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TITLE: Functionally Characterizing the Noncoding Genome of Metastatic Prostate Cancer to Identify Therapeutic Vulnerabilities

PRINCIPAL INVESTIGATOR: Matthew Freedman

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Prostate cancer (PCa) is dependent on the androgen receptor (AR) at all stages of the disease. Standard of care treatment for advanced PCa focuses on inhibition of androgen signaling. Despite initial efficacy, resistance inevitably occurs. Notably, this state, termed metastatic castration resistant prostate cancer (mCRPC), remains dependent on AR signaling. Our groups have identified a set of 17,000 gained AR binding sites that characterize mCRPC. Our proposal aims to functionally characterize these important sites through a variety of approaches. For the ease of reading, we have placed bars in the margins to identify the new additions/updates to the report.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Prostate cancer (PCa) is dependent on the androgen receptor (AR) at all stages of the disease. Standard of care treatment for advanced PCa focuses on inhibition of androgen signaling. Despite initial efficacy, resistance inevitably occurs. Notably, this state, termed metastatic castration resistant prostate cancer (mCRPC), remains dependent on AR signaling. Our groups have identified a set of 17,000 gained AR binding sites that characterize mCRPC. Our proposal aims to functionally characterize these important sites through a variety of approaches. **For the ease of reading, we have placed bars in the margins to identify the new additions/updates to the report.**

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Metastatic castration resistant prostate cancer (mCRPC), cistrome, androgen receptor binding sites (ARBS), STARR-seq, epigenomics, CRISPR, HiChIP

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goals of the proposal are as follows:

Specific Aim 1: Characterize the AR cistrome to reveal functionally active regulatory elements. This major tasks underlying this aim included STARR-seq. The milestones for STARR-seq were estimated to be completed in Year 1.

- The STARR-seq work is 90% completed. We have generated suitable STARRseq libraries and tested all gained mARBS sites for enhancer activity using a genome-wide STARRseq in our proposed model of primary PCa and mCRPC.
- The HiChIP work is 75% completed.

Specific Aim 2: Functionally test gained metastatic AR enhancers to drive castration resistance and metastatic outgrowth

The major tasks in this aim are a CRISPR dropout screen (to be completed within two years) and to perform in vivo validation of top-scoring enhancers (to be completed within four months after the initial screen).

Specific Aim 3: Identify somatic mutation that alter AR enhancer activity and characterize their function. The major tasks in this aim are to generate an AR mutated library and to validate hits. The milestones for this work are projected to be completed in years 2-3 of the proposal.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct

description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific aim 1: Test universe of 17,655 gained metastatic AR binding sites using STARR-seq
Subtask 1: Design Agilent DNA capture array, capture pooled DNA
Status: completed

- **Detailed description:** Due to recent technical innovations we chose to expand the scope of the experiments and implemented a genome-wide STARRseq library. This unpublished library has excellent ARBS coverage (>35x) and provides unbiased dataset that can be adopted to interrogate AR enhancer activity. Importantly, when compared to the smaller capture-based STARRseq activity we observed excellent correlation of AR enhancer activity (Figure 1)

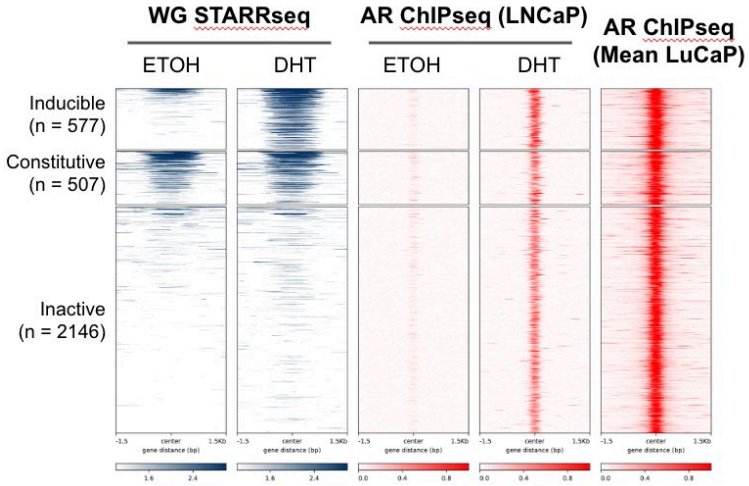


Figure 1: Comparison of primary PCa ARBS from whole-genomc STARRseq to capture-based annotations.

Subtask 2: Clone into STARR-seq plasmid
Status: completed

- **Detailed description:** Cloning was completed in the whole genome library preparation (see Subtask 1)

Subtask 2: Test for AR-driven enhancer activity in cell lines and analyze data
Status: Ongoing

- **Detailed description** With this library we have tested all gained metastatic AR binding sites (ARBS) for enhancer activity in our proposed model of primary PCa (LNCaP). In line with our hypothesis, we observed minimal AR-driven enhancer activity in these gained sites (Figure 2). Of the >17,000 gained ARBS tested only 473 (~2%) had AR-driven enhancer activity.

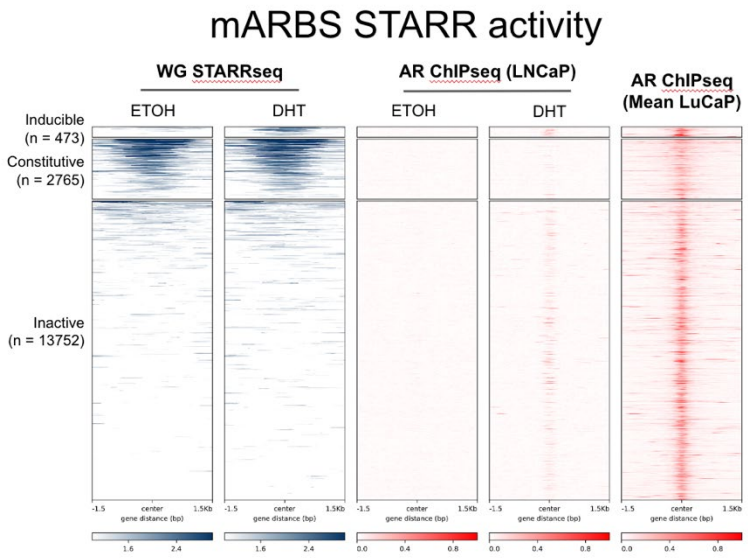


Figure 2: AR enhancer activity across all gained mARBS in a model of primary PCa (LNCaP).

Status update subtask 2:Test for AR-driven enhancer activity in mCRPC cell lines and analyze data

- There are only a handful of few AR+ PCa cell lines and most do not phenocopy the metastatic AR binding sites (mARBS) observed in clinical samples. This has led to alternative preclinical models that better phenocopy mCRPC including LuCaP patient derived xenografts (PDX). These PDXs have been extensively used to investigate mCRPC progression as they better match the epigenetic alterations and AR cistrome. However, while these PDXs better model clinical mCRPC they are both very expensive and challenging to genetically manipulate. To overcome this, several AR+ LuCaP mCRPC PDX models were recently adopted to grow as *in vitro* cell lines (unpublished). In preliminary studies we demonstrated that expression and AR/FOXA1/H3K27Ac binding of the cell line closely phenocopy the mCRPC cistrome (Figure 6). Given the strong correlation to the clinical cistrome we chose to use these cell lines to identify active mCPRC enhancers.
- Working with several of these LuCaP cell lines (n=4) we extensively optimized electroporation conditions to identify the conditions required for STARRseq a very large-scale experiment. From the different models we chose to use LuCap35CR for all subsequent work as they closely mimic the mCRPC cistrome, have demonstrated AR regulated transcription (Figure 3) and can be electroporated with >75% efficiency. Further we demonstrated that DNA transfection does not induce IFN-gamma response in these LuCaP 35CR cells which is a potential confounding response that can introduce bias in STARRseq (Figure 4). After optimization of transfection conditions we tested all AR binding sites (ARBS) for enhancer activity in LuCaP 35CR using a genome-wide STARRseq library (>2x10^8 cells/replica; three biological replicas). These experiments were completed (Table 1) and STARRseq libraries have been submitted for next generation sequencing. The resulting data will be processed with our previously optimized bioinformatic pipeline.

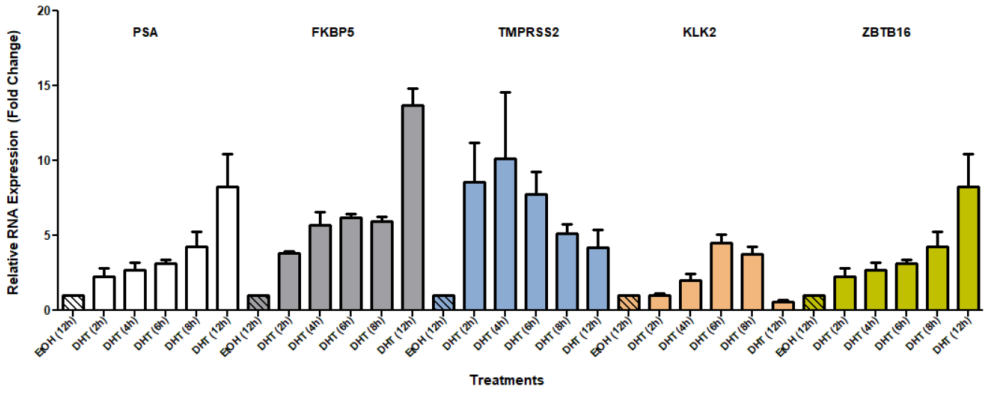


Figure 3: Quantification of canonical AR regulated genes in LuCaP 35CR following treatment with 10 nM DHT (2, 4, 6, 8, 12 hrs).

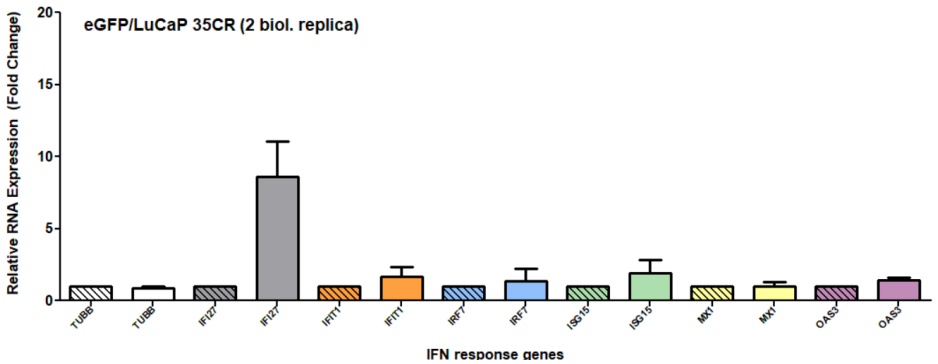


Figure 4: Quantification of IFN-gamma response genes in LuCaP 35CR following DNA electroporation

Table 1: Whole genome STARRseq in LuCaP

Biol. Replica #	Treatment Conditions (48h/48h/ 6hr)	Total Cell # electro. /Replica	Voltage	P.W (ms).	Pulse #	OGWG (Reddy) Input Lib DNA : Cell#	Cell viability after electroporation	# of Cells harvested
Rep.1 #1	10% CSS 100% EtOH	~500m	1325V	11	4	5:1	~73%	~115m ~89% viability
Rep.1 #2	10% CSS 10nM DHT	~500m	1325V	11	4	5:1	~73%	~120m ~94% viability
Rep.2 #3	10% CSS 100% EtOH	~500m	1325V	11	4	5:1	~72%	~181m ~91% viability
Rep.2 #4	10% CSS 10nM DHT	~500m	1325V	11	4	5:1	~72%	~188m ~92% viability
Rep.3 #5	10% CSS 100% EtOH	~500m	1325V	11	4	5:1	~75%	~114m ~89% viability
Rep.3 #6	10% CSS 10nM DHT	~500m	1325V	11	4	5:1	~75%	~112m ~86% viability

Specific aim 2,

Subtask 1: design guide RNAs (gRNAs) across 17,655 enhancers
Status: completed

- **Detailed description**: The design of optimal CRISPR guide RNAs is a yield of continuous development. To maximize on-target efficiency and limit off-target effects of the sgRNAs, we made use of a newly developed tools to enhance CRISPR efficiency (PMID: 34050182) and decrease off-target effects (PMID: 30367669). Based on these criteria, we designed optimized sgRNAs targeting each of the 17655 metastasis-specific ARBS, with 4 sgRNAs for each site. In addition, 600 controls were designed 10kb upstream (n=300) or downstream (n=300) of the ARBS, as well as 100 non-targeting controls from the Brunello library. As positive controls, promoter-targeting sgRNAs were included targeting essential genes (PLK1, AR, FOXA1, HOXB13). CRISPRi performance was assessed by targeting the enhancer of NKX3.1, after which cut&tag for H3K9ac and H3K27ac was performed along with ATAC-seq to determine chromatin accessibility (see Figure 5). Clear increase of H3K9me3 signal was observed at the targeted enhancer element, with a decreased H3K27ac signal both at the KNX3.1 enhancer and promoter regions. Based on this, we conclude that the CRISPRi targeting was effective, without regional suppression or indirect promoter targeting.

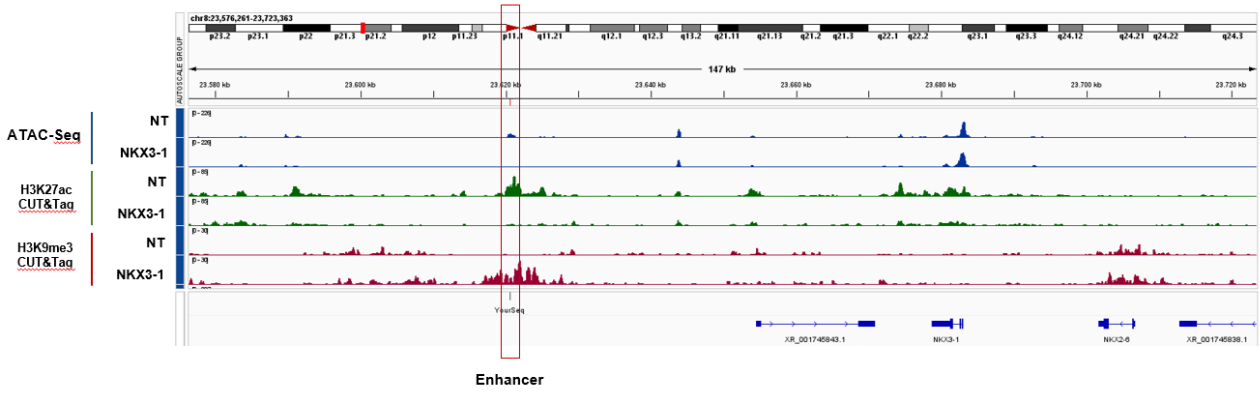


Figure 5: perturbation of the NKX3.1 enhancer by CRISPRi.

Subtask 2: Perform H3K27 ChIP seq on two hormone-sensitive (LNCaP, LAPC4) and three hormone-resistant cell lines (LNCaP-abl, 22Rv1, and VCaP)

Status: completed

Detailed description: Using wet lab and computational pipelines optimized by our teams (PMID: 32690948) to perform high-quality ChIP-seq analyses, H3K27ac ChIP-seq was successfully performed on two hormone-sensitive (LNCaP, LAPC4) and three hormone-resistant cell lines (LNCaP-abl, 22Rv1, and VCaP). For each cell line and each ChIP-seq condition, 3 biological replicates were generated, to facilitate statistical analyses downstream.

Status update subtask 2: When modelling castration resistance in prostate cancer, we're mostly dependent on hormone-sensitive cell lines that were rendered castration resistance, through long-term depletion of hormones, in vitro. In order to perform our CRISPRi screen in the clinically most-relevant setting, we decided to include a panel of newly generated cell lines, the 'LUCaP' cell lines, that were generated from mCRPC PDX derived tissue samples, in which the initial 17.655 mARBS's were identified.On these cell lines, we performed ChIP-seq for AR, FOXA1, H3K27ac and ATAC-seq. (Fig 6). Importantly, the mARBS were clearly represented in the LuCAP-derived cell line models, rendering these relevant model systems to perform our CRISPRi screen in.

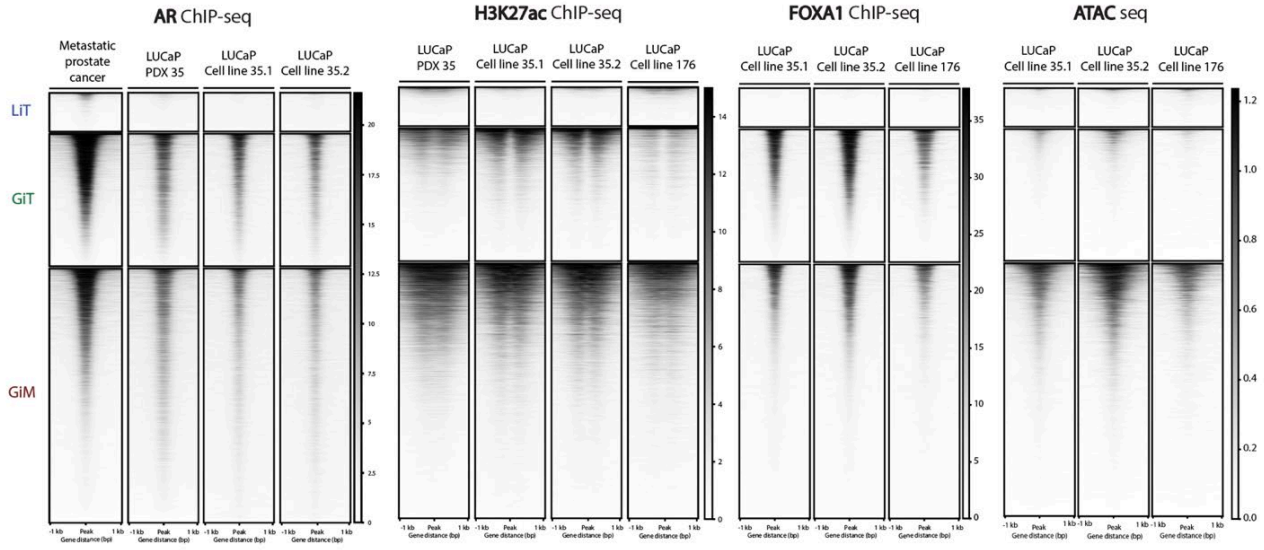


Figure 6: Tornado plots visualizing AR, H3K27ac and FOXA1 ChIP-seq signal and ATAC-seq signal in different subset's of ARBS (LiT, GiT and GiM's) in fragments per kilobase per million reads mapped in metastatic prostate cancer epithelium, LUCaP 35 PDX tissue and the different LUCaP cell lines. Data are centered at AR peaks depicting a 1-kb window around the peak center for three biological individual replicates.

Subtask 3: Conduct pooled CRISPR/Cas9 screen targeting mARBS.

Status: ongoing

Detailed description: The CRISPR library has been designed, generated and cloned as a pool. After QC-sequencing of the library over the next week, virus generation will be performed.

In parallel, challenges have been addressed in relation to the most-suitable CRISPRi system. We initially focused our attention to a Cas9 construct, which contained a tandem repeat of 10x KRAB-binding elements ("Sun-tag" CRISPRi system, Figure 7), to ensure strong enhancer suppression. While the system was clearly potent and strongly suppressed enhancer action (Figure 5), cellular fitness was clearly negatively affected in stably transduced lines. Therefore, two alternative CRISPRi systems were explored: CRISPRi-mCherry and CRISPRi-BFP, both containing one single KRAB, as a fusion to the Cas9 protein (Figure 6). All three constructs were capable to target and suppress the accessibility of the NKX3.1 enhancer (as measured by ATAC-seq ;Figure 8A,B) and to decreased

NKX3.1 expression of mRNA level (Figure 8C), with the BFP-CRISPRi construct performing best. Notably, no negative effect on cellular fitness was observed for the BFP-CRISPRi transduced cells, in contrast to the "Sun-tag" CRISPRi system. Subsequently, BFP-CRISPRi virus was generated and transduced in castration resistant cell lines, FACS-sorted on BFP (Figure 8D).

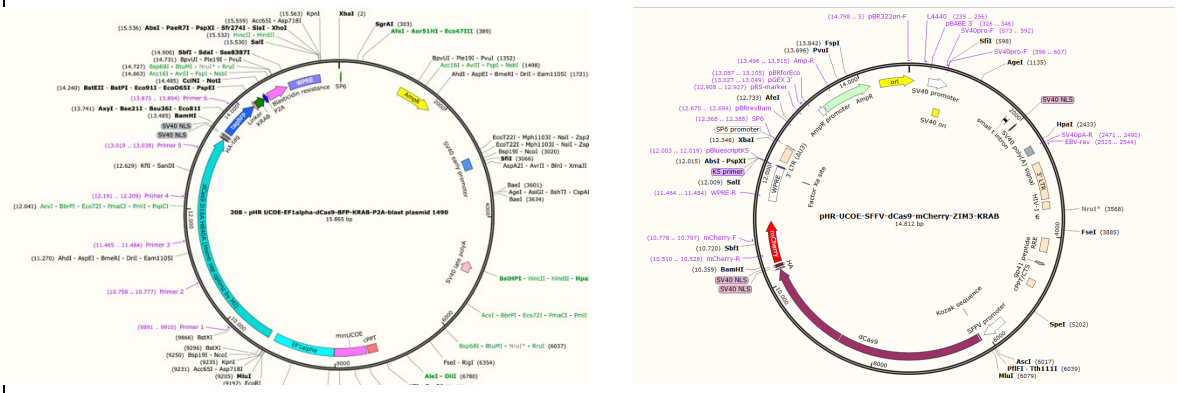


Figure 7: Vector maps of the BFP (left) and mChery (right) expression CRISPRi constructs

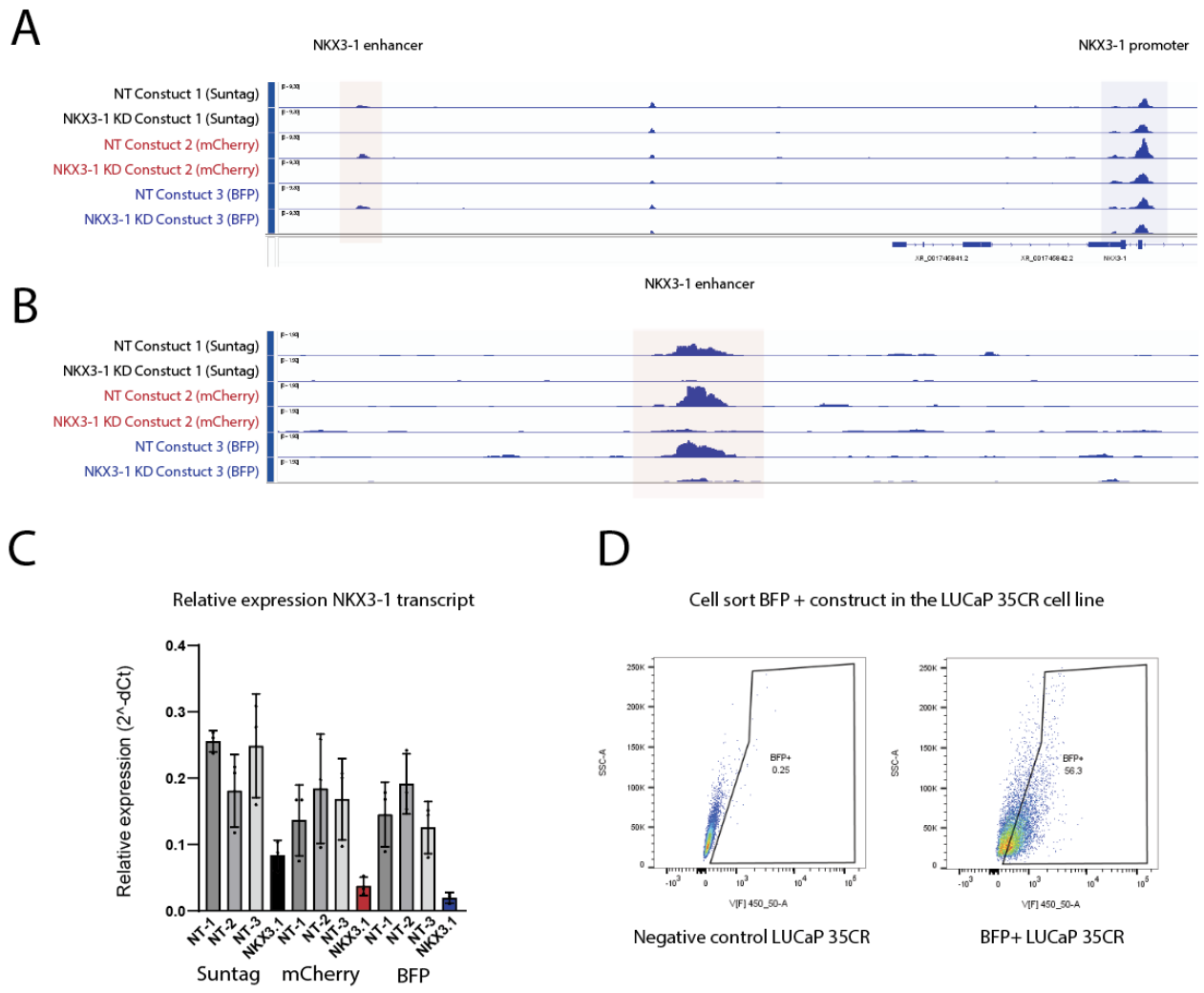


Figure 8: CRISPRi performance and cell line optimalization

A. ATAC-seq in LNCaP cells, transduced with three different CRISPRi constructs (Suntag, with tandem repeat of 10 KRAB-binding domains, and two single KRAB-Cas9 fusions). Depicted are control versus NKX3-1 enhancer sgRNA.
B. Zoom in on the KNX3.1 enhancer region. All three effectively blocking accessibility of the NKX3.1 enhancer regions, as shown by ATAC-seq
C. RT-QPCR analyses on NKX3.1 mRNA, depicting knockdown of NKX3.1 expression for all three constructs, but with BFP ('B') construct performing best.
D. Cell line sorting for the BFP-CRISPRi construct in the castration resistant LuCaP35CR model.

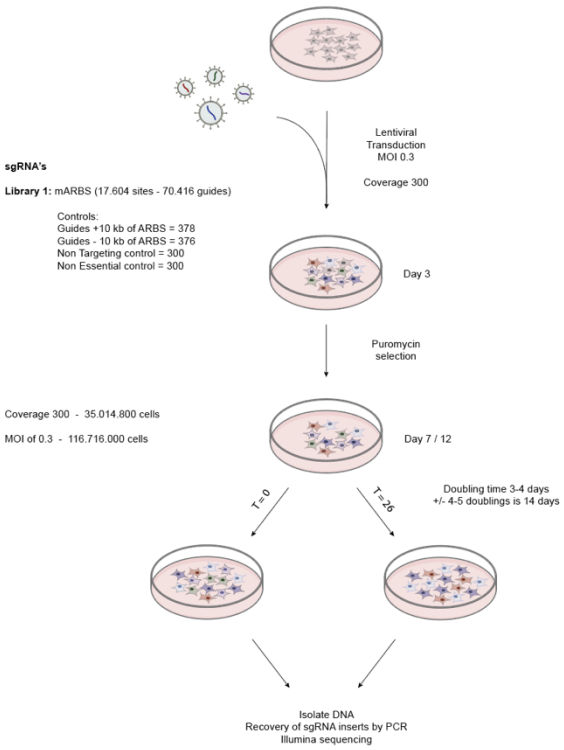


Figure 9: overview of CRISPRi screen.

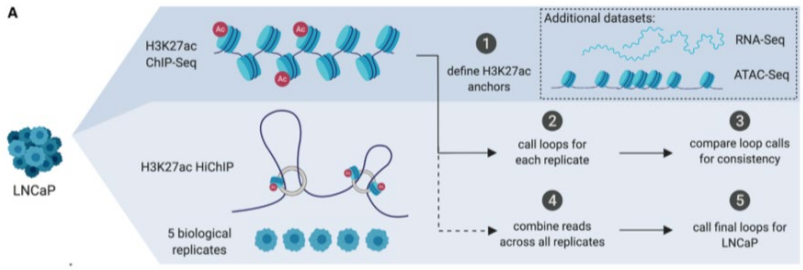
With both the cell lines, as well as the complex CRISPRi library targeting all m-ARBS, being generated and extensively tested for performance, we are now set to perform the CRISPRi-targeting screen in the most-optimal manner. For this, we will lentivirally transduce castration resistant cells (MOI: 0.3, coverage 300x), select for puromycin for the sgRNA-expressing cells, and test cells for proliferation capacity in absence of hormone (for flowchart, see Figure 9).

Major Task 2: Perform HiChIP

Status: Ongoing

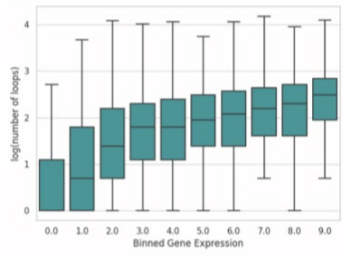
Subtasks 1 and 2: Perform chromosome conformation capture and ChIP
Detailed description: We completed H3K27ac and H3K4me3 HiChIP. AR HiChIP is underway and we aim to complete it in the next reporting period (SA1/MT2). The HiChIP assay is labor-intensive requiring a significant amount of time. We were delayed in the AR HiChIP due to COVID-related restrictions at our institution.

- Briefly, for each HiChIP experiment, we cross-linked 10 million cells and digested with the MboI restriction enzyme. Biotinylated dATP is incorporated into the digested sample and then ligated. Immunoprecipitation is then performed with the appropriate antibody (H3K27ac, H3K4me3, and AR). Samples are decrosslinked, pulled down with streptavidin beads and treated with transposase to create libraries. These libraries are then sent for sequencing.
- We have performed extensive quality control analyses for H3K27ac HiChIP. Five biological replicates were performed. Please note that these analyses were performed ahead of schedule.
-



Overview of H3K27ac HiChIP experiment and analysis workflow

- We identified a total 126,280 loops using an FDR < 0.01 using FitHiChIP.
- Gene connectivity, defined as the number of loops per gene promoter is moderately correlated with gene expression activity as measured by RNA-seq (ρ=0.49; p value < 2.2e-16).



Gene connectivity and gene expression in LNCaP. We took the union of 17,690 genes with looping counts in LNCaP and 20,114 genes with expression counts in LNCaP, dropping all genes that do not have both looping and expression information. We binned the remaining 13,274 genes into deciles (1,327 genes per decile) **a.** X-axis is the binned gene expression (FPKM) of the LNCaP genes; Y-axis represents the number of loops.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

- In Dr. Freedman's group, the project has provided the opportunity to increase training for Dr. Ji-Heui Seo, a research scientist in the laboratory. She has supervised and mentored Mr. Matthew Davidsohn and Ms. Gita Lakshminarayanan, two research technicians in the laboratory for the HiChIP and the ChIP-seq work.

- In Dr. Lack's group (UBC, Canada) the project supported training opportunities for Dr. Flora Huang, a research scientist. During this time Flora supervised a full-time undergraduate student (Mohammed Al-Musawi).

- In dr. Zwart group (NKI, Amsterdam, the Netherlands), Emma Minnee is appointed as PhD student, spearheading the experiments and analyses thereof. Ms Minnee had the opportunity to participate in, and present posters on, numerous conferences and seminar series, including the EMBO Nuclear Receptor meeting 2022, Gordon Research Conference Hormone-dependent Cancers 2023, Dutch Endocrine Meeting 2021, and the Dublin Hormone Receptor meeting 2022. On-site organized and online courses on computational biology and R programming have enabled ms Minnee to further develop her drylab capacities. Finally, Ms. Minnee developed her mentoring and educational skills, by supervising MSc student Audrey Lacoste in the lab.

Personal development:
Flora Huang (UBC, Canada)
Seminars/Meetings;
- Participating/presenting in group meetings (every week)
  - One-on-one mentoring session with Dr. Lack (every week)
  - Participating/presenting in Journal Clubs (every 2nd week)
  - Attending Vancouver Prostate Centre International Seminars (every week)

Ji-Heui Seo (DFCI, USA)
Promotion to Research Scientist

Ms. Gita Lakshminarayanan (DFCI, USA)
Matriculation to MD/PhD program

Mr. Matthew Davidsohn (DFCI, USA)
Matriculation to MD program

Emma Minnee (NKI, Amsterdam):

Board memberships;
Feb. 2023-now PhD student Council member NKI
May 2023-now NKI translational Research Board member

Conferences;

Nov. 2021 *Oncode* - CGC Annual Conference: Basic biology meets Cancer research, Amsterdam

Nov. 2021	28th Annual Prostate Cancer Foundation (PCF) Scientific Retreat, online
Nov. 2021	13th Nuclear Receptor Network Meeting (NRRN), Leiden
July. 2022	*Oncode* - CGC Annual Conference: Basic biology meets Cancer research, Amersfoort
Sep. 2022	EMBO Nuclear Receptors, Malta (*poster pres.)*
Okt. 2022	Dublin Steroid Cancer Conference, Dublin (*poster pres.)*
Nov. 2022	*Oncode* - CGC Annual Conference: Basic biology meets Cancer research, Amsterdam
Nov. 2022	Nuclear Receptor Research Network annual scientific meeting, Utrecht (*poster pres.*)
Feb. 2023	Dutch Endocrine Society Meeting, Noordwijderhout (*poster pres.*)

Courses/Masterclasses;

Jan. 2022	Oncode Masterclass "Gene editing and its application 2.0"
Jun. 2022	FlowJo course (NKI)
Jul. 2022	Introduction to R for Data Analysis course (NKI, 5 days)

Seminars/Meetings;

March. 2022	Lunch meeting with the international speaker: Luca Magnani – '*Tumor dormancy and cancer evolution: a breast cancer perspective.'*
Nov. 2022	Lunch meeting with the international speaker: Jason Carroll – '*The role of nuclear receptors and pioneer factors in cancer.'*
March. 2023	Lunch meeting with the international speaker: Mathieu Lupien – '*Chromatin variants as genetic determinants of prostate cancer development.'*

- Participating/presenting in group meetings (every week)
  - One-on-one mentoring session with dr. Zwart (every week)
  - Participating/presenting in Journal Clubs (every 2nd week)
  - Attending NKI International Seminars (every week)
  - Attending NKI Research Club (twice a week)

Supervision;

Feb. – Jun. 2023	Audrey Lacoste, Master Student *Development of Drugs and Health Products,* University of Paris-Seclay in France.
Apr. – May 2023	Maxime Steinmetz, Master Student *Biomedical Sciences*, University of Amsterdam

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these

project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The Zwart lab participates in a Patient Engagement Program, involving patients (including prostate cancer patient) in our group meetings, and interactions with researchers. In these events, the project and its implications have been extensively discussed.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- We will complete the AR HiChIP experiment in the LNCaP cell line
- Bioinformatic analyses will be performed to systematically identify enhancer-promoter interactions at our set of 17,655 metastatic AR binding sites that we previously identified.
- The plans have remained unchanged as the project is still on-track.
- Completed analysis of STARRseq from mARBS for enhancer activity in models of mCRPC
- Integrate publicly available whole-genome PCa sequencing with STARRseq/HiChIP to stratify potentially critical non-coding variant
- Perform HiChIP on 35CR LuCaP cell line
- In the next, reporting period, we aim to have finalized the enhancer CRISPR screen, and identified those AR bound enhancers that are essential for tumor cell proliferation in the mCRPC setting. We aim to have hits validated independently and tested multiple cell line models of mCRPC.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

- The androgen receptor (AR) plays a critical role in prostate cancer initiation and progression. The AR is a protein called a transcription factor that works by turning on and off sets of genes. Our groups have defined the set of AR binding sites across the human genome in normal prostate tissue, localized prostate tumors, and metastatic prostate tumors. We identified a unique set of 17,000 AR binding sites in the metastatic state. This proposal seeks to functionally characterize these sites. A greater understanding of these sites and what programs they enact will set a foundation for diagnosis and treatment of this disease.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

- The algorithms and computational tools generated in this project will be rendered publicly available, to benefit researchers of other disciplines
- We anticipate this project to serve as blueprint for other researchers and may inspire others, when searching for the impact of non-coding mutations in cancer.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

- Note that the pandemic affected (and still affects) our Institutional policies including how many people can be at the bench at one time. Therefore, we are operating at ~65% capacity. As can be seen by the data generated, we have done our best to overcome this limitation and will continue to generate and analyze data while adhering to Institute policy.
- The initially preferred CRISPRi system worked, but appreaed to negatively impact the fitness of our cells, hampering the screen. Therefore, alternative CRISPRi systems have been tested, selected, optimized and transduced. This delayed the onset of the actual screen, but greatly improved the suitability of the model system, in which the screen will be performed.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  1. Qiu X, Boufaied N, Hallal T, Feit A, de Polo A, Luoma AM, Alahmadi W, Larocque J, Zadra G, Xie Y, Gu S, Tang Q, Zhang Y, Syamala S, Seo JH, Bell C, O'Connor E, Liu Y, Schaeffer EM, Jeffrey Karnes R, Weinmann S, Davicioni E, Morrissey C, Cejas P, Ellis L, Loda M, Wucherpfennig KW, Pomerantz MM, Spratt DE, Corey E, Freedman ML, Shirley Liu X, Brown M, Long HW, Labbé DP. MYC drives aggressive prostate cancer by disrupting transcriptional pause release at androgen receptor targets. Nat Commun. 2022 May 13;13(1):2559. doi: 10.1038/s41467-022-30257-z. PMID: 35562350; PMCID: PMC9106722.; yes

  2. Giambartolomei C, Seo JH, Schwarz T, Freund MK, Johnson RD, Spisak S, Baca SC, Gusev A, Mancuso N, Pasaniuc B, Freedman ML. H3K27ac HiChIP in prostate cell lines identifies risk genes for prostate cancer susceptibility. Am J Hum Genet. 2021 Dec 2;108(12):2284-2300. doi: 10.1016/j.ajhg.2021.11.007. Epub 2021 Nov 24. PMID: 34822763; PMCID: PMC8715276.; yes

  3. Kneppers J, Severson TM, Siefert JC, Schol P, Joosten SEP, Yu IPL, Huang CF, Morova T, Altıntaş UB, Giambartolomei C, Seo JH, Baca SC, Carneiro I, Emberly E, Pasaniuc B, Jerónimo C, Henrique R, Freedman ML, Wessels LFA, Lack NA, Bergman AM, Zwart W. Extensive androgen receptor enhancer heterogeneity in primary prostate cancers underlies transcriptional diversity and metastatic potential. Nat Commun. 2022 Nov 30;13(1):7367. doi: 10.1038/s41467-022-35135-2. PMID: 36450752; PMCID: PMC9712620.; yes

- **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None

- **Other publications, conference papers and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

None

publications already specified above in this section.

None

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

<u>Example:</u>

Name: *Mary Smith*

Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Matthew Freedman
Project role:	Principal Investigator
Researcher Identifier (ORCID ID)	0000-0002-0151-1238
Nearest person month worked:	1
Contribution to the Project:	Overall PI, responsible for project direction and lab supervision
Name:	Nathan Lack
Project role:	Sub Site PI
Researcher Identifier (ORCID ID)	0000-0001-7399-5844
Nearest person month worked:	2.4
Contribution to the Project:	Resposible for STARRseq and TOTEM mutagenesis
Name:	Wilbert Zwart
Project role:	Sub Site PI
Researcher Identifier (ORCID ID)	0000-0002-9823-7289
Nearest person month worked:	1.2
Contribution to the Project:	Responsible for ACURO approval, and experiments related to CRISPR-Cas9
Name:	Emma Minnee
Project role:	Graduate Student
Researcher Identifier (ORCID ID)	**0000-0003-1012-4800**
Nearest person month worked:	12
Contribution to the Project:	Ms Minnee is performing experiments on CRISPRi perturbations in prostate cancer cells, optimizes cell lines, designing the CRISPRi library, performs the screen and analyses the hits
Name:	Ji-Heui Seo
Project role:	Research Scientist
Nearest person month worked:	1.1
Contribution to the Project:	Dr. Seo designed, performed, and analyzed all of the HiChIP experiments
Name:	Sandor Spisak
Project role:	Instructor
Nearest person month worked:	1.8
Contribution to the Project:	Dr. Spisak assisted with experiments and data reporting.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Freedman:
R01CA204954 ended 3/31/21
W81XWH21-1-0339 awarded 8/15/21
Movember PCF Challenge Award funded 10/1/21
Lack:
Prostate Cancer Canada ended 3/31/22
Turkish Scientific and Technological Research Council ended 8/31/21
Turkish Scientific and Technological Research Council awarded 10/1/21
Zwart:
N/A

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

University of British Columbia – work is funded through a subagreement to support Nathan Lack's research

Netherlands Cancer Institute – work is funded through a subagreement to support Wilbert Zwart's research

These collaborations were included in the original application and responsibilities were outlined in the SOW.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

N/A

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

None