AWARD NUMBER: W81XWH-19-1-0565

TITLE: Defining and Functionally Characterizing the Epigenome in Lethal Prostate Cancer

PRINCIPAL INVESTIGATOR: Mathew Freedman, MD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute

REPORT DATE: October 2020

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2020	ANNUAL	09/30/19–09/29/20

4. TITLE AND SUBTITLE
Defining and Functionally Characterizing the Epigenome in Lethal Prostate Cancer

5a. CONTRACT NUMBER
W81XWH–19–1–0565

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mathew Freedman, MD

E-Mail: MFREEDMAN@PARTNERS.ORG

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dana-Farber Cancer Institute
450 Brookline Ave
Boston, MA 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

Prostate cancer (PCa) is dependent on the androgen receptor (AR) at all stages of the disease. The centrality of the clinical role of this hormone-driven transcription factor (TF) in PCa renders it an ideal tumor type in which to study epigenetics. Using chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) in human radical prostatectomy (RP) specimens, we charted the AR cistrome – the universe of all AR binding sites in the genome. We observed that the AR cistrome undergoes significant alterations during the transition from localized to metastatic disease that are strikingly consistent across patients. This finding underlies our hypothesis that aberrant epigenetic signaling helps drive prostate PCa progression and provides the foundation for a deeper interrogation into the PCa epigenome across disease states in vivo. As part of this project, we have begun to define the genome-wide landscape of active enhancers and open across PCa states. The contents of these maps, in turn, guide screens that will identify regulatory elements associated with treatment resistance and key proteins binding to clinically relevant enhancers. Characterizing changes in the epigenome and its associated transcriptional programs will identify new therapeutic targets as well as biomarkers for therapy response and patient prognostication.

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	19	USAMRMC

TABLE OF CONTENTS

Page

REPORT OUTLINE

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:**

Prostate cancer (PCa) is dependent on the androgen receptor (AR) at all stages of the disease. The centrality of the clinical role of this hormone-driven transcription factor (TF) in PCa renders it an ideal tumor type in which to study epigenetics. Using chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) in human radical prostatectomy (RP) specimens, we charted the AR cistrome – the universe of all AR binding sites in the genome. We observed that the AR cistrome undergoes significant alterations during the transition from localized to metastatic disease that are strikingly consistent across patients. This finding underlies our hypothesis that aberrant epigenetic signaling helps drive prostate PCa progression and provides the foundation for a deeper interrogation into the PCa epigenome across disease states in vivo. As part of this project, we have begun to define the genome-wide landscape of active enhancers and open across PCa states. The contents of these maps, in turn, guide screens that will identify regulatory elements associated with treatment resistance and key proteins binding to clinically relevant enhancers. Characterizing changes in the epigenome and its associated transcriptional programs will identify new therapeutic targets as well as biomarkers for therapy response and patient prognostication.

2. **KEYWORDS:**

Prostate cancer; Epigenetics; transcription factor; enhancers; gene regulation; androgen receptor

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - The ultimate goals of this proposal are to comprehensively characterize the epigenetic landscape in advanced PCa in order to gain insights into key mechanisms driving lethal, treatment-resistant disease. Ultimately, these intergenic sites can be rationally targeted. The anticipated outcomes are that we will identify **areas of vulnerability (Aims 1)** that are **functionally relevant (Aim 2)** and the **transcription factors** that activate them **(Aim 3)**. The aims of the proposal require specific domains of expertise and to address these points we have assembled an outstanding team with the appropriate scientific depth to go from target identification to analysis of function.
  - Aim 1 will generate the most biologically and clinically informative epigenomic ChIP-seq datasets to date in advanced PCa. Aim 1 will also use RNA-seq, combining transcriptional analysis with ChIP to help link the regulatory elements with their target genes. Aim 2 will utilize the powerful tools of genome editing to identify regulatory elements that are functionally relevant in the development of advanced PCa and Aim 3 will identify candidate transcription factors activating disease-specific enhancers. The outcome of this study will be a compendium of candidate regulatory elements that influence PCa progression.
- **What was accomplished under these goals?**

Aim 1: To characterize the landscape of open chromatin and active enhancers in the progression from hormone-sensitive Prostate Cancer (PCa) to enzalutamide resistant metastatic castration resistant prostate cancer (mCRPC)

Major Task 1: Collect and assemble 150 metastatic tumors

Subtask 1: From the DFCI Gelb Center and the Netherlands Cancer Institute (NKI), identify and collect metastatic prostate cancer biopsies isolated from 50 men with newly diagnosed metastatic prostate cancer (month 1-24)

Subtask 2: From the DFCI Gelb Center and the Netherlands Cancer Institute (NKI), identify and collect metastatic prostate cancer biopsies isolated from 50 men with metastatic castration-resistant prostate cancer prior to initiation of second-line treatment and 50 resistant to enzalutamide. (month 1-24)

- Major subtasks completed. We now successfully collected 30 metastatic prostate cancer biopsies from newly diagnosed patients at the DFCI Gelb Center (subtask 1) and 50 biopsy samples from mCRPC patients at the NKI. These include 20 treatment-naive (10 DFCI, 10 NKI), and 20 enzalutamide resistant (10 DFCI, 10 NKI) cases, as well as 40 men with metastatic castration-resistant prostate cancer prior to initiation of second-line treatment (NKI).

- In the first year of the reporting period, we focused on our initial cohort of epigenomes, which included samples collected as part of this aim. These initial results were published in *Nature Genetics* in August 2020. **We generated and analyzed 268 epigenomes in specimens derived from human tissue** (Table 1).

Table 1. Specimens in the project to date, specified by tissue and epigenetic mark

	AR	FOXA1	HOXB13	H3K27Ac	H3K4me2	H3K4me3	H3K27me3	ATAC	All marks
Total	59	42	42	86	8	10	11	10	268
Normal prostate epithelium	13*	14	14	37+	4	3	4	4	93
Primary prostate tumor	31*	13	13	32	4	7	7	6	113
mCRPC†	15	15	15	17	0	0	0	0	62
Median no. ChIP-seq or ATAC-seq peaks (range)	20,619 (1,577–73,723)	37,691 (3,174 – 99,041)	47,338 (1,709 – 90,075)	34,609 (2,337 – 127,042)	69,558 (41,095 – 83,869)	33,215 (28,952 – 38,447)	25,4148 (112,809 – 316,413)	48,139 (25,324 – 60,232)	

* Includes seven normal prostate and 13 primary tumor AR ChIP libraries published previously – Pomerantz et al, *Nat Genet*, 2015.

\+ Includes H3K27Ac ChIP-seq performed in a specimen derived from human fetal urogenital sinus – Guo et al, *PLoS One*, 2012.

† ChIP-seq experiments performed using PDXs derived from human mCRPC with the exception of two H3K27Ac ChIP-seq specimens derived from patient mCRPC liver biopsies

Major Task 2: Epigenetic characterization of metastatic samples

Subtask 1: Chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) for Histone 3 Lysine 27 acetylation (H3K27Ac). (month 3-24)

Subtask 2: ATAC-seq (month 3-24)

- ChIP-seq and ATAC-seq for the samples collected above is underway, with the goal of meeting the 24-month goal despite significant challenges due to the laboratory shutdowns as described below.

- In 2020, we analyzed the data generated to date. We evaluated AR binding in the transition from normal prostate epithelium to localized hormone-sensitive PCa to metastatic castration-resistant disease. **Comparison of the normal prostate, localized hormone-sensitive tumor and metastatic castration resistant PCa (mCRPC) cistromes demonstrated distinct reprogramming of the AR cistrome** (Figure 1). Using a stringent threshold, we identified 17,655 ARBS consistently enriched in the transition from localized PCa to mCRPC (met-ARBS). We similarly performed H3K27Ac ChIP-seq – a mark of active enhancers and promoters – across these clinical states (mCRPC-specific sites are called met-K27ac). Unsupervised principal components analysis of primary tumor versus mCRPC showed clear separation between clinical subtypes (Figure 1). Importantly, genome-wide H3K27Ac in biopsies taken directly from patient mCRPC tumors clustered with the mCRPC PDXs. The majority of met-K27ac peaks overlapped with the met-ARBS peaks (64.9% peak overlap; p-value, <2.2E-16).

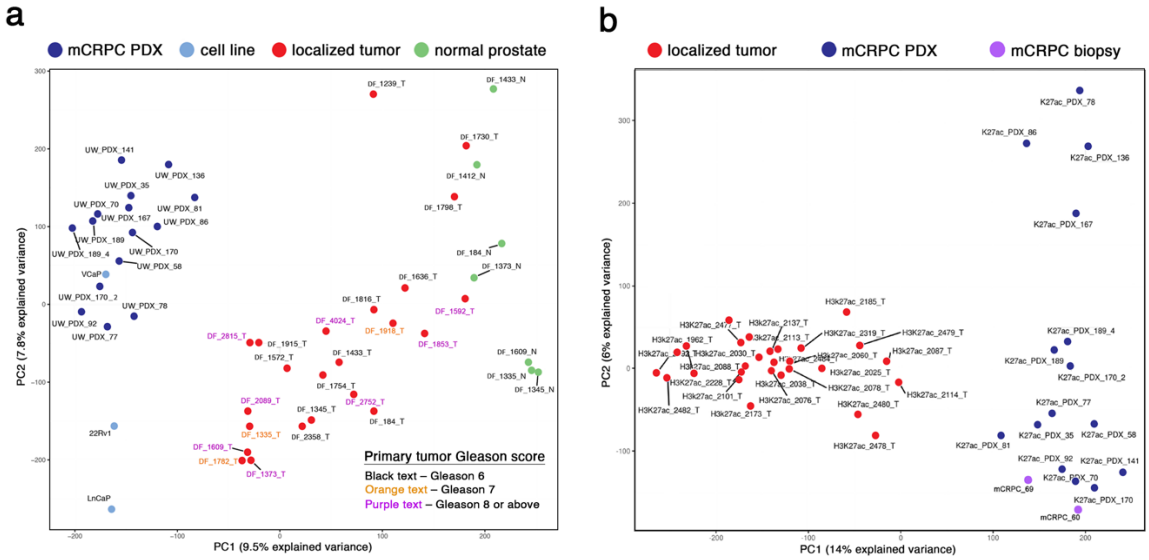

Fig. 1. The AR cistrome and genome-wide H3K27Ac are systematically reprogrammed during prostate cancer progression (a) Principal component analysis (PCA) reveals distinct AR binding patterns across prostate states. Each dot represents the genome-wide AR cistrome in an individual specimen (seven normal prostate epithelium, 23 primary PCa tumors, 15 PDX tumors derived from patient mCRPC, three PCa cell lines derived from metastatic tissue. (b) PCA reveals distinct H3K27Ac binding patterns between primary tumors and mCRPC. Each dot represents genome-wide H3K27Ac signal in an individual subject (24 primary PCa tumors, 15 PDX tumors derived from patient mCRPC, two metastasis specimens biopsied directly from patients with mCRPC).

- To evaluate how well these differential regulatory sites correlate with transcriptional differences, we accessed a publicly available transcriptomics dataset of metastatic prostate versus localized prostate tumor tissue. We rank-ordered differentially expressed genes and then projected onto this distribution the set of transcriptional start sites (TSSs) that contain a met-K27ac site. Transcripts overexpressed in metastases were highly enriched for met-K27ac TSS (p-value, <0.00001; Fig. 2). **The findings demonstrate that newly activated enhancers in mCRPC direct transcript levels of target genes**.

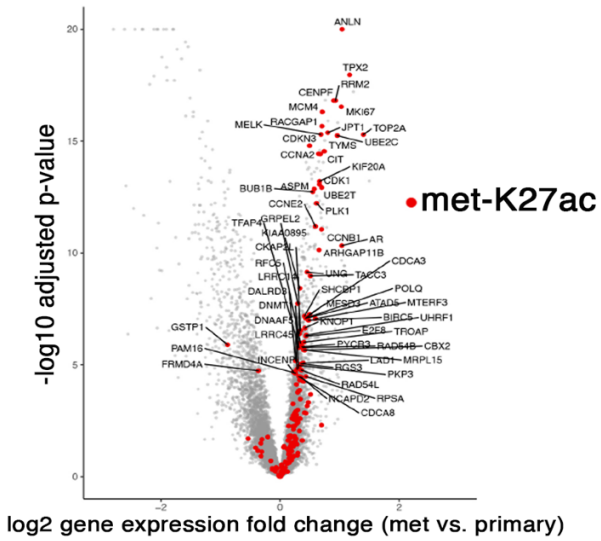

Fig. 2. Newly activated enhancers coincide with genes upregulated in mCRPC. Genes whose expression is upregulated in metastasis compared to primary tumor are enriched for met-K27ac peaks (p-value <0.00001). Each dot represents a gene. Red dots are genes with a met-K27ac in the TSS.

- To test whether other prostate relevant TFs also underwent reprogramming, we performed FOXA1 and HOXB13 ChIP-seq in 14 normal prostate, 13 localized PCa and 15 mCRPC PDX specimens. In stark contrast to AR, the FOXA1 and HOXB13 cistromes demonstrated dramatically less reprogramming during disease progression. Notably, only 306 FOXA1 and 47 HOXB13 peaks were enriched in mCRPC relative to primary disease, compared with 17,655 AR sites. We next focused on the sets of AR sites reprogrammed from normal to primary tumor (n = 9,179, as previously described) and met-ARBS (n = 17,655). Specifically, we evaluated FOXA1 and HOXB13 binding, ATAC-seq, and DNA methylation at these sites. Strikingly, in both normal and primary tumor specimens, FOXA1 and HOXB13 are already present at these 'sentinel' sites where AR is destined to bind (Figure 3). Chromatin was accessible and the DNA was relatively hypomethylated at these loci as well. **The data demonstrate that reprogrammed AR sites during transformation and metastasis are not formed *de novo*, but rather that AR binds to pre-marked, sentinel sites**.

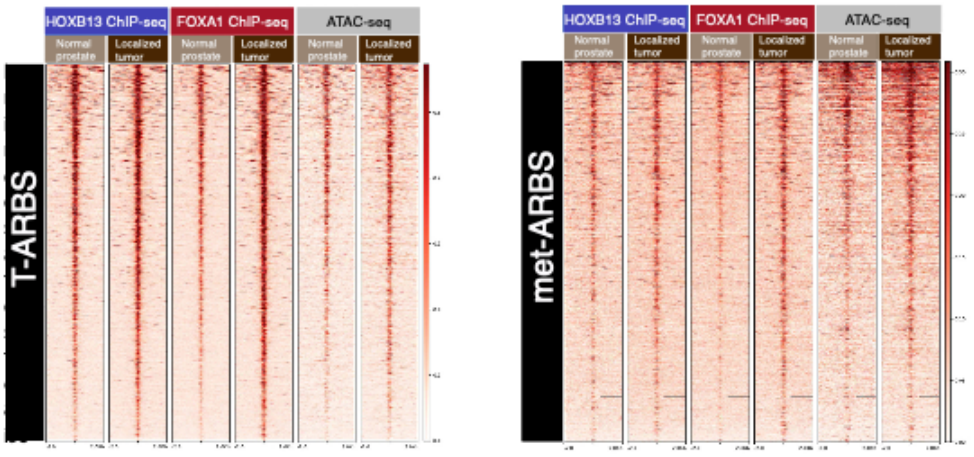

Fig. 3. During PCa progression, AR is re-programmed to pre-marked sites. Heat map indicating transcription factor and ATAC-seq signal intensity in normal prostate epithelium and primary prostate tumor. At left, the 9,179 AR T-ARBS. At right, the 17,655 met-ARBS. Each horizontal line represents a four kilobase (kb) locus. Shade of red reflects average binding intensity at that site across all subjects in the cohort.

- We characterized the TF DNA binding motifs present within met-ARBS, comparing the gained sites to shared AR sites. The most significantly enriched motif associated with met-ARBS was ZEB1 (Zinc Finger E-Box Binding Homeobox 1), a well-described TF involved in mediating epithelial to mesenchymal transition (EMT) in PCa (p=1x10-155)[1,2]. To ascribe putative biological functions to the met-ARBS, the 17,655 met-ARBS were subjected to the Genomic Regions Enrichment of Annotations Tool (GREAT)[3]. Strikingly, the gene ontology (GO) biological processes included "somatic sex determination" (p-value, 1.4 x 10-49), "activation of prostate induction" (p-value, 2.5 x

10-45) and “epithelial cell differentiation involved in prostate gland development” (p-value, 5.0 x 10-20), suggesting that the met-ARBS cistrome is reactivating prostate developmental programs. Similarly, GREAT analysis of met-K27ac revealed multiple GO terms associated with prostate gland organogenesis, such as “epithelial cell maturation involved in prostate gland development” (p-value, 4.9 x 10-38). Next, we investigated similarities between the prostate metastatic epigenome and a large panel of fetal and adult epigenomes. To this end, we assessed the correlation between the set of met-K27ac sites and a series of K27ac epigenomes generated in fetal (N=10 tissue types)[4,5] and adult tissue types (N=27)[4] (Figure 4). The tissues that were most similar to the met-K27ac sites were fetal urogenital sinus (UGS) followed by the fetal tissues most developmentally related to the prostate. sites. **These data indicate that regulatory elements commissioned during prostate cancer progression resurrect prostate-specific fetal tissue developmental programs**. The prostate metastatic epigenomic program is active during development, becomes quiescent in normal prostate and localized prostate tumors, and is reactivated in advanced disease.

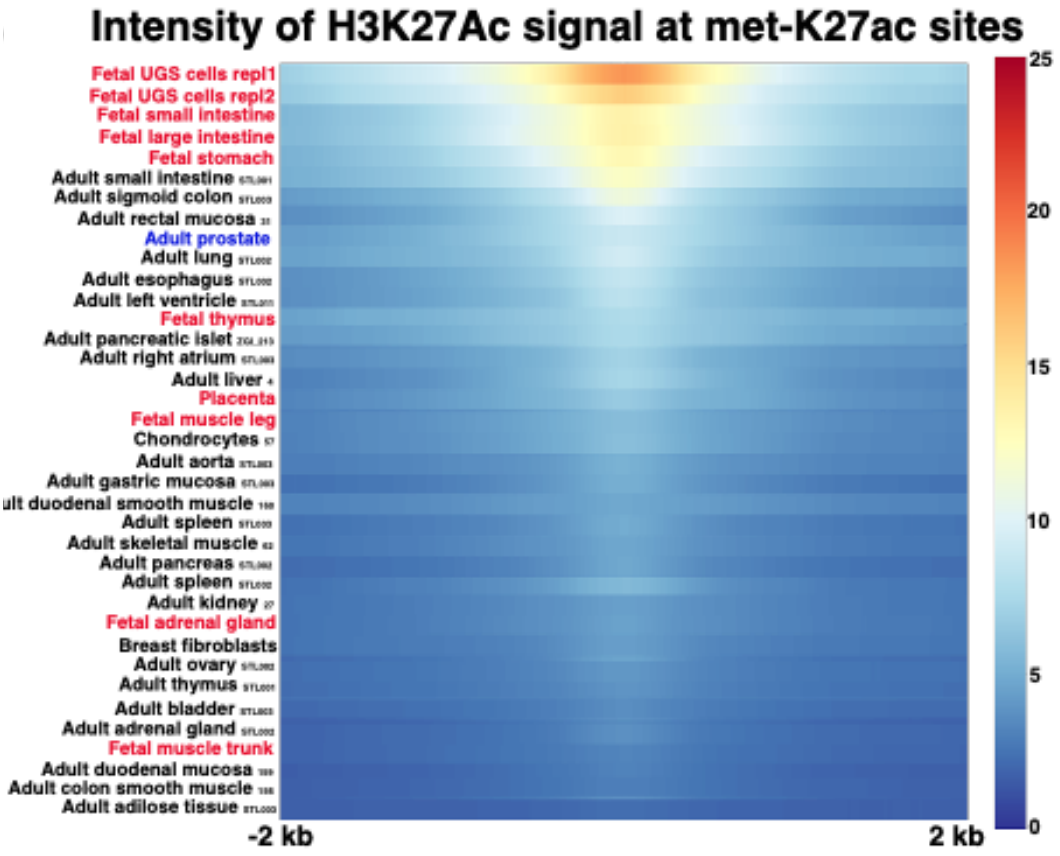

Fig. 4. Regulatory sites activated in mCRPC resurrects prostate developmental programs. Across 37 human adult and fetal cell types, met-K27ac is most strongly associated with fetal urogenital sinus. Cell type listed at left (adult tissues are followed by Roadmap Epigenomics Project identification codes). Urogenital sinus sample was performed in replicate. Heat map indicates H3K27Ac binding intensity met-K27ac sites across a 4 kb interval.

- Previously, we discovered somatic activation of a distal, functionally relevant enhancer that regulates the AR gene[6]. The enhancer region contains recurrent tandem duplications in a whole-genome sequencing (WGS) mCRPC dataset and an H3K27Ac signal that was substantially stronger in mCRPC compared with primary PCa. As part of this project, we ssought to similarly discover other somatically-acquired enhancers in advanced PCa.

- We first intersected the mCRPC-specific H3K27Ac loci with regions containing recurrent structural variants in the WGS dataset from Viswanathan et al, reasoning that recurrent somatic copy number alterations provide a biologically accepted framework for regions under selective pressure[7]. We rank ordered the genomic segments by frequency of overlap between structural variation and met-K27ac sites. Among the top ranked regions were genomic segments containing the genes AR, MYC, FOXA1, HOXB13, and NKX3-1. The genetic regions tended to be large and contained multiple genes. The HOXB13 segment, for example, was 986 kb and contained over 20 genes (Fig. 5).
- To identify enhancer-promoter interactions, we performed H3K27Ac and H3K4me3 HiChIP in LNCaP cells (Fig. 5). Based on looping interaction, co-localization with met-K27ac sites, and recurrence of H3K27Ac signal across a majority of specimens, we prioritized specific candidate enhancers for functional evaluation. Candidate enhancers were functionally evaluated using CRISPR interference (CRISPRi). Site-specific suppression of each putative regulatory element resulted in significantly decreased expression of NKX3-1, HOXB13 and FOXA1. Furthermore, CRISPRi-targeting of each individual enhancer for FOXA1 and HOXB13 decreased LNCaP cell proliferation (Fig. 5).

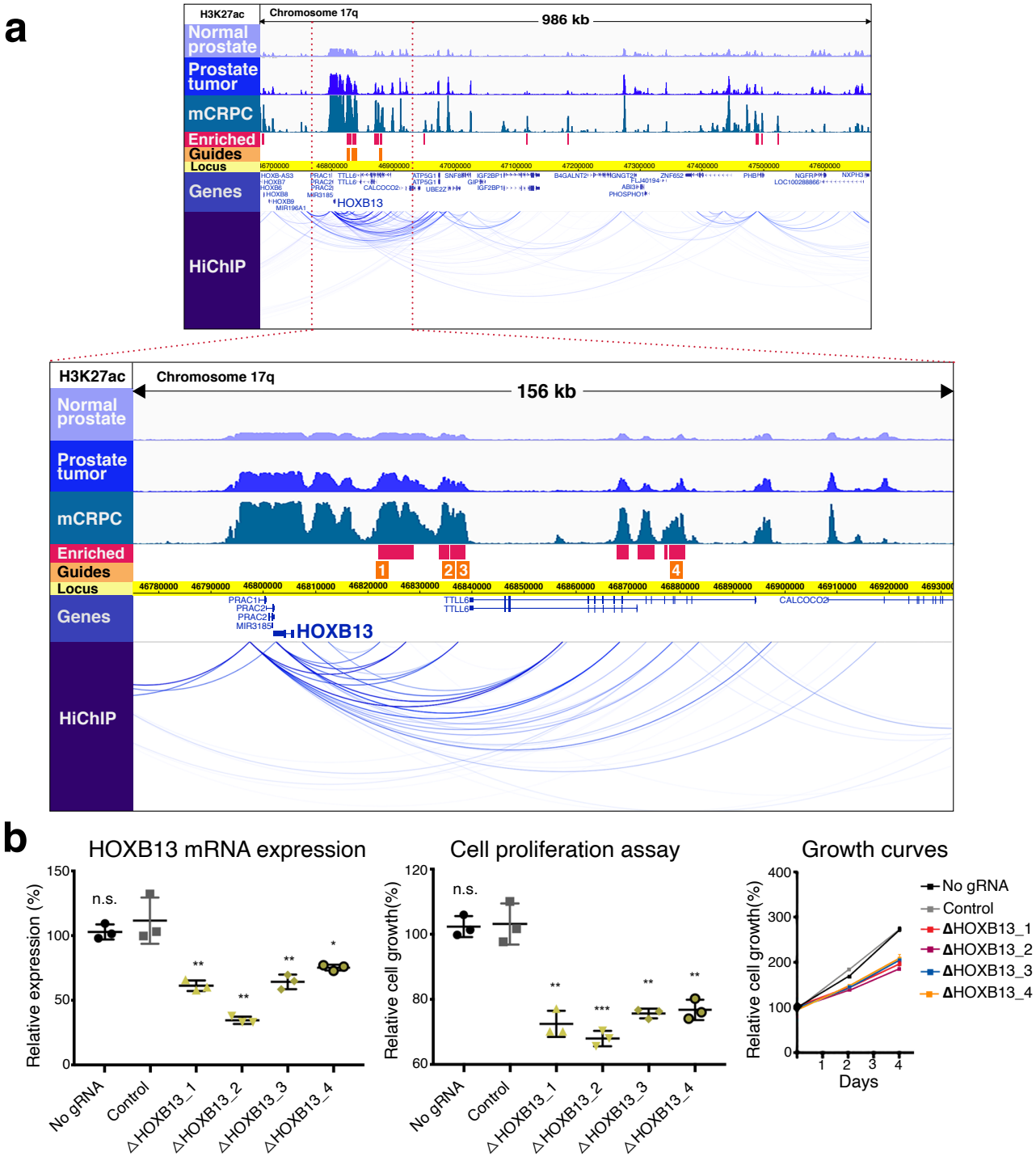

Fig. 5. Functionally relevant mCRPC enhancers are identified by interrogating epigenetic datasets across clinical states. (a) At top, H3K27Ac tracks in a 986 kb region containing HOXB13 identified by integrating ChIP-seq and WGS data, as described. Intensity of ChIP-seq signal was averaged across all DFCI normal prostate, primary prostate tumor and mCRPC specimens, respectively. HiChIP track depicts chromatin looping in the LNCaP cell line. Blue bars show H3K27Ac sites meeting criteria for mCRPC enrichment (met-K27ac). Orange bars depict the locus against which guide RNAs (gRNAs) were designed. Below, magnification of a 156 kb region (bound by red-dotted lines in the upper picture) where met-K27ac and HiChIP signals were strongest. (b) Functional interrogation of candidate metastasis-specific enhancers. Left, LNCaP HOXB13 expression in controls (no gRNA and gRNA targeting unrelated gene HPRT1) and after transduction with each gRNA depicted in (b). Middle and right, LNCaP cell proliferation over the course of four days. Each shape represents an independent experiment, center line indicates mean, error bars indicate ± s.d. Using student's t-test, two-sided – n.s. not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

- **The data indicate that gain of H3K27 acetylation coinciding with somatic DNA amplification identifies *metastasis-specific regulatory elements***. The subsequent Aims

of this project involve deep interrogation of the enhancers discovered using the methodology developed in year 1.

Major Task 3: RNA-seq of metastatic samples. Isolation of RNA, library preparation and Illumina high-throughput sequencing at DFCI (month 3-24)

- For 50 men with metastatic castration-resistant prostate cancer prior to initiation of second-line treatment (NKI), RNA-seq and WGS was performed for 20 samples with sufficient tissue available. For DFCI samples, we will plan to perform RNA-seq in the upcoming year

Specific Aim 2: To perform epigenome-wide Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based screens across the genome to identify regulatory elements and transcription factors (TFs) associated with enzalutamide resistance in model systems..

Major Task 1: Define functional enhancer landscape for enzalutamide resistance.

Subtask 1: Generate enzalutamide resistant LNCaP clones (month 1-3).

Subtask 2: Perform H3K27 ChIP seq on 5 enzalutamide resistant clones and 5 enzalutamide sensitive clones. (month 3-6).

Subtask 3: Conduct pooled CRISPR/Cas9 screen targeting differentially activated enhancers. (month 6-10)

Subtask 4: Validate gRNAs that score in pooled screen (month 10-12).

- H3K27ac ChIP-seq data generated and analyzed for three enzalutamide resistant clones and 5 sensitive clones.
- Three Enzalutamide resistant prostate cancer cell lines are available and fully annotated in our labs. An additional two models are being generated.
- Updates after year 1: postponed due to COVID-19. Subtask will be initiated once the last two enzalutamide resistant clones are available.
- Updates after year 1: postponed due to COVID-19 (see above).

Major Task 2: Identify genes regulated by altered genome (months 12-24)

Specific Aim 3: To use novel technology (GloPro) to identify the key proteins binding to clinically and functionally relevant enhancers.

Major Task 1: Identify trans-acting factors at the androgen receptor (AR) enhancer

Subtask 1: Optimize guide RNAs (gRNAs) and reaction conditions for localization of dCas9-APEX to AR enhancer in cells

Subtask 2: Perform streptavidin pulldown and mass spectrometry to identify candidate trans-acting factors

Subtask 3: Validate binding of individual factors by ChIP-QPCR

Subtask 4: Confirm effect on AR mRNA by suppression of candidate TF by RNAi

- Cell lines with gRNAs and reaction conditions for localization of dCas9-APEX performed in cells
- During the past year, the lead scientist, Dr. Sam Myers, obtained a faculty position at the La Jolla Institute for Immunology. Dr. Myers confirmed his commitment to performing this work.

Major Task 2: Identify *trans*-acting factors at enhancer identified by Aims 1 and 2 (months 12-36)

Major Task 3: Corroborate candidate *trans*-acting factors to tumor samples (months 12-36)

- **What opportunities for training and professional development has the project provided?**
  - The project has provided the opportunity to mentor three post-docs in the lab – Sylvan Baca, M.D., Sarah Alaiwi, M.D. and Amin Nassar, M.D. In the group of dr. Zwart at the Netherlands Cancer Institute, three researchers were trained with this project: , Yanyun Zhu MSc. , Tesa Serverson PhD. , and Simon Linder, MSc. Dr. Baca is a medical oncology fellow at Dana-Farber Cancer Institute. He is in the midst of his post-doctoral triaing in genomics and bioinformatics. He conducted much of the bioinformatics work described above under the mentorship of the PIs on this project. Drs. Abou Alaiwi and Nassar are both internal medicine residents at Brigham and Women's Hospital. As part of this project, they mastered ChIP-seq in human tissue specimens and worked with the bioinformatics team to analyze the data.
- **How were the results disseminated to communities of interest?**
  - The data were presented that the Prostate Cancer Foundation annual Coffey-Holden Conference in 2019. Upon publication of the data, articles describing the findings were published for lay audiences in the *Harvard Gazette* and *Cancer Therapy Advisor.*
  - Upon publication of the data, dr. Zwart was interviewed for the largest Dutch news website (https://www.nu.nl/gezondheid/6066269/dna-handleiding-zorgt-voor-doorbraak-onderzoek-uitzaaiing-prostaatkanker.html) and national radio (https://www.nporadio2.nl/nieuws/29247/onderzoeker-wilbert-zwart-over-nieuwe-ontdekking-op-het-gebied-van-uitgezaaide-kanker).
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Now that we have begun to define the universe of enhancers associated with progression to mCRPC, we will next focus on the epigenetic landscape of enzalutamide-resistant disease. Concurrently, we will functionally characterize the newly discovered non-coding drivers of PCa progression. Our HChIP-seq data (above) revealed the enhancer landscape in PCa pathogenesis, with both loss and gain of enhancers. We will use these enhancer profiles to perform an in vitro screen in prostate cancer cell lines. To determine the functional significance of these epigenetic alterations, we will perform an unbiased pooled CRISPR/Cas9-based screen to systematically suppress each differentially activated enhancer. Recruitment of a nuclease dead version of Cas9 fused to the Kruppel-associated box repressor domain (dCas9-KRAB) results in loss of H3K27ac and silencing of the enhancer. We will clone a pool of gRNAs targeting each enhancer into a lentiviral expression system. We will use five gRNAs per enhancer targeting the center of each H3K27Ac ChIP-seq peak. We anticipate approximately 4000 enhancers to be differentially activated in tumors versus normal tissue based on our preliminary results, which is well within the range typically done with genome-scale screens involving

20,000 genes and libraries consisting of 100,000 gRNAs. As a result, we will have generated the first comprehensive functional enhancer landscape required for proliferation of primary and metastatic and treatment-resistant PCa.

- With the identification of the mCRPC-specific cistromes of AR and H3K27ac, computational approaches can be performed to analyze the primary DNA sequence at these specific reprogrammed regulatory regions. Through this approach, candidate transcription factors can be identified that are responsible for driving activity or these mCRPC-specific enhancers. In the next reporting period, we aim to use this approach to identify these potential 'mCRPC-specific acting transcription factors', and perturb their expression in parental LNCaP, castration resistant derivates and enzalutamide resistant cells, using CRISPR/Cas9 or siRNA approaches.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  - The epigenome plays foundational roles in prostate cancer development, progression, and treatment. Epigenetic regulatory elements direct the expression or silencing of genes within the vast genome, determining the character and responses of the cell. The self-playing piano is a metaphor for the relationship between the genome and the epigenome. The genome can be considered to be the piano itself, with the potential to play any song. Similarly, every cell in our body contains the same DNA sequence, with the potential to express any of the various sets of genes that characterize each of our highly diverse cell types. The epigenome is represented by the music roll, the spooled sheet whose pattern of holes determines the song to be played. In the cell, epigenomic elements orchestrate a particular gene expression program. Tools to deeply annotate the epigenome in human specimens have only recently matured. Our team has constructed the most extensive library of prostate cancer epigenetic elements to date.

  - DNA sequencing studies across thousands of prostate cancer patients in multiple studies have revealed surprisingly few recurrent genetic mutations. As a result, there are few promising targets in the prostate cancer genome for therapy in a majority of patients. The *epigenome*, on the other hand, is highly dynamic. We have discovered that the prostate epigenome undergoes distinct changes during prostate tumor formation and metastasis. These changes are remarkably consistent across cases. They denote, to our knowledge, the most highly consistent molecular changes in prostate cancer progression. This finding potentially exposes fundamental vulnerabilities in the prostate cell that are universal in the prostate cancer patient population.

  - Our findings also support a long-held hypothesis regarding metastasis formation. We provide striking evidence that prostate cancer cells re-activate the epigenetic paths that the embryonic prostate cell formerly traversed during organ development in the womb. Specifically, the androgen is reprogrammed to sentinel sites in the genome where the mCRPC cell appears to commandeer the regulatory programs of its embryonic ancestors Understanding these epigenomic changes across clinical states presents potential opportunities for clinical translation. For example, the trans-acting factors essential for mCRPC-specific enhancer function may be targeted; or, mCRPC-specific enhancers themselves may be targets for therapy. More fundamentally, as the mechanisms responsible for epigenetic plasticity are better understood, blocking access to latent embryonic programs or "re-reprogramming" the cell to a more differentiated state (e.g., differentiation therapy) may be possible.

- **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?**
  - Through interviews and communication through the lay media, the project had impact on patients diagnosed with metastatic prostate cancer and their families, by communicating the scientific progress has been made for their specific phase of the disease and the international research activities that are being performed to ultimately improve their outcome.

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
     - The COVID-19 pandemic had a significant impact on our work this year. By the winter/spring 2019-20 we had generated our epigenomics and genomics in human specimens. As we were preparing to perform the functional work outlined in Aim 2 of the project, our laboratories at Dana-Farber Cancer Institute and the National Cancer Institute were closed. Both institutions placed strict restrictions on access to the laboratory for activities unrelated to direct patient care. Our team of investigators gained full access to the laboratory and tools necessary to continue our work by early September. We have resumed the work described above and have begun processing data generated pre-quarantine that have not yet been analyzed.
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - Nothing to Report
   - **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
6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**

- **Journal publications.**

  Nat Genet. 2020 Aug;52(8):790-799. doi: 10.1038/s41588-020-0664-8. Epub 2020 Jul 20.

  Prostate cancer reactivates developmental epigenomic programs during metastatic progression

  Mark M Pomerantz, Xintao Qiu, Yanyun Zhu, David Y Takeda, Wenting Pan, Sylvan C Baca, Alexander Gusev, Keegan D Korthauer, Tesa M Severson, Gavin Ha, Srinivas R Viswanathan, Ji-Heui Seo, Holly M Nguyen, Baohui Zhang, Bogdan Pasaniuc, Claudia Giambartolomei, Sarah A Alaiwi, Connor A Bell, Edward P O'Connor, Matthew S Chabot, David R Stillman, Rosina Lis, Alba Font-Tello, Lewyn Li, Paloma Cejas, Andries M Bergman, Joyce Sanders, Henk G van der Poel, Simon A Gayther, Kate Lawrenson, Marcos A S Fonseca, Jessica Reddy, Rosario I Corona, Gleb Martovetsky, Brian Egan, Toni Choueiri, Leigh Ellis, Isla P Garraway, Gwo-Shu Mary Lee, Eva Corey, Henry W Long, Wilbert Zwart, Matthew L Freedman

- **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

- **Inventions, patent applications, and/or licenses**

  Nothing to Report

- **Other Products**
  The epigenetics data generated by the project thus far has been made publicly available via the NCBI Gene Expression Omnibus upon publication of our manuscript.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

8. Name:	Matthew Freedman, M.D.
Project Role:	PI

Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-0151-1238
Nearest person month worked:	XX CM
Contribution to Project:	Dr. Freedman has led overall study design, data generation and data analysis. He directs the database and biobank that provides the materials for the project.
Funding Support:	

9. Name:	Mark Pomerantz, M.D.
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0003-4914-1157
Nearest person month worked:	XX CM
Contribution to Project:	Dr. Pomerantz has been involved in overall study design, data generation and data analysis
Funding Support:	

Name:	David Takeda, M.D.
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-5986-1169
Nearest person month worked:	XX CM
Contribution to Project:	Dr. Takeda is leading the functional molecular biology experiments
Funding Support:	

Name:	Wilbert Zwart, M.D.
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-9823-7289
Nearest person month worked:	XX CM

Contribution to Project:	Dr. Zwart has been involved in overall study design, data generation and data analysis
Funding Support:	

- **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?**
  - The Netherlands Cancer Institute
  - The National Cancer Institute

10. **SPECIAL REPORTING REQUIREMENTS**
    - **COLLABORATIVE AWARDS:** Nothing to Report
    - **QUAD CHARTS:** Nothing to Report
11. **APPENDICES:**

ADDITIONAL NOTES:

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

References:

1. Hanrahan K, O'Neill A, Prencipe M, et al. The role of epithelial-mesenchymal transition drivers ZEB1 and ZEB2 in mediating docetaxel-resistant prostate cancer. *Mol Oncol.* 2017;11(3):251-265.
2. Dai Y, Wu Z, Lang C, et al. Copy number gain of ZEB1 mediates a double-negative feedback loop with miR-33a-5p that regulates EMT and bone metastasis of prostate cancer dependent on TGF-beta signaling. *Theranostics.* 2019;9(21):6063-6079.
3. McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology.* 2010;28(5):495-501.
4. Roadmap Epigenomics C, Kundaje A, Meuleman W, et al. Integrative analysis of 111 reference human epigenomes. *Nature.* 2015;518(7539):317-330.
5. Guo C, Liu H, Zhang BH, Cadaneanu RM, Mayle AM, Garraway IP. Epcam, CD44, and CD49f distinguish sphere-forming human prostate basal cells from a subpopulation with predominant tubule initiation capability. *PLoS One.* 2012;7(4):e34219.
6. Takeda DY, Spisak S, Seo JH, et al. A Somatically Acquired Enhancer of the Androgen Receptor Is a Noncoding Driver in Advanced Prostate Cancer. *Cell.* 2018;174(2):422-432 e413.
7. Viswanathan SR, Ha G, Hoff AM, et al. Structural Alterations Driving Castration-Resistant Prostate Cancer Revealed by Linked-Read Genome Sequencing. *Cell.* 2018;174(2):433-447 e419.

1. Hanrahan K, O'Neill A, Prencipe M, et al. The role of epithelial-mesenchymal transition drivers ZEB1 and ZEB2 in mediating docetaxel-resistant prostate cancer. *Mol Oncol.* 2017;11(3):251-265.
2. Dai Y, Wu Z, Lang C, et al. Copy number gain of ZEB1 mediates a double-negative feedback loop with miR-33a-5p that regulates EMT and bone metastasis of prostate cancer dependent on TGF-beta signaling. *Theranostics.* 2019;9(21):6063-6079.
3. McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology.* 2010;28(5):495-501.
4. Roadmap Epigenomics C, Kundaje A, Meuleman W, et al. Integrative analysis of 111 reference human epigenomes. *Nature.* 2015;518(7539):317-330.
5. Guo C, Liu H, Zhang BH, Cadaneanu RM, Mayle AM, Garraway IP. Epcam, CD44, and CD49f distinguish sphere-forming human prostate basal cells from a subpopulation with predominant tubule initiation capability. *PLoS One.* 2012;7(4):e34219.
6. Takeda DY, Spisak S, Seo JH, et al. A Somatically Acquired Enhancer of the Androgen Receptor Is a Noncoding Driver in Advanced Prostate Cancer. *Cell.* 2018;174(2):422-432 e413.
7. Viswanathan SR, Ha G, Hoff AM, et al. Structural Alterations Driving Castration-Resistant Prostate Cancer Revealed by Linked-Read Genome Sequencing. *Cell.* 2018;174(2):433-447 e419.
1. Hanrahan K, O'Neill A, Prencipe M, et al. The role of epithelial-mesenchymal transition drivers ZEB1 and ZEB2 in mediating docetaxel-resistant prostate cancer. *Mol Oncol.* 2017;11(3):251-265.
2. Dai Y, Wu Z, Lang C, et al. Copy number gain of ZEB1 mediates a double-negative feedback loop with miR-33a-5p that regulates EMT and bone metastasis of prostate cancer dependent on TGF-beta signaling. *Theranostics.* 2019;9(21):6063-6079.
3. McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology.* 2010;28(5):495-501.
4. Roadmap Epigenomics C, Kundaje A, Meuleman W, et al. Integrative analysis of 111 reference human epigenomes. *Nature.* 2015;518(7539):317-330.
5. Guo C, Liu H, Zhang BH, Cadaneanu RM, Mayle AM, Garraway IP. Epcam, CD44, and CD49f distinguish sphere-forming human prostate basal cells from a subpopulation with predominant tubule initiation capability. *PLoS One.* 2012;7(4):e34219.
6. Takeda DY, Spisak S, Seo JH, et al. A Somatically Acquired Enhancer of the Androgen Receptor Is a Noncoding Driver in Advanced Prostate Cancer. *Cell.* 2018;174(2):422-432 e413.

7. Viswanathan SR, Ha G, Hoff AM, et al. Structural Alterations Driving Castration-Resistant Prostate Cancer Revealed by Linked-Read Genome Sequencing. *Cell.* 2018;174(2):433-447 e419.