewing sarcoma. The dependence of the tumor for this oncoprotein renders EWS-FLI1 an ideal drug target. Although EWS-FLI1 downstream signaling and binding partners have been extensively studied, the mechanisms regulating EWS-FLI1 protein stability remain elusive. Here, we identify SPOP and OTUD7A as *bona fide* E3 ligase and deubiquitinase, respectively, that control EWS-FLI1 protein turnover in Ewing sarcoma. SPOP targets a phospho-"VTSSS" degron generated by CK1, to recognize, ubiquitinate and degrade EWS-FLI1. Opposing this process, OTUD7A deubiquitinates and stabilizes EWS-FLI1, also dependent on the "VTSSS" degron. Depletion of OTUD7A in multiple Ewing sarcoma cell lines led to reduced EWS-FLI1 protein abundance and impeded Ewing sarcoma growth *in vitro* and in mice. We performed an AI-based virtual drug screen using a 4 million small molecule compound library and identified a lead compound 7Ai as a potential OTUD7A catalytic inhibitor. 7Ai efficiently reduced EWS-FLI1 protein levels and retarded Ewing sarcoma growth. Our study supports therapeutically targeting OTUD7A as a novel strategy to treat Ewing sarcoma bearing EWS-FLI1 fusion, and may also be applicable to other cancers with aberrant FLI1 expression.