AWARD NUMBER: W81XWH-19-1-0503

TITLE: Therapeutic Strategies to Disrupt Cx26-FAK-NANOG Complex to Attenuate Cancer Stem Cell Self-Renewal and Triple-Negative Breast Cancer Progression

PRINCIPAL INVESTIGATOR: Ofer Reizes, Ph.D.

CONTRACTING ORGANIZATION: Cleveland Clinic

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14. ABSTRACT

Triple-negative breast cancer is the most aggressive breast cancer subtype and is resistant to therapies. Our objective is to neutralize cancer stem cells, which are thought to underlie resistance to chemotherapeutics, as well as recurrence and metastasis. In parallel, we seek to minimize collateral damage to normal non-cancer cells. We identified that the protein connexin 26 (Cx26) is necessary and sufficient for the survival of cancer stem cells in triple-negative breast cancer models. While Cx26 was previously proposed to be a tumor suppressor, epidemiological studies suggest otherwise, as patients with high Cx26 had a poorer prognosis. Our studies indicate that Cx26 promotes cancer stem cell survival by forming a protein complex with the transcription factor NANOG, a master regulator of cancer stem cell function, and focal adhesion kinase in triple-negative breast cancer but not in other breast cancers. Our objective is to prevent this complex from forming and thereby inhibit cancer stem cell survival and growth. We will develop a therapeutic strategy to target complex formation that will be tested in pre-clinical models.

15. SUBJECT TERMS

NONE LISTED

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- **INTRODUCTION.** Triple-negative breast cancer (TNBC), the most aggressive breast cancer subtype, is associated with high rates of recurrence and metastasis as there are no clinically targeted therapies[1-5]. Toxic chemotherapeutic agents are the primary treatment regimen, highlighting the need for new targeted therapies. TNBC contains self-renewing, therapeutically resistant cancer stem cells (CSCs) that are responsible for tumor progression and metastasis[6-9]. The molecular circuitry that underlies stem cell pluripotency includes key transcription factors that are essential self-renewal signaling nodes and are highly expressed in TNBC[10, 11]. <u>To effectively target CSCs, it is essential to disrupt these signaling networks. However, as CSCs are maintained by pluripotency transcription factors, direct targeting remains a critical barrier.</u> We developed a reporter system based on the expression of the promoter of NANOG, a pluripotency transcription factor, to enable rapid and robust assays for studying and disrupting CSC signaling nodes[12]. In collaboration with Dr. Justin Lathia and his lab, we focused on the connexin family of proteins, which we found to be essential for TNBC CSC self-renewal despite a previously hypothesized tumor-suppressor function for some subunits[13, 14]. In a recently published report[15], we found that connexin 26 (Cx26) was elevated in TNBC compared with normal mammary tissue and enriched in CSCs compared with their non-CSC progeny in TNBC cell lines and patient-derived xenograft models. In functional studies, we demonstrated that Cx26 was necessary and sufficient for CSC maintenance and regulated NANOG protein stability. In TNBC, Cx26 localized to an intracellular membrane-bound vesicle in complex with the pluripotency transcription factor NANOG and focal adhesion kinase (FAK). <u>**Hypothesis:**</u> Based on published and preliminary data, **we hypothesize that self-renewal and tumor growth can be reduced by disrupting the Cx26/NANOG/FAK complex.**

- **KEYWORDS:** Cancer stem cells, gap junction, connexin-26, triple negative breast cancer

- **ACCOMPLISHMENTS:**

  - **What were the major goals of the project?**

    - **Specific Aim 1** will test the hypothesis that the Cx26/NANOG/FAK complex is essential for NANOG stability, activation, and maintenance of self-renewal.

    - **Specific Aim 2** will test the hypothesis that disrupting the integrity of the Cx26/NANOG/FAK complex attenuates self-renewal and tumor growth.

    - See Document 1_SOW

- **What was accomplished under these goals?**

- Major activities accomplished during this reporting period include:
    - New improved analysis of peptide binding via SPR, deeper in vivo analysis of MDA-MB-231 tumors: H&E analysis, staining for Ki67 and vimentin, RNAseq analysis of tumors, and IHC of primary TNBC patient specimen.
- Specific objectives accomplished include: manuscript in preparation
- Significant results:

Specific Aim 1: Soon after project initiation, we discovered the antibodies against NANOG, Cx26, and FAK for western blot and co-immunoprecipitation studies were no longer commercially available. Our efforts in this aim have continued to focus on screening multiple antibodies for these studies (Major Task 1, Subtask 1), as well as working with CCF Core facilities to generate a specific antibody ourselves. As indicated below, going forward we are generating TNBC cells expressing myc-Cx26 via CRISPR. This work is ongoing.

We are also working to validate these antibodies to assess complex member expression in tissue microarray samples (Major task 4, Subtask 1). We have validated Cx26 and FAK in luminal breast cancer tissue, but due to the lack of a specific antibody for NANOG protein, we have moved our efforts to staining sequential tissue sections for each complex member instead of at once (**Figure 1**).

Immunohistochemical analysis of patient specimen (Major task 4, subtask 1). Part of this work entails assessing the Cx26/NANOG/FAK complex in formalin fixed paraffin embedded (FFPE) tissues via immunohistochemistry to assess the expression of each component of the complex in well annotated invasive breast carcinomas. Archival FFPE tissues from the Department of Anatomic Pathology were identified to include breast carcinomas that represent the major molecular subtypes (luminal A, luminal B, basal-like/TNBC and HER2 positive) as previously determined by immunohistochemistry (estrogen receptor, progesterone receptor, HER2 with reflex HER2 FISH as needed and Ki67). Cx26, NANOG and FAK antibody clones were worked up independently on appropriate FFPE control tissues as suggested from the Human Protein Atlas and antibody manufacturer suggestions (**Figure 1**).

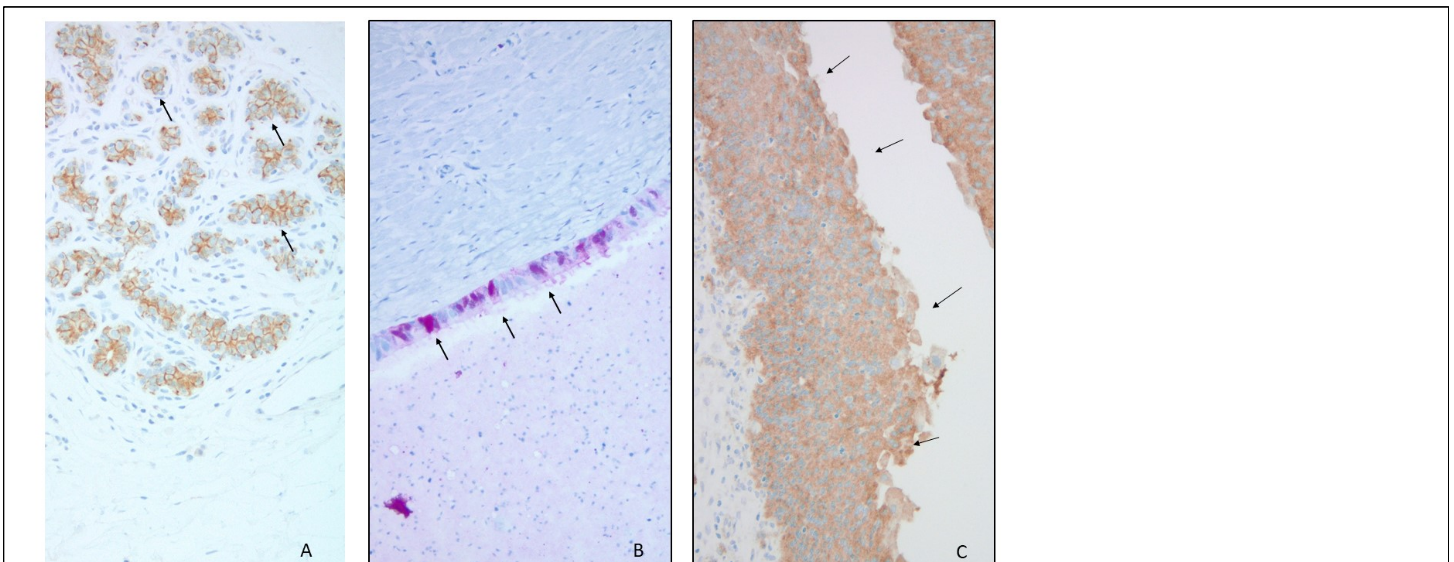

Figure 1. Composite image of control tissue staining. A) Cx26 (brown chromagen) showing membranous expression within the epithelium only of a normal breast terminal duct lobular unit. B) NANOG immunostain (purple chromagen) showing nuclear immunoreactivity within rare glandular cells of the epididymis. C) FAK (brown chromagen) showing cytoplasmic immunoreactivity within anal squamous mucosa.

After optimization of the antibodies, we believed that using a multiplex approach utilizing three different colored chromagens would allow for evaluation of protein expression within the same tissue and on a single slide. Although the theory of this was promising, despite numerous different chromagen color combinations, the

resultant runs provided inconsistent results within the control tissues as well as within the breast carcinoma samples (**Figure 2**). Often one of the colors was masked or diminished such that accurate evaluation was limited. To remedy this, we are stepping away from the multiplex and are now running each antibody individually in serial sections of the tissues of interest. These serial sections are 4 microns in thickness and can be whole slide imaged and synchronized, so that the same tissue areas may be evaluated in tandem.

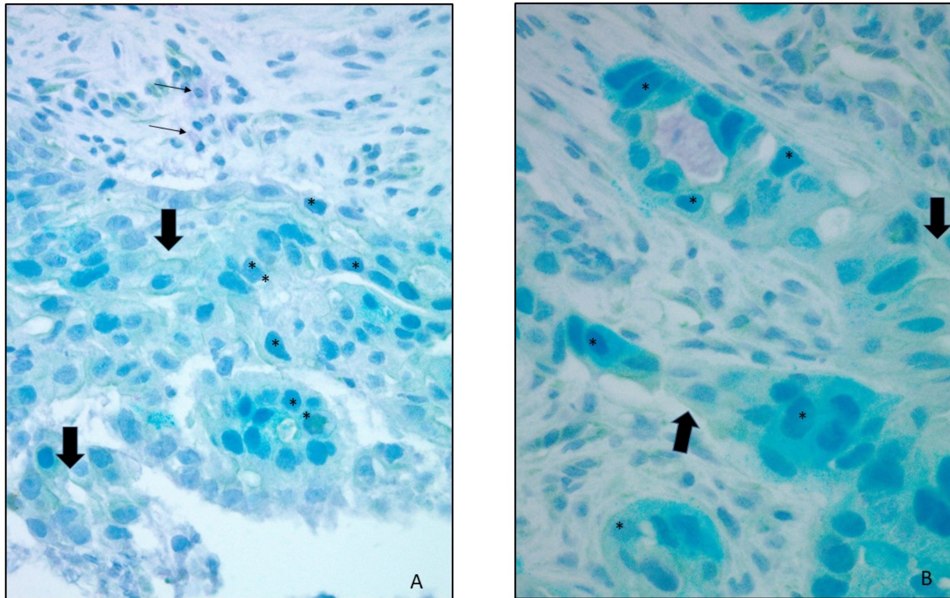

Figure 2. Composite image of multiplex immunohistochemistry with Cx26 (green chromagen), NANOG (teal chromagen) and FAK (purple chromagen). A) Example of a triple negative breast cancer with NANOG immunoreactivity (nuclear staining within neoplastic cells denoted by *) with faint barely perceptible Cx26 cytoplasmic staining and possible membranous staining (block arrows within neoplastic cells) and rare inflammatory cells with FAK expression within cytoplasm (thin arrows.). B) Different example of a triple negative breast cancer with NANOG immunoreactivity (nuclear staining within neoplastic cells denoted by *) with Cx26 cytoplasmic staining (block arrows within neoplastic cells) and no identifiable FAK expression

Specific Aim 2: Most activities have focused on this aim. We reassessed binding affinity of peptides a new surface plasmon resonance Biacore200 machine to both FAK and NANOG proteins (Major task 5, Subtask 1 and Major task 6, Subtask 1 and 2), (**Figure 3**).

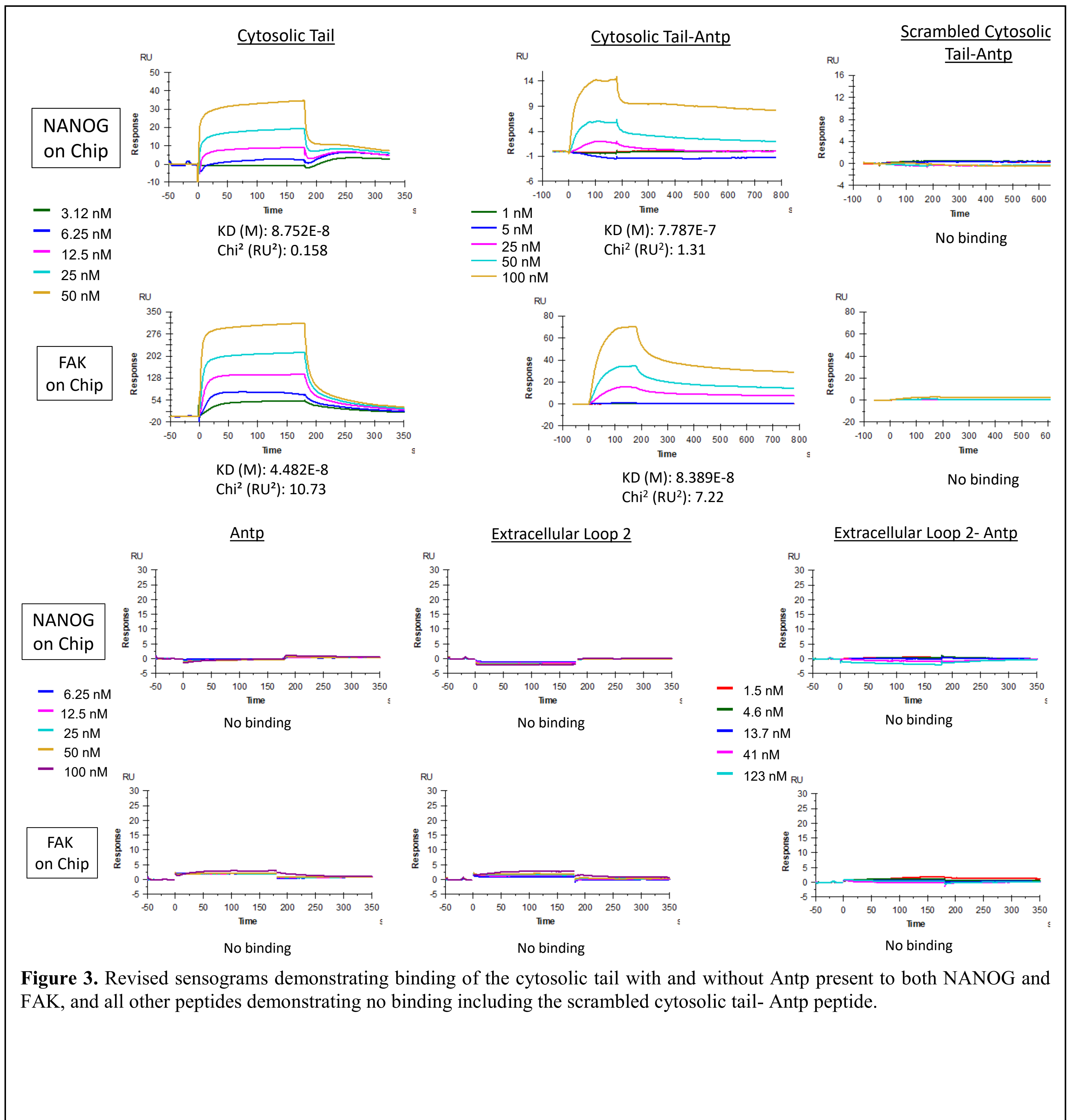

Figure 3. Revised sensograms demonstrating binding of the cytosolic tail with and without Antp present to both NANOG and FAK, and all other peptides demonstrating no binding including the scrambled cytosolic tail- Antp peptide.

Tumors harvested from vehicle, scrambled cytosolic tail-Antp and cytosolic tail-Antp treated mice were stained and scored for necrosis via H&E and Ki67, as well as Vimentin via IF staining for mesenchymal phenotype (**Figure 4**). These findings are highly significant as they point to the necrotic state of the tumor as well as loss of mesenchymal character of the tumor. First, we observed significant reduction in tumor viability in Antp-cyto tail tumors based on H&E analysis of the tumors (**Fig. 4A**). Vehicle and Scrambled Antp-cyto tail showed no difference in extent of necrosis. We analyzed the tumors for Ki67, marker of tumor proliferation, and similar to the necrosis analysis, we found reduced Ki67 staining in Antp-cyto tail treated tumors with no significant difference in Ki67 staining in vehicle or scrambled control treated tumors (**Fig 4B**).

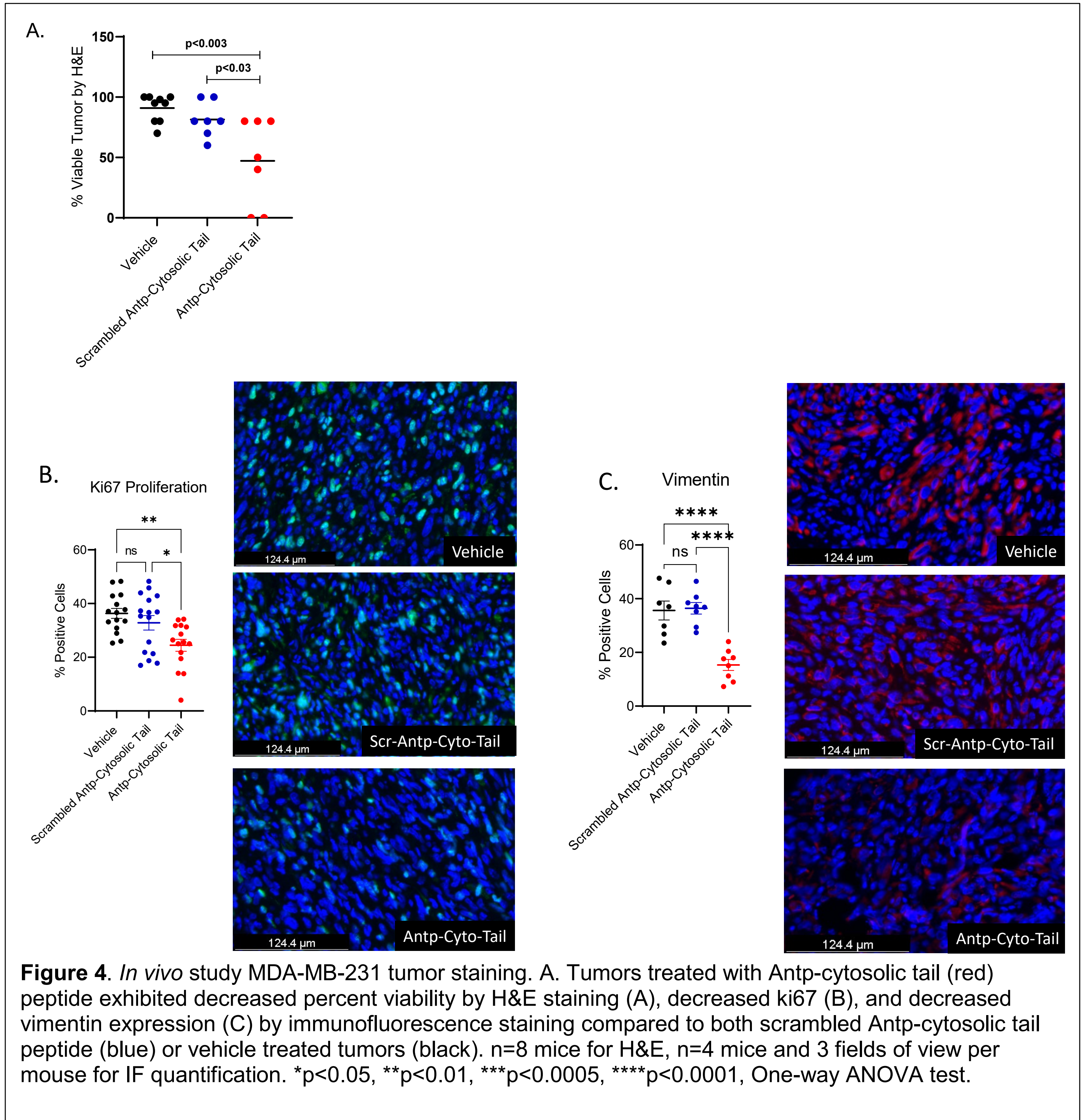

Figure 4. *In vivo* study MDA-MB-231 tumor staining. A. Tumors treated with Antp-cytosolic tail (red) peptide exhibited decreased percent viability by H&E staining (A), decreased ki67 (B), and decreased vimentin expression (C) by immunofluorescence staining compared to both scrambled Antp-cytosolic tail peptide (blue) or vehicle treated tumors (black). n=8 mice for H&E, n=4 mice and 3 fields of view per mouse for IF quantification. *p<0.05, **p<0.01, ***p<0.0005, ****p<0.0001, One-way ANOVA test.

Additionally, RNAseq was performed in Antp-cytosolic tail peptide compared to scrambled Antp-cytosolic tail peptide treated tumors (**Figure 5**). *Due to the pandemic, the RNAseq studies were delayed but sequencing is complete and we present some of the key findings from the analysis.* As such, the functional studies based on RNAseq findings are postponed. However, we performed bioinformatic analysis of the differentially expressed genes. The volcano plot and heat maps indicate that overall the changes between Antp-cytosolic tail vs Scrambled peptide were relatively small. This finding is not surprising as the most significant aspect of the tumor study was reduced tumor growth.

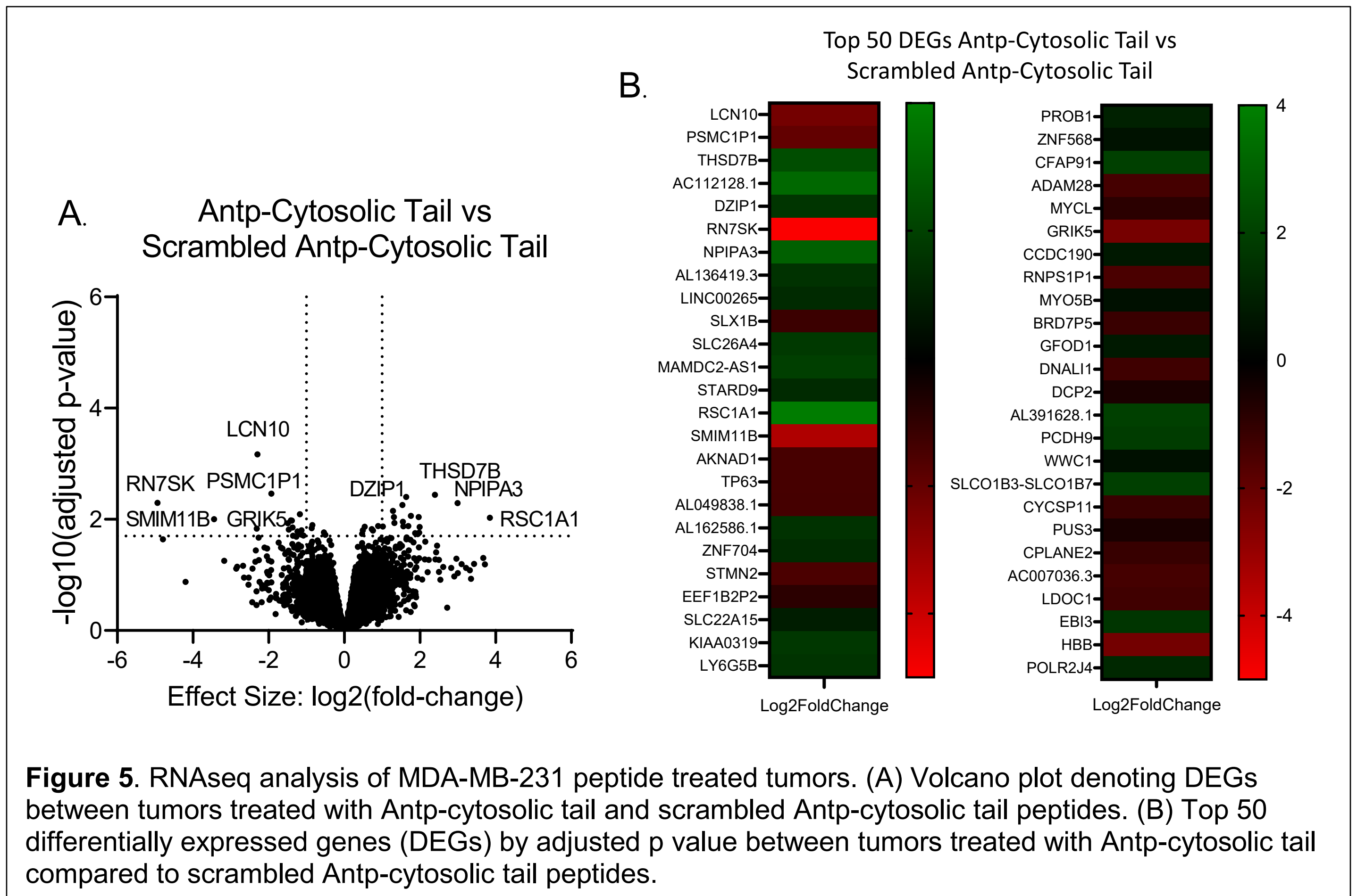

Figure 5. RNAseq analysis of MDA-MB-231 peptide treated tumors. (A) Volcano plot denoting DEGs between tumors treated with Antp-cytosolic tail and scrambled Antp-cytosolic tail peptides. (B) Top 50 differentially expressed genes (DEGs) by adjusted p value between tumors treated with Antp-cytosolic tail compared to scrambled Antp-cytosolic tail peptides.

To gain mechanistic understanding of the effects of the Antp-cytosolic tail peptide in TNBC cells, we examined whether treatment with the tail peptide affects NANOG stability. Our published observations (preliminary data in the original application) indicated that TNBC cells, which contain the Cx26/NANOG/FAK complex, also contain higher levels of NANOG protein compared to luminal breast cancer cells. Based on these data, we hypothesized that disruption of the complex via peptide treatment may destabilize NANOG, thus reducing NANOG protein levels and increasing turnover. To investigate whether this was the case, we performed a cycloheximide treatment to block novel protein synthesis and analyzed NANOG levels via immunoblot. Some of our experiments have shown a decrease in NANOG levels after Antp-cytosolic tail treatment, suggesting that disruption of the complex via peptide treatment destabilizes NANOG (**Fig. 6A**). However, other experiments have failed to show any change with peptide treatment (**Fig. 6B**). We are currently working to optimize these experiments.

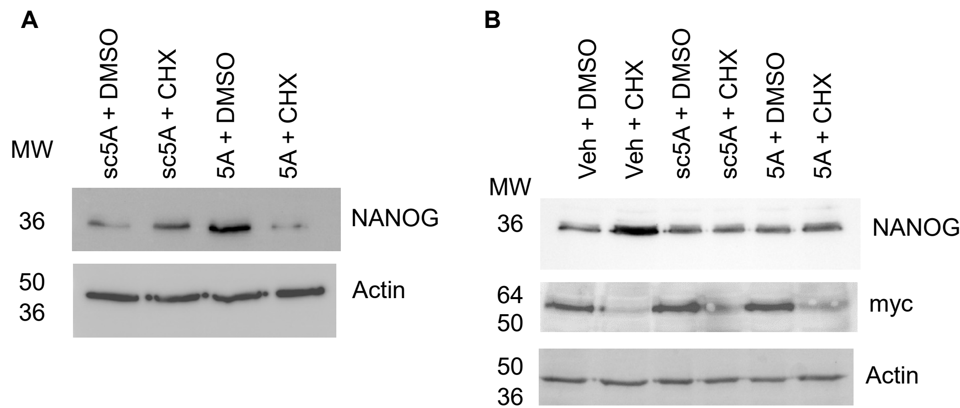

Figure 6. Stability of NANOG after treatment with antennapedia-tagged Cx26 cytosolic tail peptide. Cells were treated with vehicle (Veh), 10 µm Antp-scrambled peptide (sc5A), or 10 µm Antp-cytosolic tail (5A) for 3 days and then treated with cycloheximide (CHX) for 5 hours. Nuclei were isolated, and equal amounts of nuclear lysates were subjected to immunoblotting for NANOG, Actin as a loading control, and myc as a positive control for protein degradation. Some experiments, as in **A,** showed a decrease in NANOG protein stability after peptide treatment, whereas others, as in **B**, showed that NANOG protein stability did not change with peptide treatment.

What opportunities for training and professional development has the project provided?

Professional Development:

Emily Esakov: oral and poster presentation at the International Gap Junction Conference in Victoria Canada as well as poster presentations at the LRI Research Day

- **How were the results disseminated to communities of interest?**
    - Nothing to Report
- **What do you plan to do during the next reporting period to accomplish the goals?**
    - During the next reporting period the team involved in the project will work diligently and efficiently to accomplish the goals and objectives. We will plan experiments according to the SOW and troubleshoot with the help of experienced colleagues in the event any problems arise experimentally.

- **IMPACT:**
    - **What was the impact on the development of the principal discipline(s) of the project?**
        - *Nothing to Report*
    - **What was the impact on other disciplines?**
        - *Nothing to Report*
    - **What was the impact on technology transfer?**
        - *Nothing to Report*

- **What was the impact on society beyond science and technology?**
    - *Nothing to Report*

- **CHANGES/PROBLEMS:**
  - **Changes in approach and reasons for change**
  - **Actual or anticipated problems or delays and actions or plans to resolve them**
    - An unexpected delay occurred due to validated antibodies no longer being manufactured by Santa Cruz. We continue to screen alternate antibodies as well as made an anti-Cx26 antibody through our Hybridoma Core facility to be used in the co-immunoprecipitation studies outlined. None of these have been successful to date. As an alternative approach, the introduction of a MYC tag to Cx26 will be completed using a CRISPR/Cas9 system previously utilized in our lab. We will work with Synthego Corp. who has developed an efficient, automated platform for CRISPR editing of cell lines. This will allow for an increased editing efficiency and quality to ensure the maintenance of pluripotency for our breast cancer cell lines remains intact. We will receive the Cx26 tagged cell lines in a clonal format and utilize robust antibodies for MYC to optimize our immunoprecipitation of the Cx26/NANOG/FAK complex. This will provide us an opportunity to perform the studies outlined in Major Task 1-3 in the SOW.
    - The most significant impact is due to the Covid-19 pandemic. We experienced significant delays due to staffing required to work remote. This impacted our ability to implement some of the proposed studies for year 2. This was most apparent in the progress on immunohistochemical studies of complex formation in TNBC specimens. Staffing in pathology was impacted by the demands of the clinical service. As indicated in the report, we attempted to perform complex in FFPE sections. The lack of ability to perform the study has resulted in our decision to change our strategy and stain adjacent sections for the individual components and reconstruct digitally. This is in progress.
  - **Changes that had a significant impact on expenditures**
    - *Nothing to report*
  - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    - *Nothing to report*
  - **Significant changes in use or care of human subjects :** Nothing to Report
  - **Significant changes in use or care of vertebrate animals:** Nothing to Report
  - **Significant changes in use of biohazards and/or select agents:** Nothing to Report

- **PRODUCTS:**

- **Publications, conference papers, and presentations**
    - **Journal publications.** Nothing to Report
    - **Books or other non-periodical, one-time publications.** Nothing to Report
    - **Other publications, conference papers, and presentations.** Nothing to Report
- **Website(s) or other Internet site(s)** Nothing to Report
- **Technologies or techniques** Nothing to Report
- **Other Products** Nothing to Report

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
    - **What individuals have worked on the project?**

Name:	*Ofer Reizes, PhD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.8*
Contribution to Project:	*Dr. Reizes is project lead and meets weekly with the project team to review progress.*
Funding Support:	No Change
Name:	*Justin Lathia, PhD*
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2
Contribution to Project:	*Dr. Lathia provides insights on project related to connexins.*
Funding Support:	No change
Name:	*Emily Esakov, PhD*

Project Role:	Post-doctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Esakov works to complete all project aims through the development of research studies and data analysis.
Funding Support:	
Name:	Erin Mulkearns-Hubert
Project Role:	Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Mulkearns-Hubert gives valuable insight and helps with co-immunoprecipitation studies and data analysis.
Funding Support:	No change
Name:	Johnson
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Ms. Johnson provides support for all animal studies completed.
Funding Support:	No change
Name:	Ahuja
Project Role:	Technician

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Ms. Ahuja provides support for all bench studies
Funding Support:	No change

-

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

- **What other organizations were involved as partners?**

  - *Nothing to report*

STATEMENT OF WORK – 10/31/2018, updated 06/06/2021
PROPOSED START DATE September 1, 2019

Site 1: Cleveland Clinic
Lerner Research Institute
PI: Reizes

Specific Aim 1. Test the hypothesis that the formation of the Cx26/NANOG/FAK complex is essential for NANOG stability leading the activation of functions that promote pluripotency.	**Timeline % completed**	**Site 1**
Major Task 1 Is the Cx26/NANOG/FAK complex present in other breast cancer subtypes and across models?		
Subtask 1: Determine Cx26/NANOG/FAK protein complex via co-immunoprecipitation in cell line and PDX models (TNBC, ER+/PR+, HER2+) and control cell lines (mammary epithelial cells, fibroblasts).	1-4 20%	Drs. Reizes, Esakov, and Lathia
Subtask 2: Determine Cx26/NANOG/FAK protein complex via co-immunoprecipitation in CSC models derived from MDA-MB-231 and HCC70 cells.	2-6 10%	Drs. Reizes, Esakov, and Driscoll
Subtask 3: Generate additional CSC models in TNBC, ER+/PR+, and HER2+ cell lines for complex assessment in Subtask 2.	1-9 0%	Drs. Reizes and Esakov
Subtask 4: Assessment of NANOG mutants in complex formation and sequence of events leading to complex formation.	1-6 10%	Drs. Reizes and Esakov
Subtask 5: Assessment of interaction between Cx26, NANOG, and FAK via co-immunoprecipitation assays in primary tumor tissue with pathological characterization.	3-9 0%	Drs. Reizes, Esakov, and Downs-Kelly
Major Task 2 Does Cx26/NANOG/FAK complex disruption alter NANOG stability and function?		
Subtask 1: Test Cx26 mutants in TNBC and nonTNBC models (Table 1) on NANOG transcriptional activity via promoter reporter constructs and ChIP assays with established targets.	3-9 0%	Drs. Reizes, Driscoll, and Esakov
Subtask 2: Test FAK mutants in TNBC and nonTNBC models (Table 1) on NANOG transcriptional activity via promoter reporter constructs and ChIP assays with established targets.	9-18 0%	Drs. Reizes, Driscoll, and Esakov
Subtask 3: Test Cx26 knockdown TNBC and nonTNBC models (Table 1) on NANOG protein stability.	9-18 10%	Drs. Reizes, Driscoll, and Esakov
Subtask 4: Test bound and free NANOG activity using in vitro transcription assays.	9-18 0%	Drs. Reizes, Driscoll, and Esakov
Major Task 3 Does the Cx26/FAK/NANOG ternary complex alter response to chemotherapy?		
Subtask 1: Test conditions in Major Task 2 with Paclitaxel for Cx26/NANOG/FAK protein complex and NANOG function.	9-18 0%	Drs. Reizes, Lathia, Esakov and Mr. Braley

Subtask 2: Analyze tumors for complex disruption based on IP and NANOG stability.	12-20 0%	Lathia, Esakov and Mr. Braley
Major Task 4 Does the Cx26/FAK/NANOG ternary complex inform patient outcome?		
Subtask 1: Assess Cx26, NANOG, and FAK protein expression in test tissue microarray (128 samples) representing multiple breast cancer subtypes.	12-18 50%	Drs. Reizes, Esakov, and Downs-Kelly
Subtask 2: Assess Cx26, NANOG, and FAK protein expression in validation tissue microarray (50 samples) representing multiple breast cancer subtypes.	12-20 20%	Drs. Reizes, Esakov, and Downs-Kelly
Subtask 3: Analyze expression relative to breast cancer subtype and clinical outcome.	15-20 0%	Drs. Reizes, Esakov, and Downs-Kelly
Milestone(s) Achieved: (1) Defining the breast cancer subtype(s) in which the Cx26/NANOG/FAK protein complex is present and correlation to patient prognosis; (2) Evaluation of NANOG function with intact and disrupted Cx26/NANOG/FAK protein complex; (3) Co-author manuscript describing function of NANOG in the context of the Cx26/NANOG/FAK protein complex.		
Deliverables: This aim will elucidate the mechanism by which Cx26 regulates NANOG stability and determine the functional consequence on NANOG transcriptional activity. Furthermore, we will establish the subset of tumors containing the ternary complex for diagnostic and prognostic purposes. This deep biological and mechanistic understanding is necessary for future therapeutic development.		

Specific Aim 2. Test the hypothesis that disrupting the integrity of the Cx26/NANOG/FAK complex attenuates self-renewal and reduces tumor growth.		
Major Task 5 Do the identified Cx26 interacting domains bind NANOG and FAK in breast cancer cells?		
Subtask 1: Generate antennapedia-tagged peptides for co-immunoprecipitation in TNBC and nonTNBC cells (Table 1).	1-9 100%	Drs. Reizes, Lathia, Esakov, and Mr. Braley
Subtask 2: Validate peptide binding to NANOG and FAK in breast cancer cells, PDX models, and CSCs compared to mammary epithelial cells and fibroblasts and assess intracellular localization.	3-9 0%	Drs. Reizes, Lathia, Esakov, and Mr. Braley
Major Task 6 Does the cell-penetrating Cx26 blocking peptide bind to NANOG and FAK?	6-15	
Subtask 1: Synthesize peptides with antennapedia sequence to provide intracellular access.	6-12 100%	Drs. Reizes and Esakov
Subtask 2: Determine binding affinity of peptides to NANOG and FAK via surface plasmon resonance and isothermal calorimetry.	6-15 100%	Drs. Reizes, Lathia, Esakov, and Mr. Braley
Major Task 7 Do the cell-penetrating Cx26 blocking peptide alter CSC function in vitro?		
Subtask 1: Test ability of cell-penetrating peptide to disrupt Cx26/NANOG/FAK complex integrity as outlined in Major Task 1.	12-18 20%	Drs. Reizes, Lathia, and Esakov
Subtask 2: Test ability of cell-penetrating peptide to disrupt NANOG function as outlined in Major Task 2.	18-24 20%	Drs. Reizes, Lathia, and Esakov

Subtask 3: Test ability of cell-penetrating peptide to alter CSC marker expression, proliferation, survival, self-renewal, and migration/invasion.	21-30 90%	Drs. Reizes, Lathia, and Esakov
Major Task 8 Can the cell-penetrating Cx26 blocking peptide integrated into a gel carrier?		
Subtask 1: Formulate cell-penetrating peptide in a pluronic gel carrier (Pluronic F127, Sigma) or microcapsules in collaboration with the Gourdie laboratory (see letter).	1-6 100%	Drs. Reizes, Lathia, Gourdie, and Esakov
Subtask 2: Evaluate release dynamics of cell-penetrating peptide.	1-6 100%	Drs. Reizes, Lathia, Gourdie, and Esakov
Subtask 3: Validate function of cell-penetrating peptide on Cx26/NANOG/FAK complex as outlined Major Task 1.	7-12 10%	Drs. Reizes, Lathia, Gourdie, and Esakov
Major Task 9 Does the cell-penetrating Cx26 blocking peptide alter TNBC tumor growth in vivo?	1-36	
Subtask 1: Test ability of cell-penetrating peptide to alter tumor growth and metastatic activity in established xenografts from TNBC cell lines and PDX models. **In vivo calculation:** We have utilized references within the Guide for the Care and Use of Laboratory Animals from the National Research Council to estimate the minimal number of animals necessary to achieve statistical significance. The sample size is determined based on the following calculation: $N = 2[(u_a + u_b) s/d]^2$ where: N = group size, $u_a = 1.96$ ($p<0.05$), $u_b = 1.282$ (beta error=0.1), s = standard error, d = difference between the groups. Based on the data we obtained to date, standard error for tumor size is approximately 200 mm^3. To detect a >40% difference in tumor size, **we need 10 mice per group to achieve appropriate power for the study**. This group size will be used for all tumor growth studies. **To optimize usage of mice, we will only test 2 TNBC cell sources (1 cell line and PDX line) for in vivo studies before moving on to other breast cancer subtypes (ex. PR+/ER+ and HER2+ as outlined in Major Task 10).** Tumor growth: 2 TNBC cell sources x 6 experimental conditions x 10 mice per group= 120 mice Metastatic activity: 2 TNBC cell sources x 6 experimental conditions x 10 mice per group= 120 mice	1-12 50%	Drs. Reizes, Lathia, and Esakov
Subtask 2: Test ability of cell-penetrating peptide in combination with Paclitaxel to alter tumor growth in established TNBC xenografts from cell lines and PDX models. We will perturb established tumors using 2 TNBC cell sources with 4 cell-penetrating peptide complex conditions alone and in combination with Paclitaxel. 2 TNBC sources x 4 complex conditions x 2 paclitaxel concentrations x 10 mice per group = 160 mice **Total: 400 mice for Task**	24-36 0%	Drs. Reizes, Lathia, and Esakov

Table 1. Cx26 c-terminal cytoplasmic peptides
Cytosolic tail
Cytosolic tail-Antp
Antp-

Scrambled Cytosolic tail	
Mutant peptides:	
Peptide name	Peptide Sequence
xR216	XYCSGKSKKPV
xV226	RYCSGKSKKPX
xRY	XXCSGKSKKPV
xPV	RYCSGKSKKXX
216-220	RYCSGXXXXXX
221-226	XXXXXKSKKPV
A216-220	RYCSGAAAAAA
A221-226	AAAAAKSKKPV