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14. ABSTRACT

Metastatic prostate cancer is one of the most lethal form of cancer and the patients are normally treated with next generation AR targeted therapy, such as enzalutamide. However, resistance to these agents are not evitable. Our previously work revealed prostate cancer cells can gain resistance via a novel mechanism lineage plasticity, partially driven by Sox2. Therefore, this study aimed to fully understand the mechanism of lineage plasticity driven resistance and identify novel resistance biomarkers, including long non-coding RNAs. In the past one year, (1)we have revealed the detailed mechanism how TP53 and RB1 loss lead to de-repress of Sox2 driven lineage plasticity (2) we have identified Notch1 as a key regulator of lineage plasticity and examined the efficacy of treating tumors with enzalutamide plus Notch inhibitor. (3) We have identified a new biomarker, CHD1-loss, which confers lineage plasticity and resistance, and identified 4 more resistant driver genes. (4) We have analyzed patient's data and identified top altered lncRNAs in resistant tumors. (5) We have constructed CRISPR I library to screen lncRNAs crucial to drive lineage plasticity and resistance.

15. SUBJECT TERMS

Enzalutamide resistance, Lineage plasticity, Notch1, CHD1-loss, lncRNA, CRISPRi library screening

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1. INTRODUCTION

Despite the extensive research efforts and large amounts progress made in cancer research, the survival rate of many cancers remains low, with resistance to targeted therapies remaining a challenge. One possible explanation is additional genomic alterations moved the cancer cells away from the dependency on the original drug target. Metastatic prostate cancer served as a relevant model since it is one of the most lethal form of cancers and the molecular drug target is Androgen Receptor (AR). Patients with metastatic prostate cancer are often treated with next generation AR targeted therapy as "standard-of-care", such as enzalutamide and apalutamide. Although the clinical success of these new agent has transformed the treatment of metastatic prostate cancer, resistance to these agents are unfortunately inevitable. Given this, there is an urgent need to identify novel biomarkers and develop therapeutic approaches to prevent antiandrogen resistance or overcome it.

With support from a ***FY14 PCRP Postdoctoral Research Award***, we have previously identified a novel mechanism of resistance called lineage plasticity, which enabled the prostate cancer cells to escape the luminal epithelial lineage and transformed into a multi-lineage, progenitor-like status that is no longer dependent on AR. In around 10% of patients this lineage plasticity is enabled by both alterations in the loci of TP53 and RB1 and consequently activation of Sox2. This study not only generated novel insights into the mechanisms of anti-cancer therapy resistance, but also suggests potential therapeutic strategies to overcome resistance via disruption lineage plasticity.

However, the exact mechanism of how cancer cells acquired lineage plasticity remain largely unclear, while the downstream effectors of lineage plasticity are largely unknown either. Therefore, the **Aim 1** of this awarded ***2018 Idea Development Award*** study proposed to completely understand the molecular mechanism of lineage plasticity driven resistance, in an effort to develop therapeutic approach to reverse resistance. Furthermore, we have previously demonstrated a clear correlation between lncRNAs and lineage plasticity related resistance in metastatic prostate cancer, suggesting lncRNAs might be utilized as a novel class of biomarkers for antiandrogen response. Thus, the **Aim 2** of this study focus on identifying the lncRNAs related to lineage plasticity and AR targeted therapy resistance, which may lead to the identification of novel biomarkers to predict patients' response and ultimately benefit clinical practice by way of precision medicine.

Within the first year of this award, we have made significant progression on both aims, which already led to a manuscript in revision at ***Cancer Cell***. We have also constructed the CRISPRi based lncRNA library screening and in the process of analysis the results from the screening, to identify novel biomarkers for lineage plasticity and resistance. Detailed progression will be described in report below.

2. KEYWORDS

Enzalutamide resistance
Lineage plasticity
Notch1
CHD1-loss
Biomarker
lncRNA
CRISPRi library screening

3. ACCOMPLISHMENTS

3.1 Major Goal and Objective of the Project

As described in introduction, metastatic prostate cancer is one of the most lethal form of cancers and the management of metastatic prostate cancer is greatly challenged by the inevitable resistance to "standard-of-care" AR targeted therapy, such as enzalutamide and apalutamide. There is an urgent need to identify novel biomarkers and develop therapeutic approaches to prevent antiandrogen resistance or overcome it. We have previously revealed the novel mechanism of lineage plasticity driven resistance, enabled the cancer cells transition from luminal epithelial cells to multi-lineage, progenitor-like status that is no longer dependent on AR. In around 10% of patients with *TP53/RB1* alterations, this lineage plasticity is enabled via activation of Sox2.

However, there are several questions remain:

1. How does Sox2 activated and mediated the lineage plasticity program?
2. What is the downstream effector of lineage plasticity and can we inhibit it?
3. Since *TP53/RB1* only deleted in 10% of patients, are there any other genomic alterations leading to lineage plasticity?
4. What is the role of lncRNA in regulating lineage plasticity?
5. Can we identify lncRNAs as biomarker for lineage plasticity and resistance?

Therefore, the **overall goal of this study is to fully understand the mechanism of lineage plasticity mediate AR targeted therapy (antiandrogen) resistance, in effort to develop novel therapeutic agents to overcome resistance and novel predictive biomarkers to guide the effort of precision medicine**. We have separated the overall goal into two **independent** specific aims as following:

Specific Aim 1: Elucidate the exact mechanism by which RB1 and TP53/miR-34 promote SOX2 activation and identify the key downstream effectors of SOX2.

This aim can be further divided into two **independent** sub-aims and we have made significant progression on both of the sub-aims:

Sub-aim-1.1: Fully reveal the exact molecular mechanism of how *RB1* and *TP53* cooperatively suppress the activation of Sox2. This sub-aim is aligned with both the 1st and 2nd Major Tasks of Aim 1 of approved SOW.

Sub-aim-1.2: Identify the critical components of *SOX2* driven pluripotency network regulating lineage plasticity. This sub-aim is aligned with the 3rd Major Tasks of Aim 1 of approved SOW.

Specific Aim 2: Determine the function of lncRNAs in regulating lineage plasticity and antiandrogen response, in order to develop lncRNAs as potential drug targets or biomarkers

This aim can be further divided into two **independent** sub-aims and we have made significant progression on both of the sub-aims:

Sub-aim-2.1: Determine the lncRNAs enriched in prostate cancer models with increased lineage plasticity. This sub-aim is aligned with both the 1st Major Tasks of Aim 2 of approved SOW.

Sub-aim-2.2: Identify lncRNAs that regulate the gain of lineage plasticity and antiandrogen resistance. This sub-aim is aligned with the 2nd Major Tasks of Aim 2 of approved SOW.

3.2 Major Accomplishments Under These Goals

Since the two Aims and all sub-aims of this project were designed to be **independent** to lower the overall risk of this project, we have started working on all of the sub-aims and tasks spontaneously and made significant progression and accomplishments to all of these sub-aims and tasks. Therefore, these accomplishments will be described independently below, in the order of tasks in approved SOW.

3.2.1. Major Accomplishment 1: Revealed that RB1 and TP53 suppressed Sox2 cooperatively through epigenetic mechanisms in the patients with metastatic prostate cancer. These findings are aligned with sub-task1 and sub-task2 in specific aim 1 in SOW.

3.2.1-A. RB-loss de-suppress Sox2 via changing histone modification

As discussed above, my previous work has revealed this novel mechanism of lineage plasticity by which the cancer cells acquired ability to transition from luminal epithelial cell lineage to other lineages, which is partially mediated by Sox2. However, it is largely unclear how does this lineage plasticity started from the first place when TP53 and RB1 were lost in 10% of patients. Understand the upstream of lineage plasticity is particular important because it may lead to identification of novel biomarkers. Indeed, we have made significant progress to find that RB1 loss led to de-suppress of Sox2 transcription through the remove of repressive histone markers. As shown in **Figure 1**, we symmetrically investigated all the histone markers on the Sox2 locus and found significant changes in many of the repressive and active markers, including H3K4me1, H3K4me3, H3K27me3 and H3K9me3, which clearly suggested that RB1 suppress Sox2 via mediating histone modification on its promoter region.

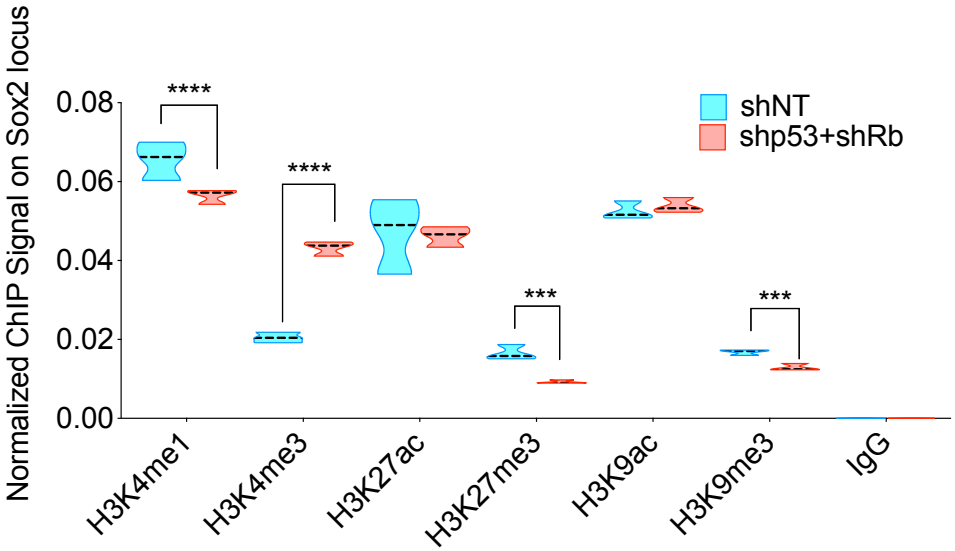


Figure 1. *Relative ChIP signals of annotated histone modification markers on the promoter region of SOX2 in wt cells vs cells with TP53/RB1 depletion. p-values were calculated using multiple t-test. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

3.2.1-B. EZH2 is the key mediator of this RB1 regulated histone modification on Sox2 transcription and lineage plasticity.

Since RB1-loss de-repress the transcription of Sox2 via histone modification, it raised the possibility that regulators of the epigenetic modification machinery would be potential therapeutic targets to restore this activation of Sox2, consequently the lineage plasticity driven resistance. Therefore, we first checked the key polycomb related epigenetic regulators, Enhancer of zeste homolog 2 (EZH2), in both the stable KD and inducible KD system we previously generated and found that EZH2 has been significantly upregulated in the cancer cells with RB1 loss, as shown in **Figure 2A** and **Figure 2B**.

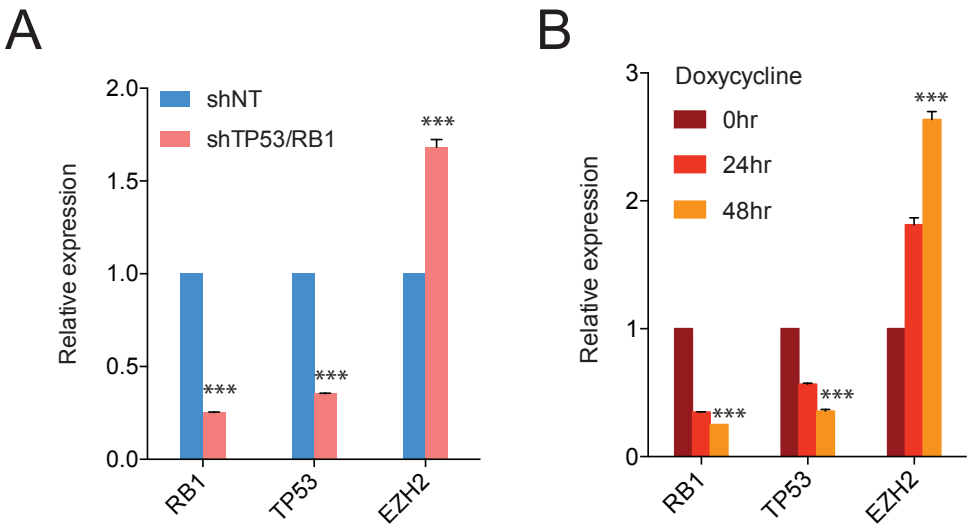


Figure 2. (A)*Relative gene expression of EZH2 in cells transduced with annotated hairpins in stable KD system. p-values were calculated using multiple t-test. (B) Relative gene expression of EZH2 in cells transduced with annotated hairpins in dox-inducible KD system. The cells were treated with doxycycline for 24-48 hours. p-values were calculated using one-way ANOVA. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

These results are particular interesting because it suggests that inhibition on EZH2 may have the therapeutic potential to restore the inhibition on Sox2 and consequently overcome the lineage plasticity driven resistance. Therefore, we examined the efficacy of EZH2 inhibitor (GSK126) on LNCaP/AR tumor cells and demonstrated that EZH2 inhibition can largely overcome the resistance to enzalutamide treatment alone *in vitro*, as shown in **Figure 3**.

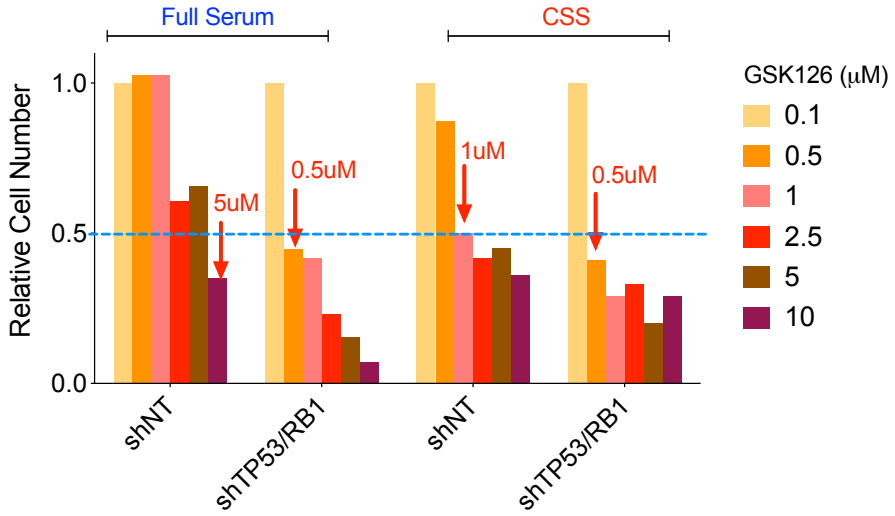


Figure 3. (A)*Normalized cell number of LNCaP/AR cells treated with different dosage of EZH2 inhibitor GSK126. The dosage needed for 50% of inhibition is labeled in red. Cells were treated either in full serum or CSS medium to mimic castration therapy in patients.*

More importantly, we have examined the key lineage marker genes in these cells being treated with EZH2 inhibitor and demonstrated that the interruption of epigenetic modification machinery indeed prohibits the gain of lineage plasticity, as shown in **Figure 4 A-E**.

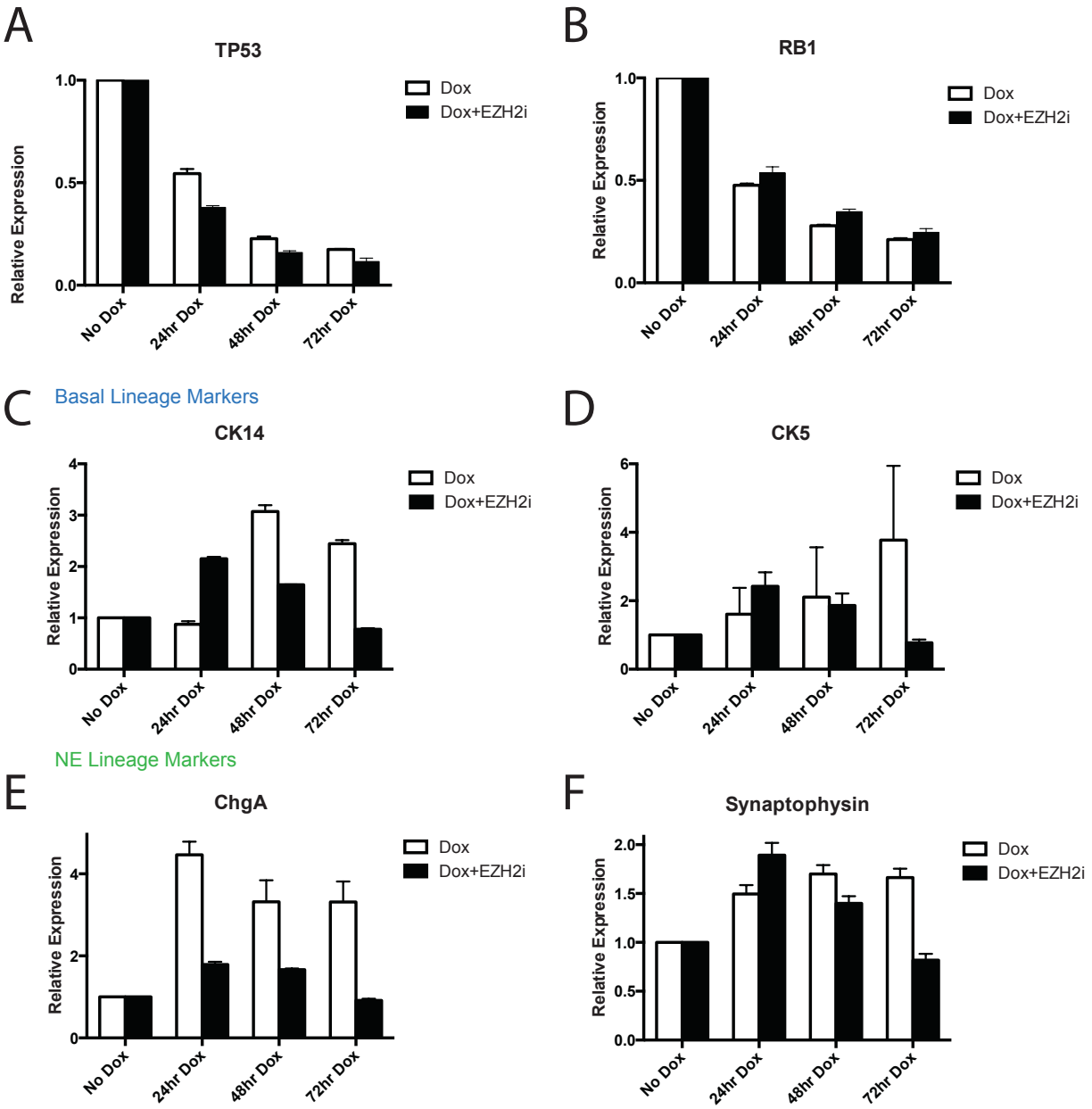


Figure 4. (A-F) *Relative gene expression of RB1 and TP53 and canonical lineage marker genes in cells transduced with annotated hairpins in a dox-inducible KD system. The cells were treated with doxycycline for 24-72 hours. For all panels, p-values were calculated using multiple t-test. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

Furthermore, we have demonstrated that the histone modification changes observed on Sox2 locus were almost completely reversed by EZH2 inhibitor, as shown in **Figure 5**. These results not only support the hypothesis that RB1-loss de-repress Sox2 transcription and lineage plasticity via dysregulation of histone modification, but also provided a possible novel therapeutic avenue to overcome this resistance with EZH2 inhibitor and enzalutamide combined therapy.

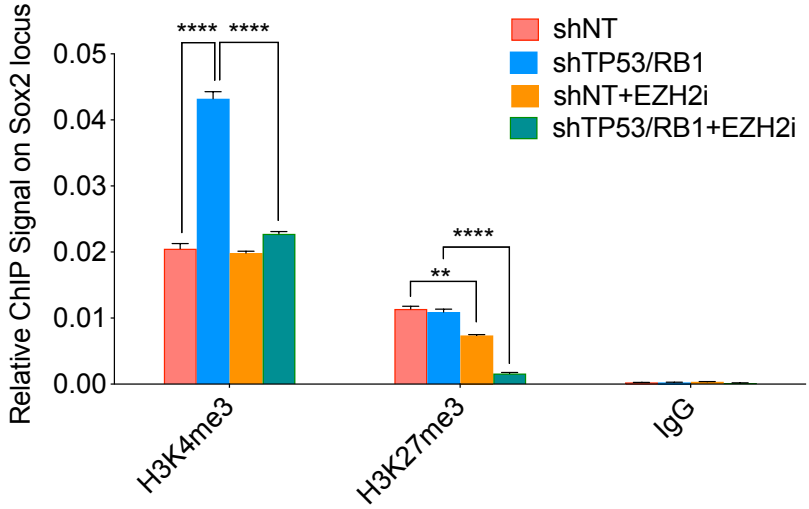


Figure 5. *Relative ChIP signals of annotated histone modification markers on the promoter region of SOX2 in wt cells vs cells with TP53/RB1 depletion, treated with either vehicle or EZH2 inhibitor GSK126. p-values were calculated using one-way ANOVA. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

<u>3.2.1-C. TP53 cooperatively suppress Sox2 translation through the activation of miR-34.</u>

Although we have revealed the key mechanism of how RB1 suppressed Sox2 via epigenetic modification, possibly in an EZH2 dependent manner, it is not clear how p53 is involved in regulating Sox2 since single deletion of either TP53 or RB1 is not sufficient to confer Sox2 upregulation nor lineage plasticity, as we previously reported. Since miR-34 is known to be one of the key downstream effectors of TP53, we examined the level of miR-34 and found significantly upregulated miR-34 in the cells with TP53 and RB1 depletion, shown in **Figure 6**.

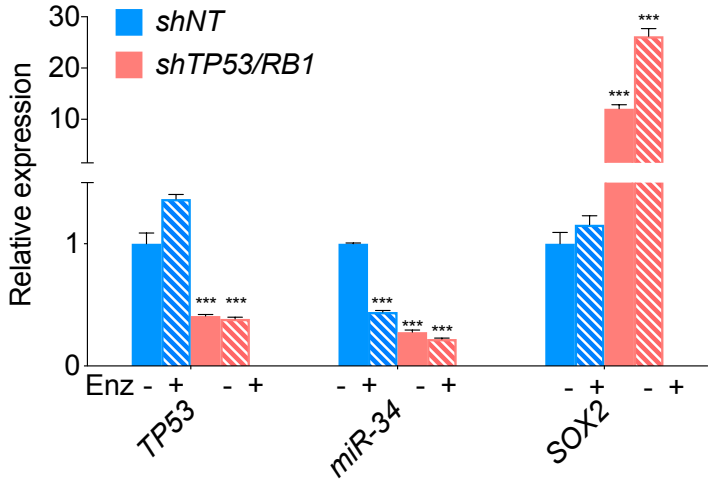


Figure 6. *Relative gene expression of TP53, miR-34 and Sox2 in cells transduced with annotated hairpins in a stable KD system. p-values were calculated using two-way ANOVA. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

Furthermore, we have overexpressed the cDNA of miR-34 in cells with shTP53/RB1 depletion in an inducible system and examined the expression of Sox2 and canonical lineage marker genes. As shown in Figure 7 below, the overexpression of miR-34 completely abolished the upregulation of Sox2, as well as the consequently gaining of lineage plasticity, which supports the hypothesis that TP53 cooperatively suppress Sox2 with RB1 via the activation of miR-34.

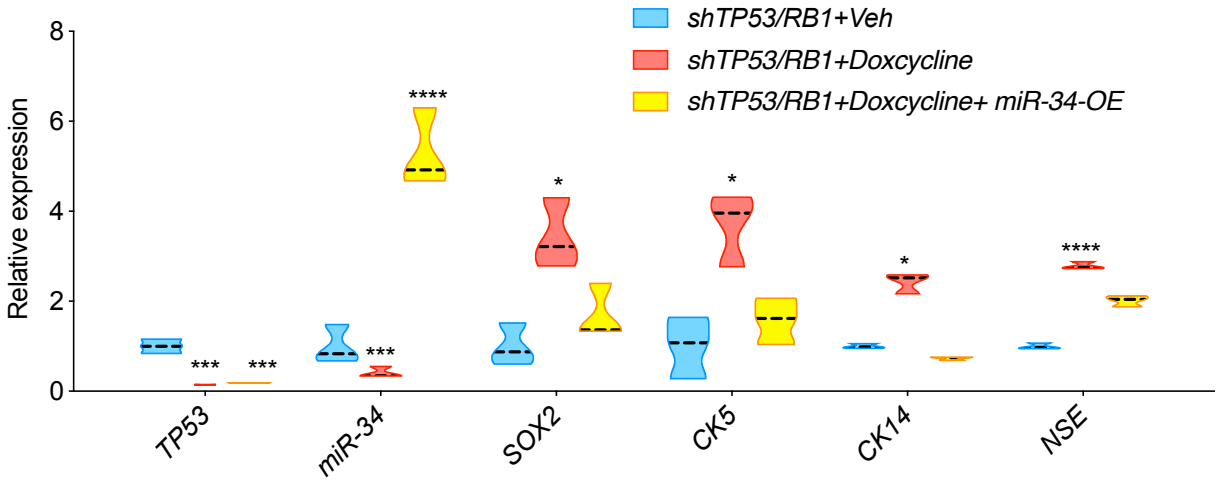


Figure 7. *Relative gene expression of TP53, miR-34, Sox2 and canonical lineage marker genes in cells transduced with annotated hairpins in a dox-inducible KD system. The cells were treated with doxycycline for 72 hours. p-values were calculated using two-way ANOVA. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

This hypothesis of miR-34 mediated suppression of Sox2 were further supported by observation in 3D-cultured organoid system. We have generated *miR-34*^{TKO} organoids (**Figure8A**) from the *miR-34*$^{TKO/TKO}$ mouse that carries targeted deletion of the entire *miR-34* family (*miR-34a, miR-34b* and *miR-34c*) and examined the expression of Sox2, as well as another key downstream effector of TP53, p21. As shown in **Figure 8B**, *miR-34*^{TKO} organoids have clearly upregulated level of Sox2 and downregulation of p21, levels of which are comparable to TP/RB1 depletion.

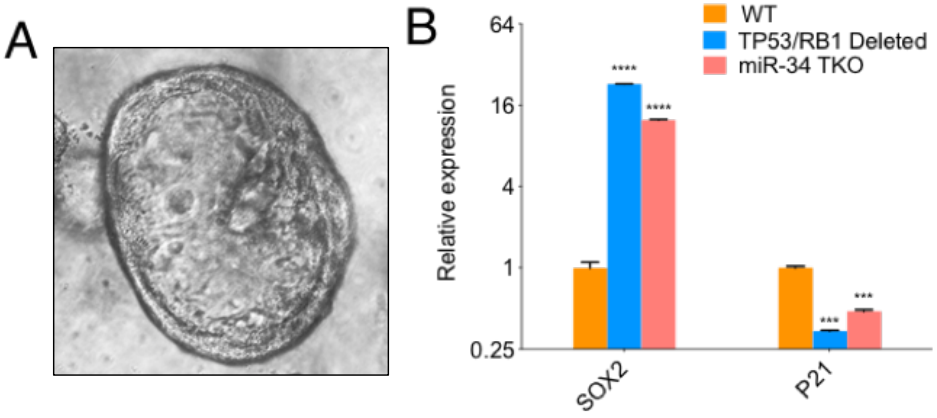


Figure 8. (A)Morphology of the *miR-34^{TKO}* organoids. (B) *Relative gene expression of TP53, miR-34, Sox2 and canonical lineage marker genes in cells transduced with annotated hairpins in a dox-inducible KD system. The cells were treated with doxycycline for 72 hours. p-values were calculated using two-way ANOVA. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

Furthermore, canonical lineage marker genes are also partially changed in these miR-34^{TKO} organoids, although at much smaller scale compared to TP53/RB1 depletion (**Figure 9**). These may due to the lack of RB1 depletion in these organoids.

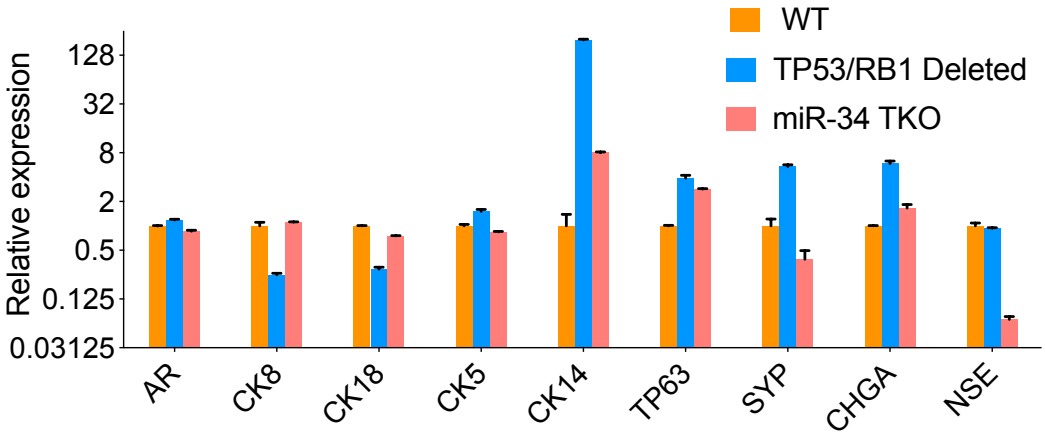


Figure 9. *Relative gene expression of canonical lineage marker genes miR-34T^{KO} organoids compare to the wildtype and TP53/RB1 depleted organoids.*

<u>3.2.1-D. Sox2 activation is sufficient to confer lineage plasticity and resistance in vitro</u>

Since we have dissected the molecular mechanism of RB1/TP53 depletion driven Sox2 activation, then the next question is whether this Sox2 activation is sufficient to confer lineage plasticity? Interestingly, we performed RNA-seq analysis on the wildtype cells and Flag-Sox2 overexpression cells and compared the transcriptome profile changes. As shown in **Figure 10**, there is dramatically overlapping between the genes changed by TP53/RB1 loss and Sox2 overexpression alone, which suggested that majority of the transcriptome changes happened after TP53/RB1 loss are due to Sox2 activation alone.

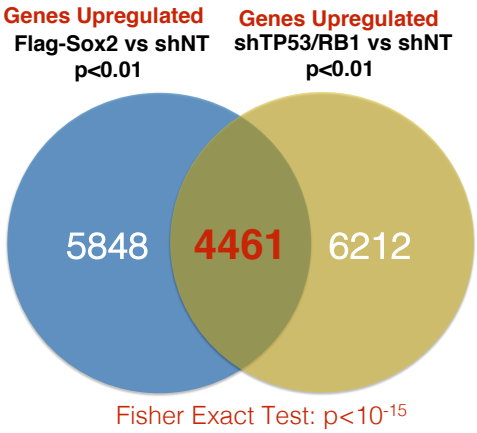


Figure 10. *Transcriptome analysis showing the most significantly changed genes between the TP53/RB1 depleted cells and the Flag-Sox2 overexpression cells.*

This transcriptome analysis is consistent with what we previously reported, in which overexpression of Sox2 alone is sufficient to cause enzalutamide resistance *in vitro*, as shown in **Figure 11**. However, Sox2 overexpression is not sufficient to confer resistance in vivo (data not shown), which may suggest that there are other factors regulate lineage plasticity. Indeed, based on the newest published SU2C patient's cohort, there are only 10% of patients have double deletion or mutations in the loci of both TP53 and RB1 (**Figure 12**), which suggest that there must be other genomic lesions confer lineage plasticity and resistance.

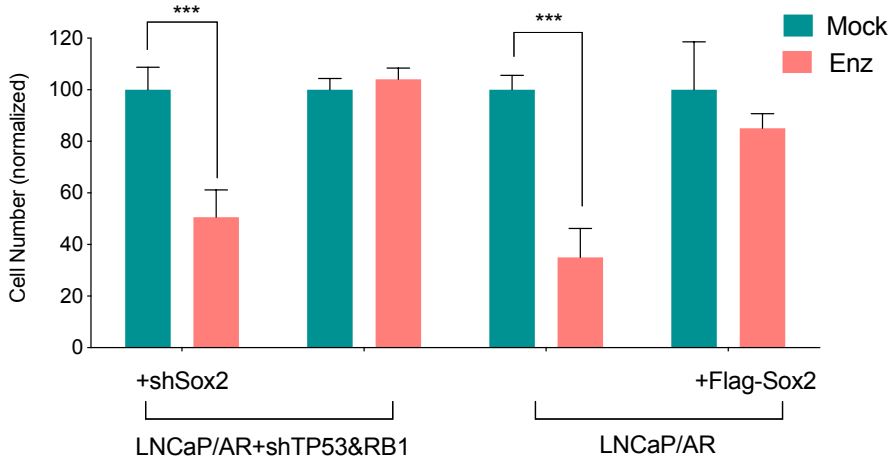


Figure 11. *Normalized cell number of LNCaP/AR cells transduced with annotated shRNAs or overexpressed plasmids. p-values were calculated by multiple t-test. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

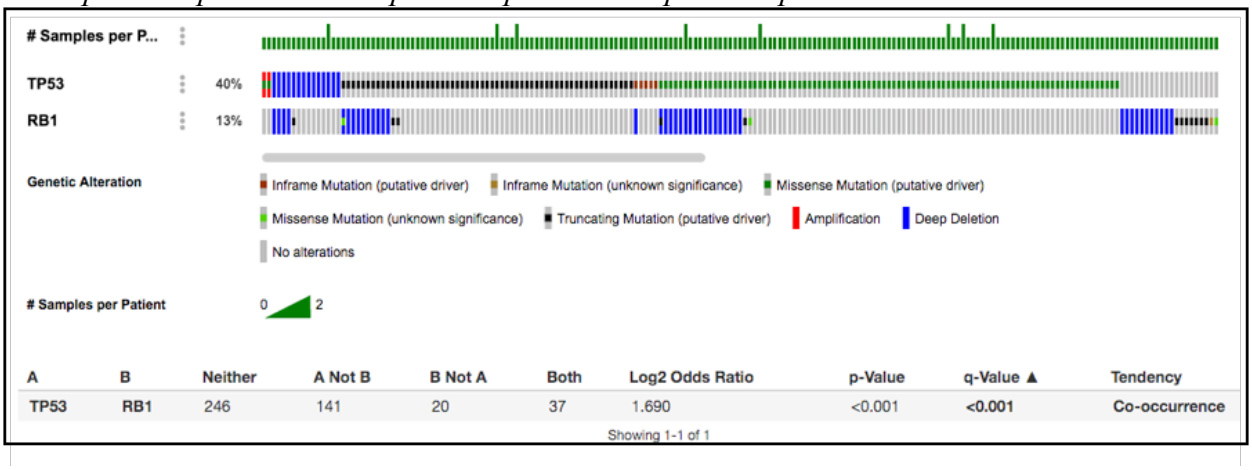


Figure 12. *OncoPrint of the TP53/RB1 alteration ratio in the newest SU2C patients cohort. Data from cbioportal.org.*

3.2.2. Major Accomplishment 2: Revealed a novel genomic alteration, CHD1-loss, as an upstream biomarker which confers lineage plasticity and resistance to AR targeted therapy. These findings are nature follow-up work following the results generated from sub-task1 and sub-task2 in specific aim 1 in SOW.

3.2.2-A. CHD1-loss confers AR targeted therapy resistance in vitro and in vivo.

As described in the end of Major Accomplishment 1, Sox2 overexpression is not sufficient to confer resistance in vivo and there are only 10% of patients have double deletion or mutations in the loci of both TP53 and RB1, which suggest that there must be other genomic lesions confer lineage plasticity and resistance. To find those additional genomic lesions lead to lineage plasticity, we thoroughly examined the LREX cells, a previously reported enzalutamide-resistant subline of LNCaP/AR cells, and unexpectedly found that there is significantly lower level of the Chromodomain helicase DNA-binding protein 1 (*CHD1*) in LREX cells (**Figure 13**). These results indicate that loss of CHD1 may be another upstream genomic event led to lineage plasticity.

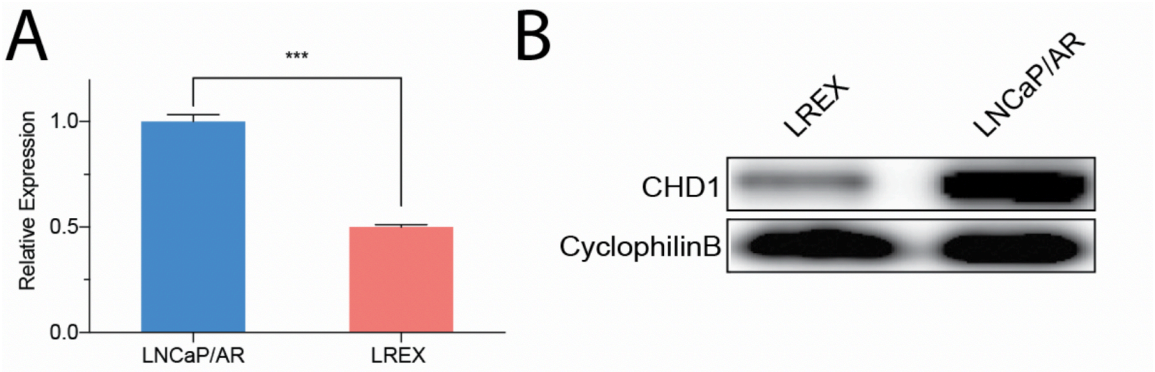


Figure 13. (A)Relative gene expression level of the *CHD1* in LNCaP/AR and LREX cells. p-value were calculated by t-test. (B) Western blot showing CHD1 protein levels in LNCaP/AR and LREX cells. Cyclophilin B serves as loading control.

In normal tissues, *CHD1* functions as a chromatin remodeler and is required to maintain the open chromatin state of pluripotent embryonic stem cells and for somatic cell reprogramming. Numerous lines of evidence from cell lines and genetically engineered mice implicate CHD1 as a tumor suppressor, including prostate cancer. However, CHD1's role in regulating lineage plasticity and AR targeted therapy is not clear. To test our hypothesis, we knockout CHD1 by CRISPR and observed significant resistance to enzalutamide in the CHD1-depleted cells, as shown in **Figure 14**.

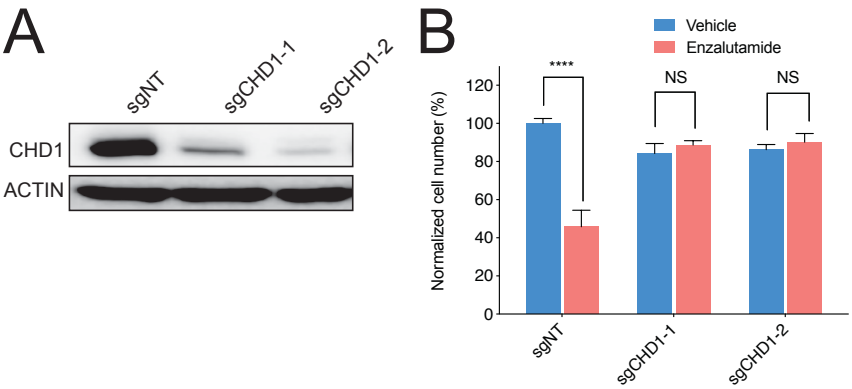


Figure 14. *(A) Western blot of CHD1 protein levels in LNCaP/AR cells transduced with annotated guide RNAs. ACTIN serves as loading control. (B) Relative cell number of LNCaP/AR cells transduced with annotated guide RNAs, normalized to sgNT+Veh group. Cells were treated with 10 μM Enz or same volume of DMSO for 7 days and cell numbers were counted. mean ± s.e.m. is represented, and p-values were calculated using multiple t tests. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

CHD1 depletion not only lead to resistance in vitro, but also in vivo as shown in **Figure 15** below. Interestingly, we did not observe increased growth after CHD1 knockdown in mice that did not receive enzalutamide treatment (**Figure 15**), which suggest that CHD1 related resistance is not due to its general function as a tumor suppressor.

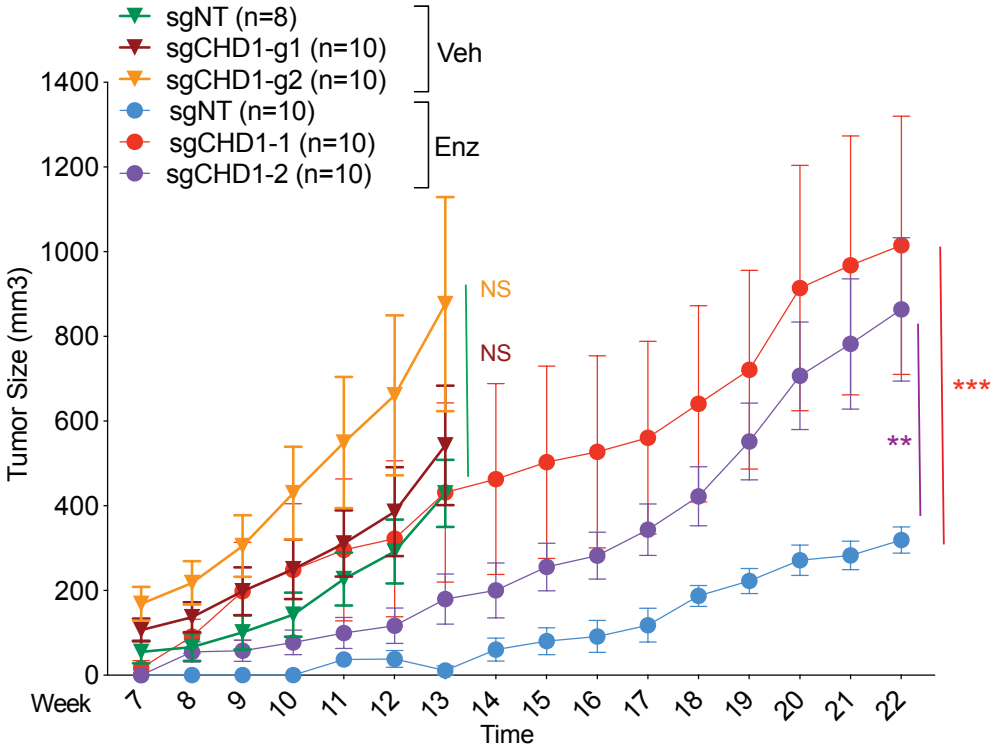


Figure 15. *(A) Tumor growth curve of xenografted LNCaP/AR cells transduced with annotated guide RNAs. Enz denotes enzalutamide treatment at 10mg/kg from day 1 of grafting. Veh denotes 0.5% CMC + 0.1% Tween 80 treatment at same dosage. mean ± s.e.m. is represented and p-values were calculated using 2-way ANOVA with Bonferroni correction. **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

3.2.2-B. CHD1-loss led to gain of lineage plasticity and resistance in similar way as TP53/RB1 depletion observed before.

To investigate the mechanism by which CHD1 loss promotes antiandrogen resistance, we first asked if AR signaling activity was restored in these enzalutamide-resistant tumors. To our surprise, we observed sustained inhibition of the AR target genes PSA, NKX3.1, TMPRSS2, NDRG1, PMEPA1 and STEAP1, indicating that canonical AR signaling is not restored (**Figure 16**). This suggested that CHD1 loss might activate transcriptional programs that relieve prostate tumor cells from their dependence on AR by reprogramming away from their luminal lineage, exactly the same as we observed in the setting of combined loss of RB1 and TP53. =

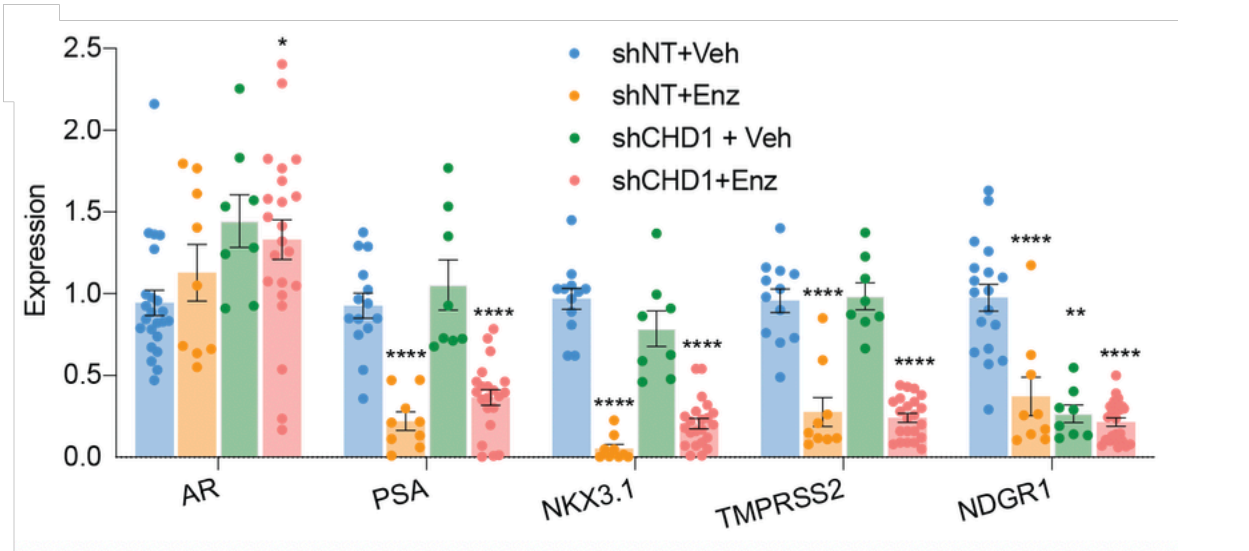


Figure 16. *Relative gene expression of AR and AR target genes in tumors collected from LNCaP/AR xenografts transduced with annotated shRNAs. mean ± s.e.m. is represented and p-values were calculated using 2-way ANOVA with Bonferroni correction.*

Since the CHD1-loss tumor exhibit very similar phenotype as the tumors with TP53/RB1 depletion, we next sought to examined the lineage changes in the cells with CHD1 depletion. To better access the lineage plasticity change, we made LNCaP/AR cells with dox-inducible shCHD1 depletion and treated the cells with different time of doxycycline and examine the expression level of canonical lineage markers. As shown in **Figure 17**, the depletion of CHD1 led to rapid (evident within only 48 hours after doxycycline-inducible CHD1 knockdown) and reversible (lineage changes back in 7 days after doxycycline withdrawal).

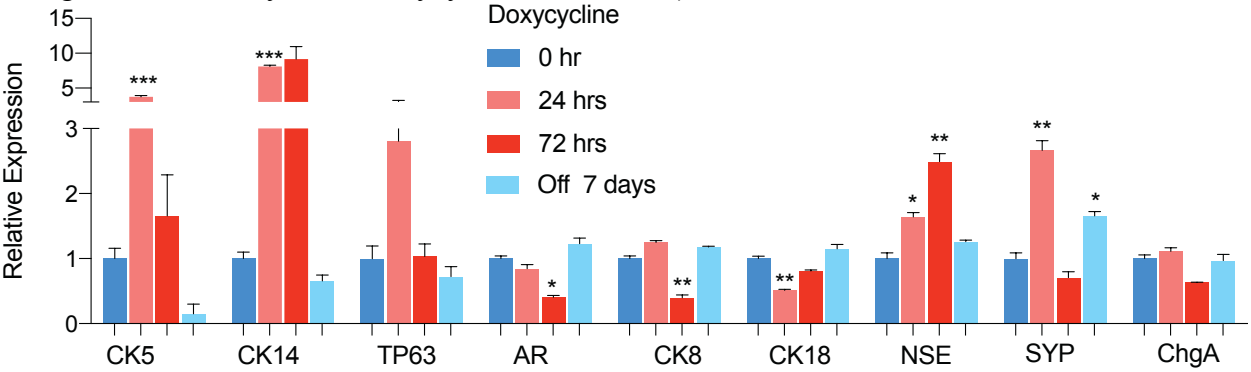


Figure 17. *Relative gene expression level of canonical lineage specific marker genes in LNCaP/AR cells transduced with annotated inducible shRNAs at various time points. p-value were calculated by 2-way ANOVA with Bonferroni correction, all compared to 0 hr condition. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

Beside the changes in canonical lineage marker genes, we also observed significant gain of genes related to epithelial-mesenchymal transition (EMT) (**Figure 18**), which may suggest that CHD1-loss has even wider influence on the program of lineage plasticity than TP53 and RB1. This is particularly interesting since there are around 10-20% patients with CHD1 loss in metastatic cohorts.

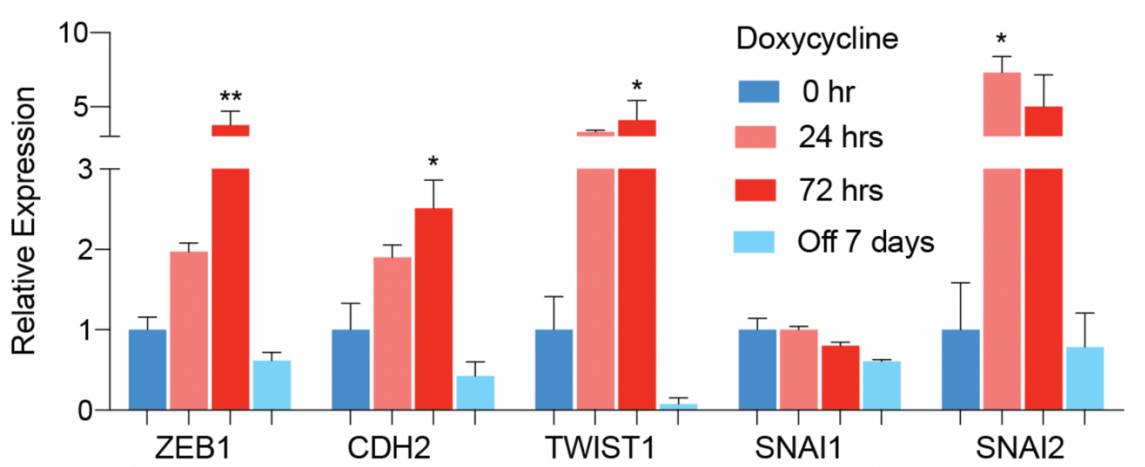


Figure 18. *Relative gene expression level of EMT marker genes in LNCaP/AR cells transduced with annotated inducible shRNAs at various time points. p-value were calculated by 2-way ANOVA with Bonferroni correction, all compared to 0 hr condition. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

More importantly, this CHD1-loss confers resistance is also reversible, as the cells are re-sensitized to enzalutamide treatment after 7 days of doxycycline withdrawal, exactly the same situation as TP53/RB1 depletion situation, as shown in **Figure 19**.

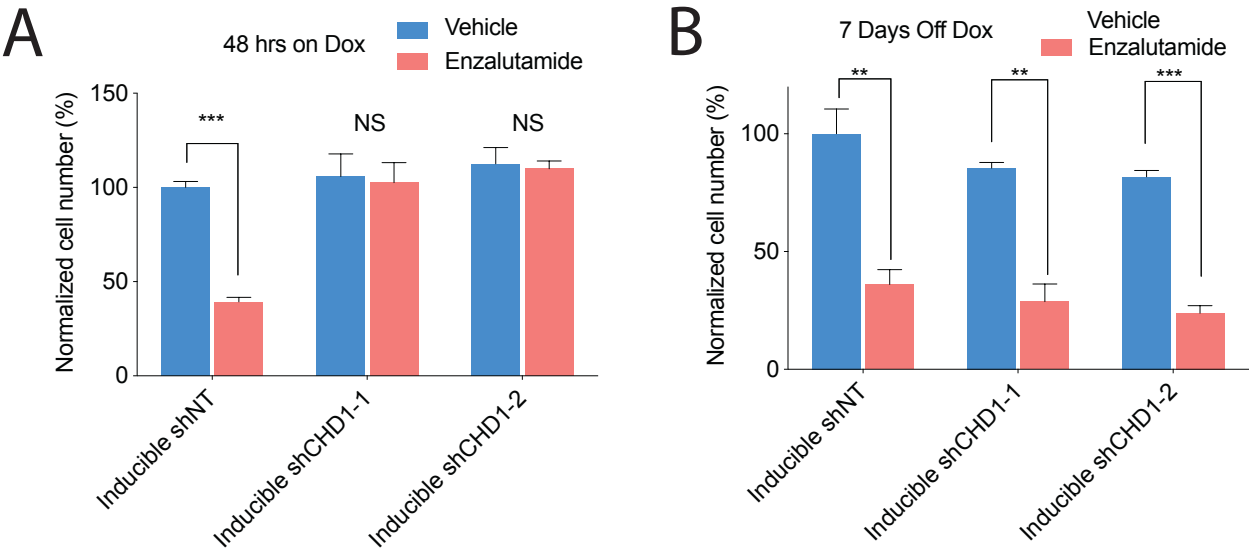


Figure 19. *(A) Relative cell number of LNCaP/AR cells transduced with annotated shRNAs in an inducible vector system, normalized to shNT+Veh. Cells were treated with doxycycline for 48 hours, and then treated with 7 days of 10μM Enz or DMSO, and cell numbers were counted. (B) Relative cell number of LNCaP/AR cells transduced with annotated shRNAs in an inducible vector system, normalized to shNT+veh. Cells were treated with doxycycline for 48 hours, remove doxycycline for 7 days, and then treated with 7 days of 10μM Enz or DMSO, then cell numbers were counted. For both panels, mean ± s.e.m. is represented, and p-values were calculated using*

*multiple t tests. For all panels, **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

3.2.2-C. CHD1-loss led to activation of Sox2, which is possible another upstream mechanism of Sox2 activation and lineage plasticity gain.

Since we observed such similar lineage plasticity and resistance in CHD1-depleted tumors as previously observed in the context of TP53/RB1 loss, we went on examined the status of Sox2 in these CHD1 depleted tumors. Remarkably, we observed significant upregulation of Sox2 in some of the CHD1-depleted tumors which are resistant to enzalutamide treatment, as shown in **Figure 20**.

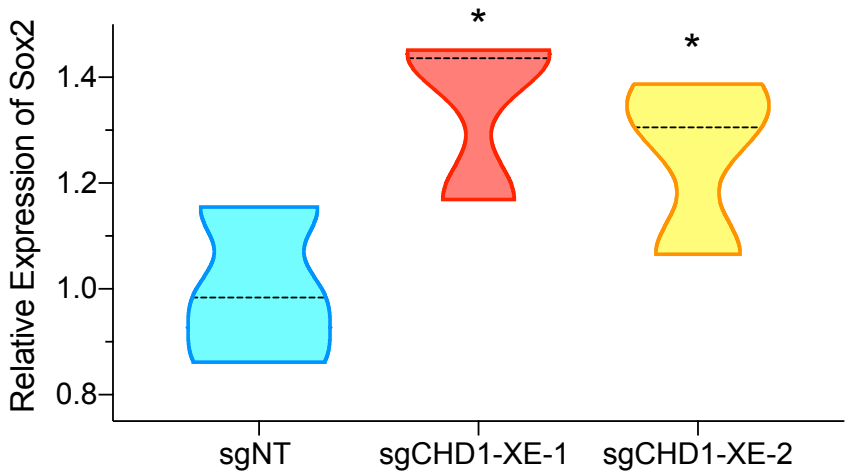


Figure 20. *Relative gene expression level of Sox2 in independently derived sgCHD1 depleted tumor xenograft cell lines, which are resistant to enzalutamide treatment. p-value were calculated by 1-way ANOVA. For all panels, mean ± s.e.m. is represented and * represents p<0.05.*

These results are particularly interesting because it provided a model that multiple upstream genomic alterations, including TP53, RB1 and our newly found CHD1 can utilize similar approach to active Sox2 and its driven lineage plasticity, as a mean for cancer cells to escape therapy. We are currently moving forward to understand the molecular details and common feature of these lineage plasticity in all the resistant models we have built.

3.2.3. Major Accomplishment 3: Identify Notch1 as the critical downstream effector of *SOX2* driven pluripotency network regulating lineage plasticity. These findings are aligned with sub-task3 in specific aim 1 in SOW.

3.2.3-A. Identify Notch1 as the top candidate downstream effector of Sox2 mediated lineage plasticity

Although reversing AR targeted therapy (antiandrogen: enzalutamide) resistance by knocking down SOX2 raises some hope that appropriate clinical interventions could prevent or overcome resistance, direct pharmacologic inhibition of SOX2 is not currently feasible. Therefore, to overcome resistance caused by SOX2-driven lineage plasticity, identifying contributions of key components of the SOX2-regulated pluripotency network in this lineage plasticity is crucial. Through RNA-seq analysis, we have identified 92 candidate genes and observed significant enrichment of the MAPK and Notch signaling pathways, including *NOTCH1*, *HES1*, *BRAF* and

MAP3K8. We have performed follow-up validation of these candidate genes and confirmed their upregulation in TP53/RB1 depleted cells, as shown in **Figure 21**.

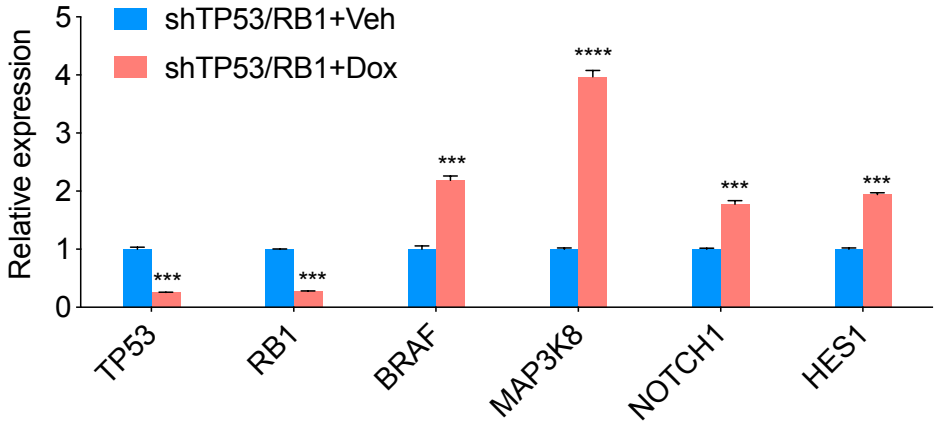


Figure 20. *Relative gene expression level of Sox2 downstream effectors in cells transduced with an inducible KD system. p-value were calculated by multiple t-test. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

To validate which of these genes are the real downstream effector, or most crucial one, we use CRISPR to knockout these genes individually and performed dose response experiments. Interestingly, the knockout of Braf or Cot both only cause partially rescue of the drug sensitivity (**Figure 21 A-B**), despite the dramatically upregulated MAPK signaling. On the other hands, both guides targeting Notch1 almost completely re-sensitize the cancer cells to enzalutamide treatment, similarly as the ones targeting Hes1, which is a downstream factor of Notch1 (**Figure 21 C-D**).

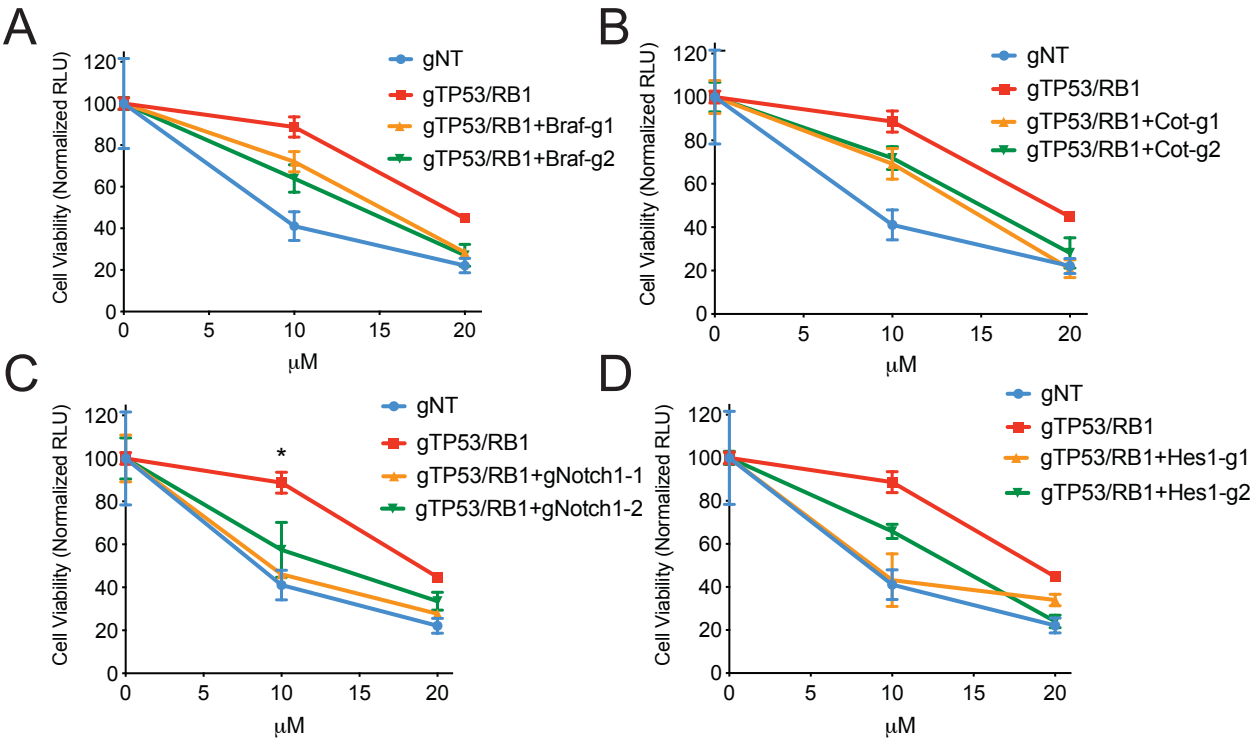


Figure 21. (A-D) *Relative cell viability as measured by Cell Titer-Glo assay, showing cells transduced by guide RNAs targeting annotated genes. p-value were calculated by one-way ANOVA. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

These data indicate that Sox2 may mediate lineage plasticity via multiple pathways and Notch1 is one of the crucial regulators, which is required for this lineage plasticity driven resistance.

3.2.3-B. Examined the efficacy using Notch1 inhibitor to treat the resistant prostate cancer cells and achieve positive results.

As described above, Notch1 is possibly one of the crucial regulators of Sox2 mediate lineage plasticity, which is required for the maintenance of AR targeted therapy resistance. Therefore, these data suggest a possible therapeutic avenue to overcome enzalutamide resistance by Notch1 inhibitor.

To test this hypothesis, we first need to establish a stable resistant cell line which has TP53/RB1 alterations and Sox2 activation, because the original cell model we built was in a multi-lineage, intermediate status and has the potential to move back to AR dependency if the selection pressure from AR targeted therapy is removed. To do so, we started to treat the sgTP53/RB1 cells with low dosage of enzalutamide to maintain selection pressure and kept the treatment for as long as 6 months. Indeed, with this prolong treatment of enzalutamide, we indeed observed stable and continues upregulation of some the canonical neuroendocrine cell lineage markers in these new cell lines named sgTP53/RB1-ER (Enzalutamide Resistant) (**Figure 22**), which make these cells good platform to test the efficacy of Notch1 inhibitor.

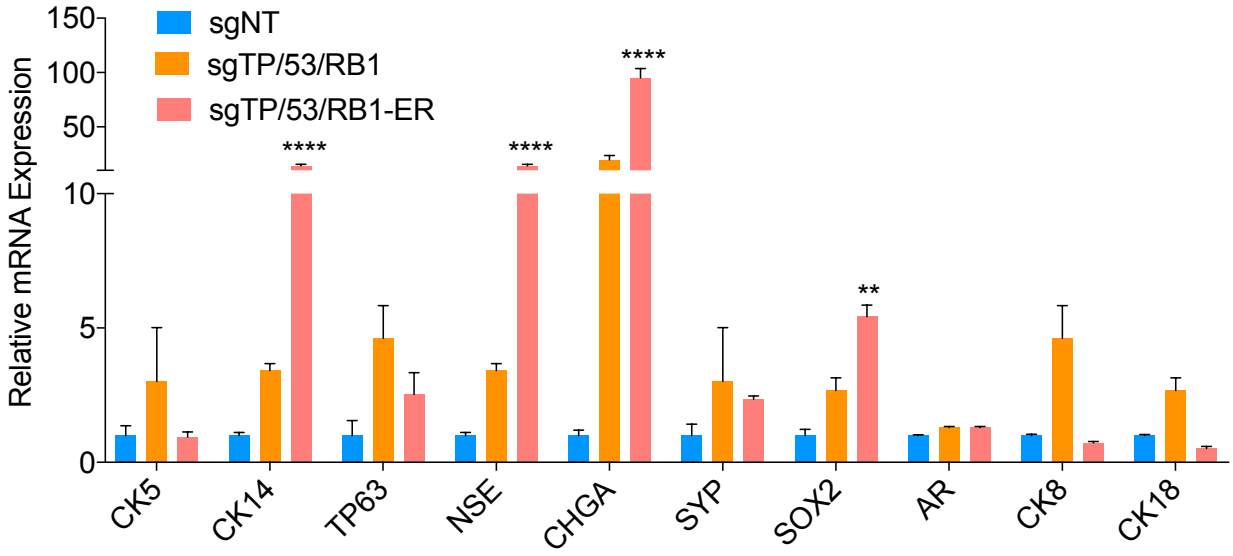


Figure 22. *Relative gene expression level of Sox2 and canonical lineage marker genes in sgTP53/RB1 cells and sgTP53/RB1-ER cells. p-value were calculated by 1-way ANOVA. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

We then gave the sgTP53/RB1 cells Notch1 inhibitor LEAF™ purified anti-human Notch1 antibody and examined the cell viability by Cell TiterGlo assay. As shown in Figure 23, the sgTP53/RB1 are completely resistant to enzalutamide treatment but very sensitive to Notch1 inhibitor. The combination of enzalutamide and Notch1 inhibitor achieved similar level of growth inhibitor as Notch1 inhibitor alone. These data provided the rationale to test Notch1 inhibitor in vivo to re-sensitize the cells.

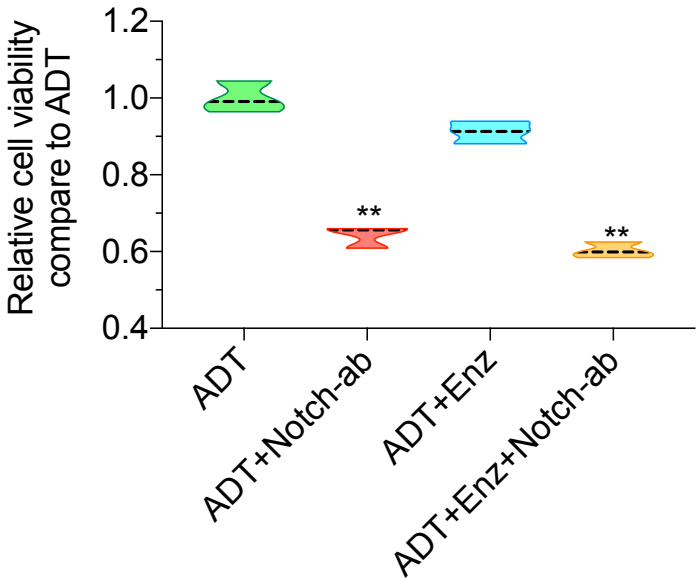


Figure 23. *Relative cell viability as measured by Cell Titer-Glo assay, showing sgTP53/RB1-ER cells treated with different combination of drugs. ADT represents androgen deprivation therapy which is achieved by CSS medium.Notch-ab represent Notch antibody. p-value were calculated by one-way ANOVA. For all panels, mean ± s.e.m. is represented and **** represents p<0.0001. *** represents p<0.001. ** represents p<0.01. * represents p<0.05.*

3.2.4. Major Accomplishment 4: Determine the lncRNAs enriched in prostate cancer models with increased lineage plasticity. These findings are aligned with sub-task1 in specific aim 2 in SOW.

As stated in introduction, long non-coding RNAs (lncRNAs) have emerged as important regulators of many physiological functions and diseases including multiple cancers. Previously reports have indicate that many lncRNAs can regulate cell fate decision, stem cell pluripotency, cell differentiation and cell reprogramming, which raise the possibility that lncRNAs may regulate lineage plasticity driven resistance.

In collaboration with Dr. Demichelis and Dr. Benelli, we analyzed three independent cohort of patients data, the IPM cohort, the old SU2C cohort and the new SU2C just published this year on PNAS (Abida et al., PNAS, 2019). We first examined the raw genomic data of all these three studies, and combined the genomic data to separate all the patients into two groups (shown in **Figure 24**), one with adenocarcinomas and one with neuroendocrine because these two types of prostate cancers have the pathological features belongs to different lineages of cancer cells.

	NE	Adeno
SU2C v2	23	309
SU2C v1	5	143
IPM-Cornell	5	23

Figure 24. *Patients separation of two cohorts based on their tumor pathological feature, adenocarcinoma (adeno) or neuroendocrine (NE) cancer.*

Then we performed AUC analysis to see if there is strong correlation between the different cohorts when comparing adenocarcinoma vs neuroendocrine cancer. As shown in **Figure 25** below, there is very strong correlation between three different patients cohort, suggesting the crucial lncRNAs are very consistent between different cohorts.

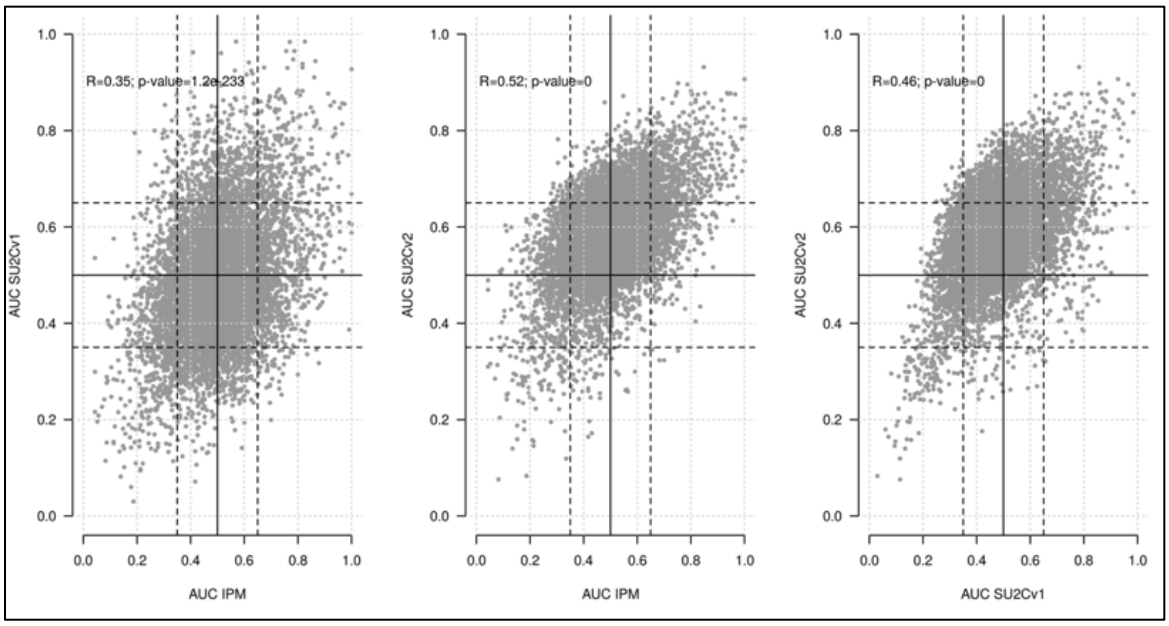


Figure 25. *AUC analysis of patients cohort showing strong correlation between different cohorts, when considering lncRNA expression. P-values were calculated by AUC analysis.*

Then we examined the genomic data of both TP53 and RB1 locus and separated all the patients into another two groups (shown in **Figure 26**), one with wildtype TP53/RB1 and one with alterated TP53/RB1 because we want to identified the top lncRNAs related to TP53/RB1 regulated lineage plasticity and resistance.

	TP53alt/RB1alt	TP53wt/RB1wt
SU2C v2	27	180
SU2C v1	63	24
IPM-Cornell	16	5

Figure 26. *Patients separation of two cohorts based on their genomic alterations in both TP53 and RB1 loci, wildtype vs alerted.*

Similarly, we performed AUC analysis to see if there is strong correlation between the different cohorts when comparing TP53/RB1 wildtype and altered cancers. As shown in **Figure 27** below, there is very strong correlation between three different patients cohort, suggesting the crucial lncRNAs are very consistent between different cohorts.

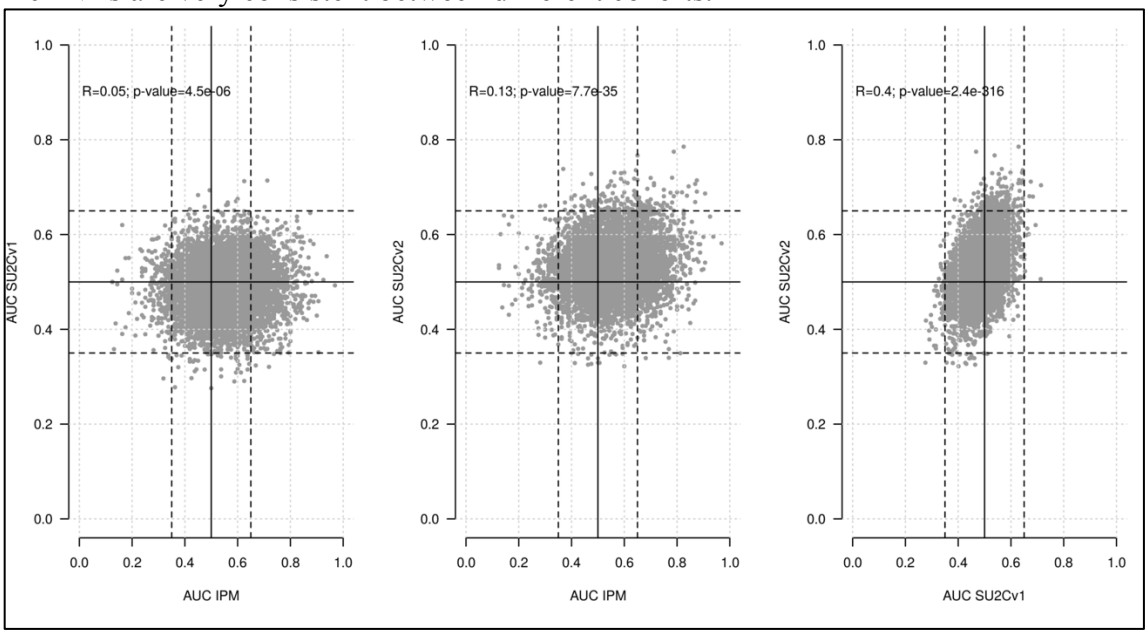


Figure 27. *AUC analysis of patients cohort showing strong correlation between different cohorts, when considering lncRNA expression. P-values were calculated by AUC analysis.*

Then we performed differentially expression analysis of all the lncRNAs and identified some of the top lncRNAs significantly expressed differently in the neuroendocrine tumors compare to adenocarcinoma (**Figure 28**), which would be greatly candidate for further investigation.

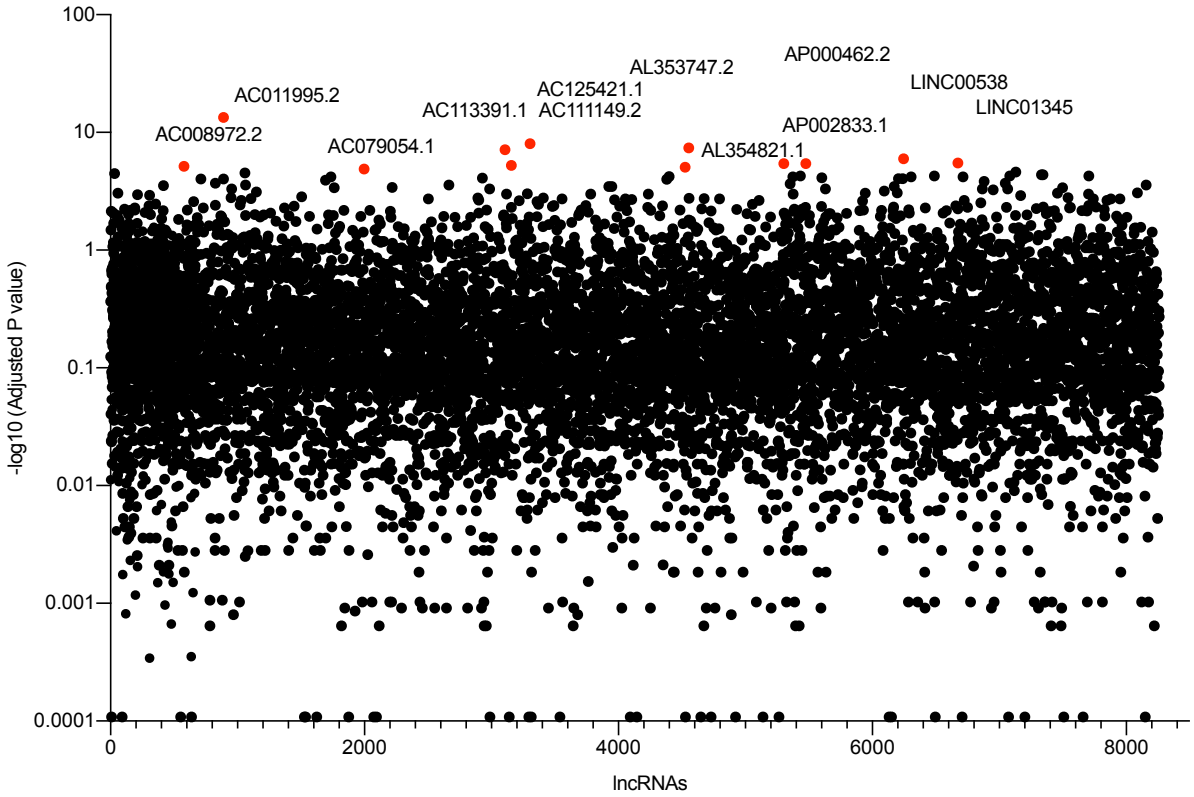


Figure 28. *-log10 of adjusted p-value of all the >8000 annotated lncRNAs, when compared the expression between adenocarcinoma patients vs neuroendocrine patients. The top possible candidate lnccRNAs with highest p value are marked as red dot.*

Based on these whole genome lncRNA analysis, we have performed validation on some of the top changed lncRNAs in both comparations. As shown in **Figure 29** below, BLACAT1 and STEAP2 are two of the major changed lncRNAs in neuroendocrine cancer compared to adenocarcinoma.

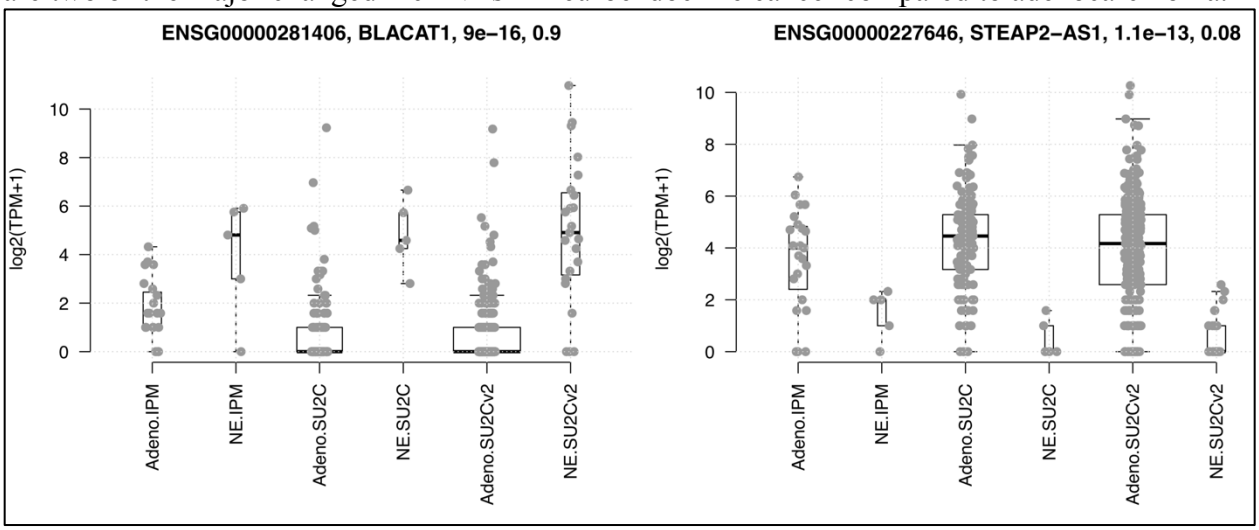


Figure 29. *Expression level shown as log2 of TPM of two top changed lncRNAs in neuroendocrine prostate cancer, compared to adenocarcinoma.*

Similarly, we have also validated some of the top changed lncRNAs in TP53/RB1 altered patients, as shown in **Figure 30** below: LINC01587 and AC098588 are two of the major changed lncRNAs in TP53/RB1 altered prostate cancer, compared to wildtype cancers.

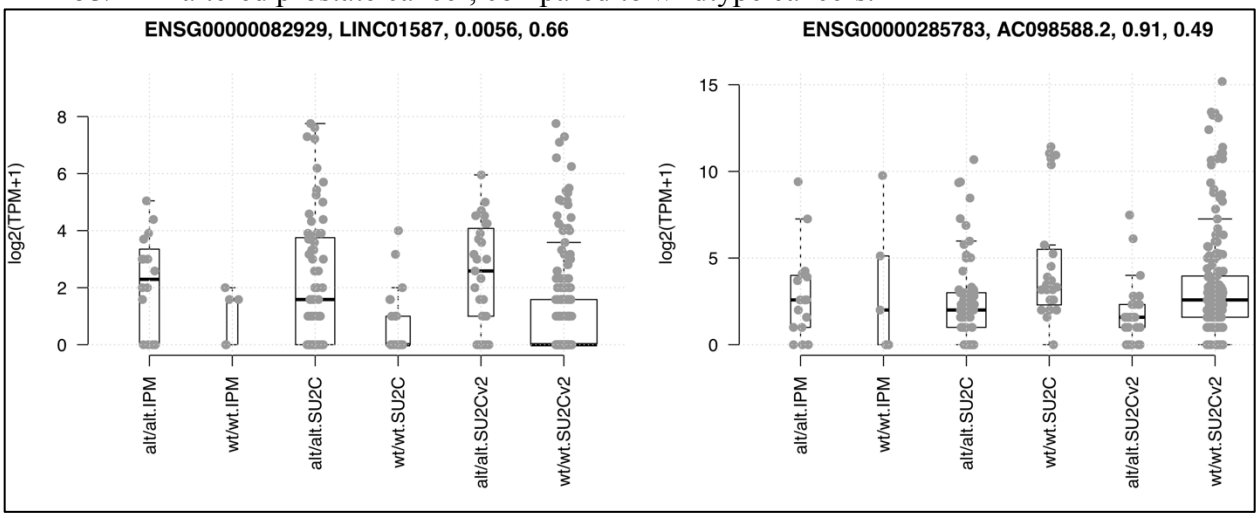


Figure 30. *Expression level shown as log2 of TPM of two top changed lncRNAs in TP53/RB1 altered prostate cancer, compared to wildtype cancers.*

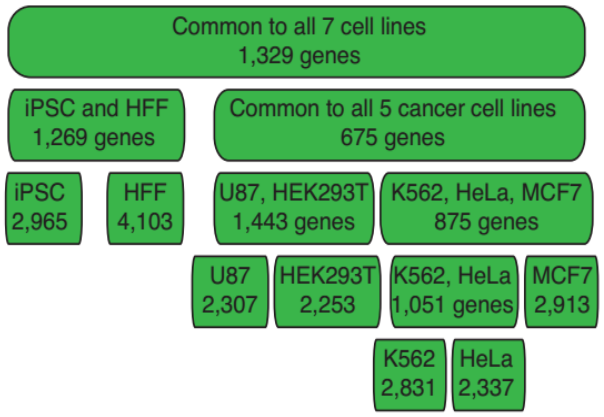
3.2.5. Major Accomplishment 5: Construct a customized CRISPRi library targeting the lncRNA candidates and perform CRISPRi-based library screen in LNCaP/AR cells. These findings are aligned with sub-task2 in specific aim 2 in SOW.

Genomic library screening has been used widely as an unbiased approach to identify novel gene or non-coding RNAs crucial to physiological or pathological functions. In the past few years, CRISPRi based whole genome library screening has demonstrated great benefit in library screening, especially in the screening of lncRNAs. Therefore, to identify lncRNAs that regulate the gain of lineage plasticity and antiandrogen resistance, we decided to first construct a CRISPRi based library targeting over 10,000 lncRNAs in the genome and perform library screening in LNCaP/AR cells for lncRNAs related to lineage plasticity and resistance. To do so, we have to achieve several key acoomplishments and we have already done several of them as following:

3.2.5-A. Construct a customized CRISPRi library targeting ~4000 lncRNAs.

We have first purchased several CRISPRisub-libraries, originally made by the Weissman lab, from addgene as shown in **Figure 31-A** below. The selected sub-libraries are based on their possible functions in tumorigenesis and cell fate regulations. The amount of lncRNAs and the sgRNA targeting them are listed in **Figure 31-B**.

A



B

Sub-library	Gene Targets	gRNAs	Control gRNAs
Common to all 7 cell lines	1329	13,548	258
Common to all 5 cancer cell	675	6903	153
U87 & HEK293T	1443	14,694	264
K562, HeLa, MCF7	875	8944	194
Total	4322	44089	869

Figure 31. *(A) Selected sub-libraries from the CRISPRi library CRiNCL (B)Numbers of gRNAs and lncRNAs within these libraries. Cited Liu et al. Science. 2017.*

Then we amplified these sub-libraries according to the published protocol. To achieve enough coverage of the library cloning, we have to greatly expand the colonies during this amplification. The final number of colonies we picked and the coverage of each sub-libraries are listed in **Figure 32** below:

library	Library sgRNA	Number of colonies (whole clonies in bioassay plate*10000)	Coverage (number of whole clonies/library sgRNA)
U87, HEK293T	14694	1237	841
K562, Hela, MCF7	8944	1100	1229
Common to 5	6903	1920	2781
Common to 7	13548	1936	1428

Figure 32. *Colonies picked and coverage of each sub-libraries.*

Then the next step, we PCR the cloned library and sent the PCR product to next generation sequencing in order to examine the coverage of correctness of the cloned library. The results of one representative PCR is shown in **Figure 33** and the correct band is marked in red.

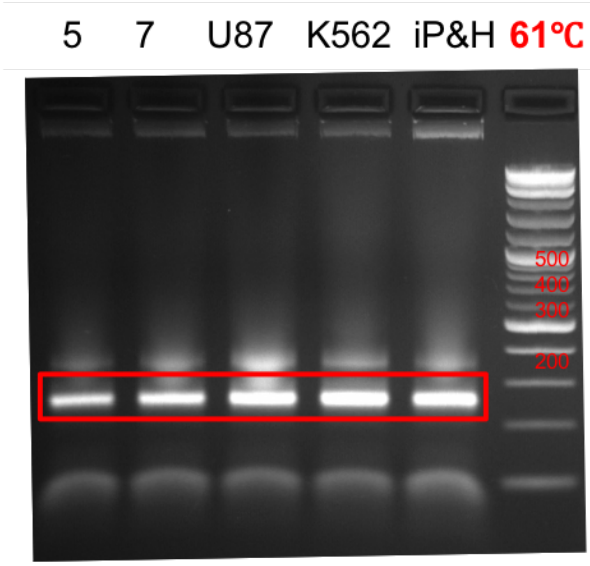


Figure 33. *Library sequencing PCR, desired band of PCR products are shown in red square.*

It took around two months for us to get all the sequencing results back and the quality of all sub-libraries we cloned are exceptional, as every library has coverage more than 99% and mapping efficiency higher than 80%. The sequencing results are shown in **Figure 34** below:

sub-library	sgRNA	Coverage representative (%)	Mapping efficiency (%)
U87, HEK293T	14694	99.932908	91.3383928
K562, Hela, MCF7	8944	99.94469	83.686001
Common to 5	6903	99.873007	83.84091
Common to 7	13548	99.91342	91.141841

Figure 34. *Results of the next generation sequencing of sub-libraries, showing the quality of each library is exceptional.*

3.2.5-B. Generate and characterize single clones used for library screening.

Since the prostate cancer cell lines are very heterogenous, which will raise serious problem when we conduct library screening because all the individual cells would evolve differently, independently to the guide RNAs transduced into the cell. Therefore, one most important step for a success library screening is generating series of single clones with high efficiency of CRISPRi mediated gene silencing and good response to enzalutamide treatment.

To generated single clones expressing good amount of deCas9, which is the key component of CRISPRi system, we first transduced LNCaP/AR cells in bulk with deCas9 contruct and the poly clonal lines are shown in **Figure 35** below:

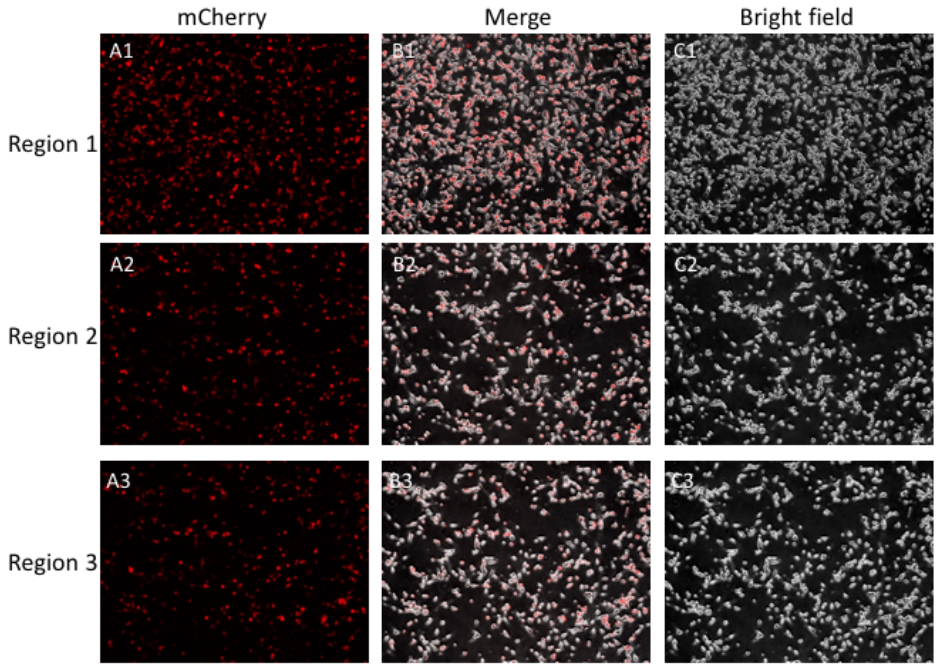


Figure 35. *Polyclonal LNCaP/AR cells transduced with CRISPRi system.*

Then we sorted the polyclonal population and cultured them in single clone for 2-3 weeks until single clone emerged into a population of cells, which can be picked up and split into single clone cell lines. The picture of several single clones we generated are shown in **Figure 36** below.

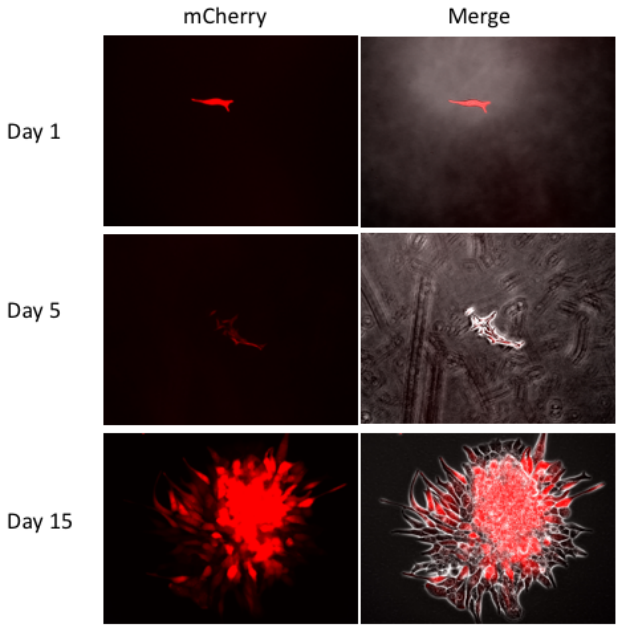


Figure 36. *Pictures of 1 single clone emerged from polyclonal cells expressed mCherry and deCas9.*

Then we characterize these single clones first by examining the expression level of deCas9 in these cells. Then we only picked the clones expressed most abundant deCas9, because the knocking down efficiency to lncRNA will be higher in these clones. The expression level of deCas9 are shown in **Figure 37** below:

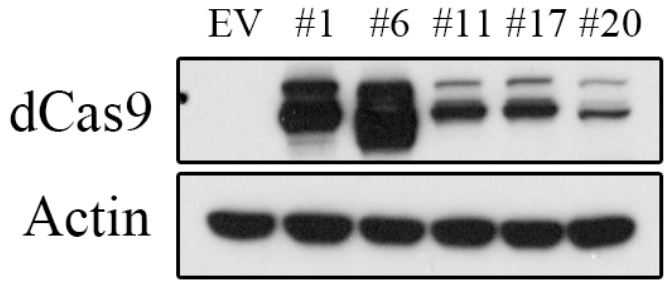


Figure 37. *Western blot showing the amount of deCas9 in different single clones.*

Then we went on to examine the response level of these single clones to enzalutamide because we have to pick a clone with good window of response for library screening. All the picked single clones were treated with enzalutamide for 7 days and responses are shown in **Figure 38** below.

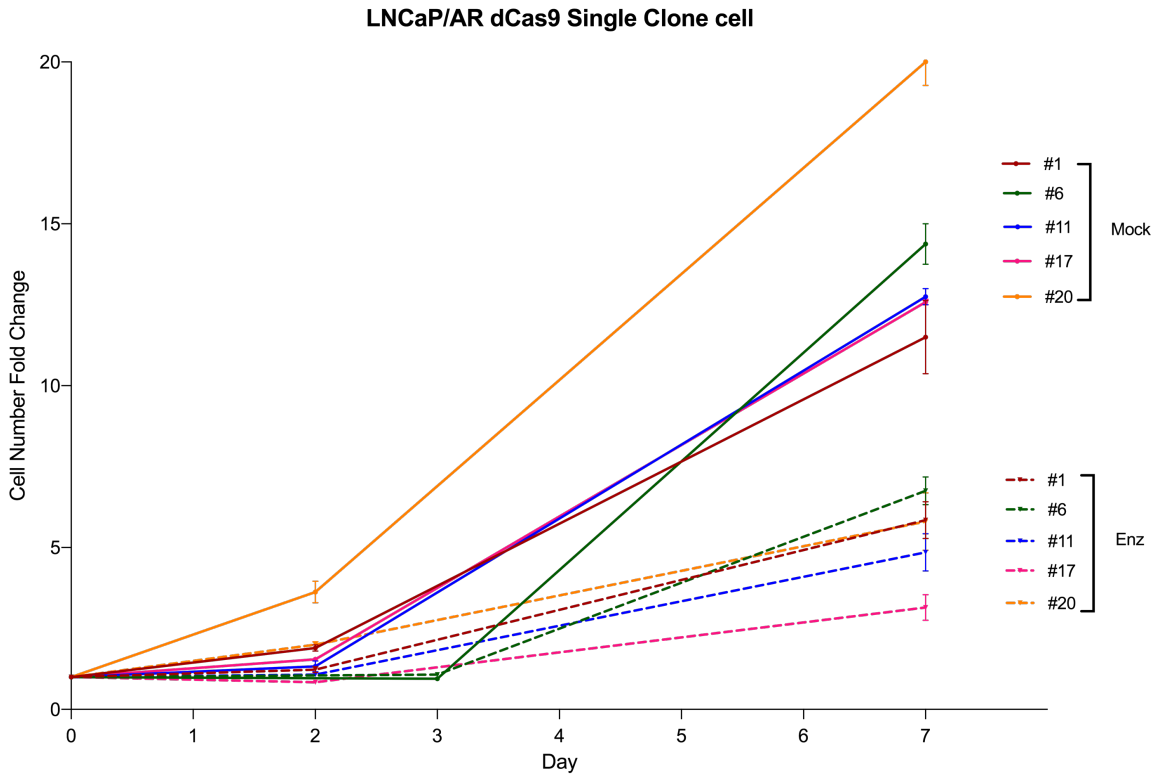


Figure 38. *Drug response curves (to enzalutamide) of different single clones expressing deCas9. Mock denotes DMSO and enz denotes enzalutamide treatment of 10μM.*

The last but most important step of characterization is validating if CRISPRi system work well in these single clones. To test that, we transduced three well characterized lncRNAs, MALAT1, PVT1 and NORAD. The CRISPRi mediated suppression of these three lncRNA in the bulk polyclonal cells are very good, as shown in **Figure 39** below:

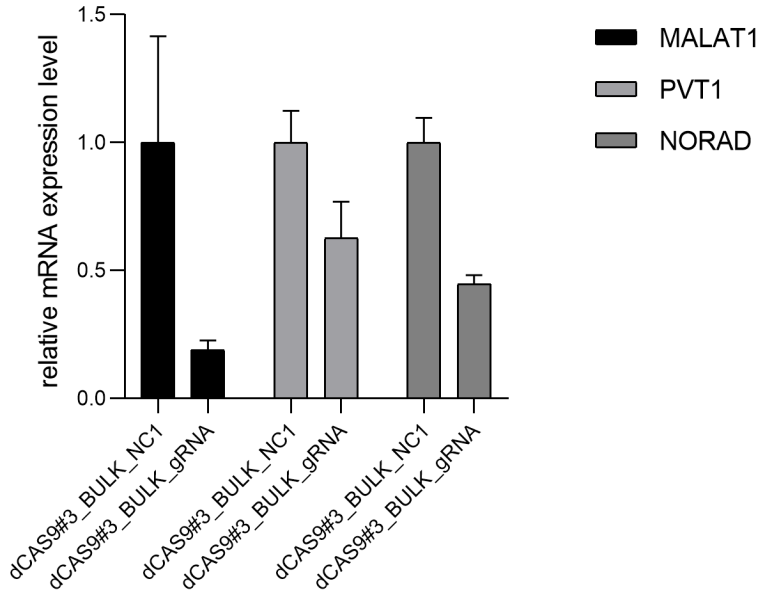


Figure 39. *Relative expression of selected lncRNAs MALAT1, PVT1 and NORAD in bulk polyclonal LNCaP/AR cell lines.*

Then we examined some of the top single clones and picked the single clone with most efficient suppression of these 2 positive control lncRNAs, as shown in **Figure 40** below.

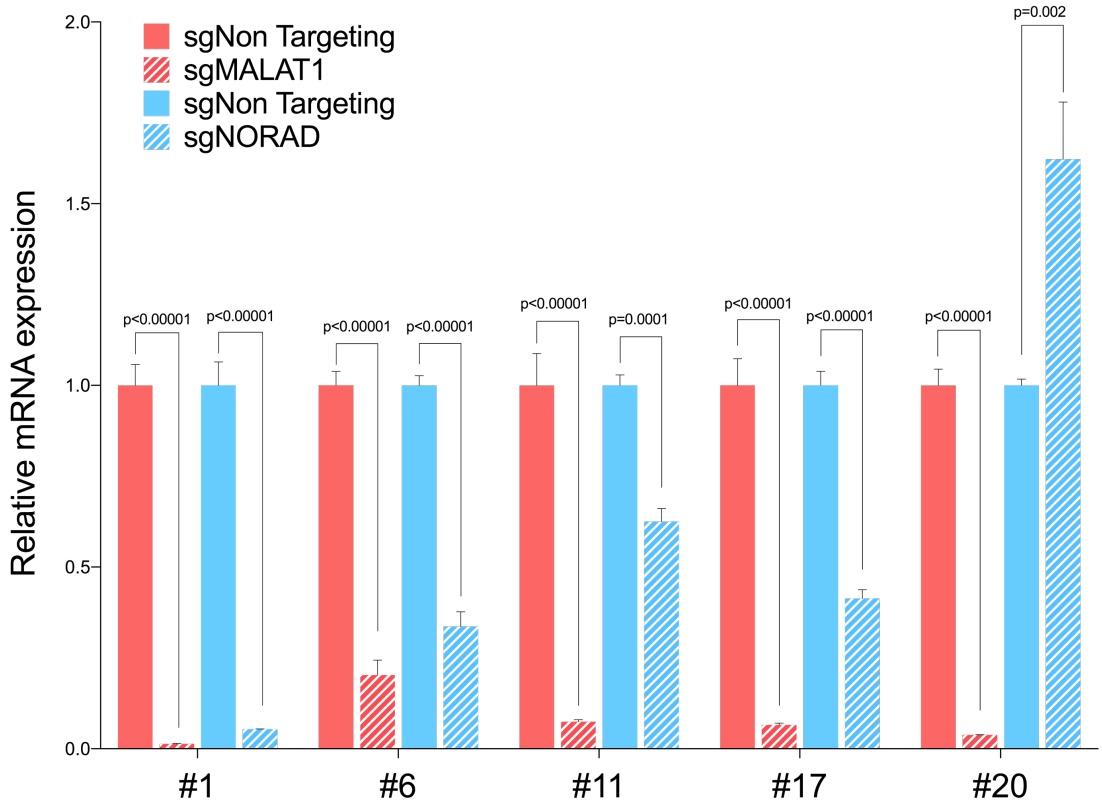


Figure 40. *Relative expression of selected lncRNAs MALAT1 and NORAD in top picked single clonal LNCaP/AR cell lines.*

3.3 Training opportunities and professional development

Nothing to Report. This award is not intended to provide training opportunities.

3.4 Results disseminated to communities of interest

Nothing to Report this period.

3.5 Plan to do during the next reporting period:

3.5.1. Validate the efficacy of Notch1 inhibitor to overcome lineage plasticity driven resistance in vivo and in more models.
As we have preliminary showed the good efficacy of using Notch1 inhibitor to overcome resistance in LNCaP/AR cells in vitro, we will then examine the efficacy in vivo as well. Furthermore, we will test the use of Notch1 inhibitor in different prostate cancer models besides LNCaP/AR to expand the influence of our finding.

3.5.2. Reveal more molecular details of the CHD1-loss mediated lineage plasticity.
Although we preliminary showed that CHD1-loss led to activation of Sox2 and increased lineage plasticity, the detailed molecular mechanism of this component of the lineage plasticity pathway is largely unclear. We will continue to reveal the molecular basis of this axis upstream of Sox2 and identify potential biomarkers for lineage plasticity.

3.5.3.Conduct a high throughput drug screening to identify new therapeutic agents.
Our work has greatly expanded the understanding of lineage plasticity driven resistance and showing that many genomic alterations, besides TP53/RB1 loss, can lead to increased lineage plasticity and resistance, such as CHD1-loss. Therefore, it would be greatly beneficial to patients and the overall impact of this project if we can find a specific inhibitor of lineage plasticity through a high throughput drug screening, using our sgTP53/RB1-RE resistant cell as a platform.

3.5.4.Conduct the lncRNA CRISPRi library screening and find key lncRNAs related to lineage plasticity and resistance.
We have already constructed the library and generated single clones express efficient CRISPRi knockdown system in the past one year. So in the following year, we will perform the library screening and identify the novel lncRNAs. The detailed library screening design and work flow is shown in **Figure 41** below.

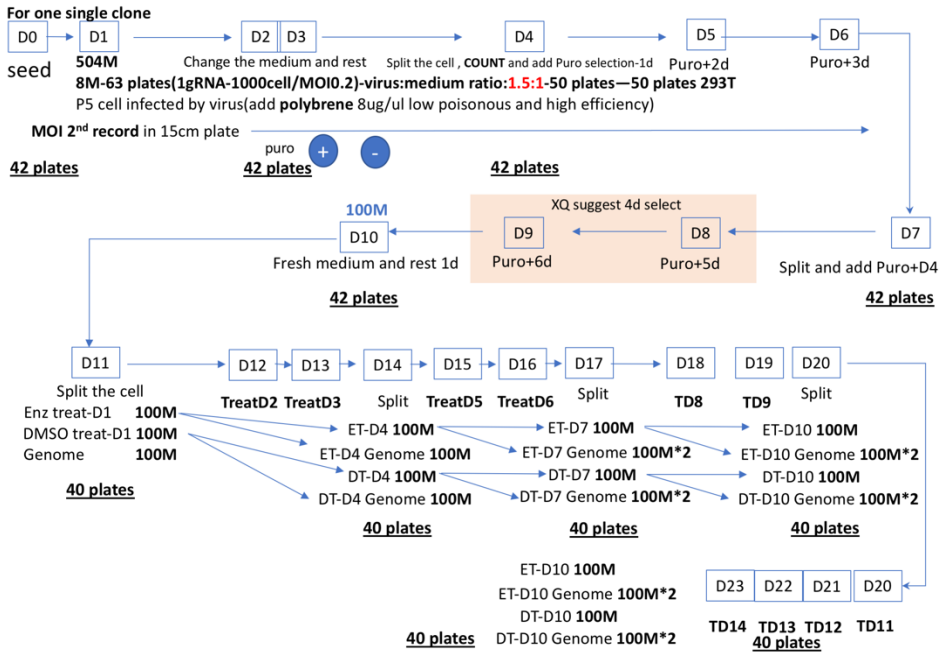


Figure 41. *Work flow and experiment design of the CRISPRi library screening in LNCaP/AR cells.*

4. IMPACT

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

4.1.1. We have comprehensively revealed that RB1 and TP53 suppressed Sox2 cooperatively through epigenetic mechanisms in the patients with metastatic prostate cancer. This finding adds great clarity to the knowledge and understanding of how genomic alterations effect the gain of lineage plasticity and resistance, which provide rationale to targeting epigenetic machinery.

4.1.2. We have tested the efficacy to targeting epigenetic machinery, such as EZH2, to overcome lineage plasticity related resistance. This finding may provide a new therapeutic avenue to overcome resistance and directly benefit patients.

4.1.3. Our finding of CHD1-loss confers lineage plasticity greatly expand the understanding of lineage plasticity beyond the scope of TP53/RB1-loss, which only happens in 10% of patients. This finding also suggests that there might be much more genomic lesions can lead to resistance through similar mechanism of lineage plasticity.

4.1.4. We have identified the most crucial mediator of Sox2 drive lineage plasticity, Notch1, and demonstrated the efficacy of Notch1 inhibitor in treating AR targeted therapy resistant tumors. This finding will likely provide rationale for novel therapeutic approach to fight AR targeted therapy resistance as well.

4.1.5. We have analyzed more than 300 patients genomic data and revealed large amount of lncRNAs differentially expressed between different type of cancers, as well as cancers with different genomic alterations, such as TP53/RB1 alterations. These results greatly expand our understanding of the roles of lncRNAs in regulating cell lineage plasticity and resistance.

4.2 What was the impact on other disciplines?

Nothing to Report this period.

4.3 What was the impact on technology transfer?

Nothing to Report this period.

4.4 What was the impact on society beyond science and technology?

Nothing to Report this period.

5. CHANGES/ PROBLEMS

5.1 Changes in approach and reasons for change.

The principle and major task of project remains the same. However, we have slightly changed some of the approaches to achieve the same goals of the project. For example, we originally designed to use CRISPR to delete RB1 gene from miR-34^{TKO} mouse organoids. However, we found the efficiency of CRISPR mediated deletion in these organoids are very low. One of the possible reasons is the low efficiency of virus transduction in 3D culture organoids. Another possible reason is lacking efficient approach to select the positive deleted clones in 3D culture system. We are still working to optimize the protocol to delete RB1 from miR-34^{TKO} mouse organoids, but since we got unexpected good results from other cancer cell line systems, we believe it is more important to move the project forward at the same time.

Another change in approach is a nature follow up on the findings from aim 1, because we found the gene deletion of CHD1 can lead to very similar activation of Sox2 and lineage plasticity. Since the loss of CHD1 are found in around 10-20% of patients, more than TP53/RB1 loss, we decided to follow up on dissecting this upstream axis of Sox2 driven lineage plasticity, in an effort to develop CHD1-loss as a novel biomarker for lineage plasticity and resistance. The manuscript reporting this exciting finding is now under revision at ***Cancer Cell.***

The last approach change is we decided to conduct a high throughput drug screening, in order to identify novel small molecules, which can reverse the lineage plasticity driven resistance. First reason for this new approach is we found that there are many more genomic alterations and pathways can active Sox2 and lineage plasticity, therefore it would be much wiser to do unbiased screen for compound targeting lineage plasticity, instead of testing existing specific drugs. Another reason is we have generated this stable resistant cell lines sgTP53/RB1-ER in the past one year, which is very resistant to enzalutamide. This line can be a great platform to perform high throughput drug screening.

5.2 Actual or anticipated problems or delays and actions or plans to resolve them.

As described above, the only technical problem we met (which is anticipated) is the genomic manipulation of 3D organoid system is much more difficult than we thought. We are still in the process of optimize the protocol to increase the efficiency of genetic modification. Another possible solution is use GEMM mouse as a platform and generated organoid which already have the desired modification. Of course, this approach is limited by the GEMM mouse resources available.

5.3 Changes that have a significant impact on expenditures.

Nothing to Report this period.

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

Nothing to Report this period

5.5 Significant changes in use or care of vertebrate animals.

Nothing to Report this period

6. PRODUCTS

6.1 Publications, conference papers and presentations

6.1.1 Publications:

(1) Zhang Z*, Zhou C*, Li X*, Barnes S, Deng S, Hoover E, Chen C, Metang L, Zhang YX, Navrazhina K, Cao Z, Wongvipat J, Choi D, Johnson N, Huang C, Wang C, Lee YS, Lee E, Yun D, Linton E, Chen X, Liang Y, de Stanchina E, Abida W, Lujambio A, Li S, Lowe S, Malladi V, Sawyers CL†, **Mu P†.** Loss of CHD1 promotes lineage plasticity and heterogynous mechanism of resistance to AR targeted therapy. ***Cancer Cell***, **in revision. *Equal contribution, †Corresponding**

(2) Hsieh JT, Lin CH, Yun EJ, Lo UG, Tai YL, Deng S, Hernandez E, Dang A, Saha D, **Mu P,** Lin H, Li TK, Shen TL, and Lai CH. The Paracrine Induction of Prostate Cancer Progression by Caveolin-1. ***Cell Death & Disease***, **in press**.

6.1.2 Conference abstract:

(1) Deng Su, Zhou Chuanli, **Mu Ping,** 04 Mar 2019. Targeting SOX2 Mediated Lineage Plasticity to Overcome Antiandrogen Resistance in Advanced Prostate Cancer. Presented at ***Nature Conference: The Tumour Cell: Plasticity, Progression and Therapy***, New York, NEW YORK.

(2) Zhou Chuanli, Deng Su, **Mu Ping**, Li Xiaoling, Zhang Zeda, 05 Mar 2019. IN VIVO SHRNA LIBRARY SCREEN IDENTIFIES CHD1 AS A MOLECULAR TUNER REGULATING CHROMATIN PLASTICITY IN PROSTATE CANCER. Presented at ***Nature Conference: The Tumour Cell: Plasticity, Progression and Therapy,*** New York, NEW YORK.

(3) **Mu Ping,** 09 Nov 2018. Identifying Novel Gene Deletions as Predicting Biomarkers of Antiandrogen Resistance in Advanced Prostate Cancer. Presented at ***Society for Basic Urologic Research 2018 Annual Meeting***, Rancho Mirage, CALIFORNIA

(4) **Mu Ping**, 24 Oct 2019. Loss of CHD1 Promotes Cancer Heterogeneity and Lineage Plasticity to Escape AR Targeted Therapy in Metastatic Prostate Cancer. Presented at ***Prostate Cancer Foundation Annual Meeting***, San Diego, CALIFORNIA.

(5) Li Xiaoling, Deng Su, **Mu Ping** 05 Mar 2019. Identifying the SYNCRIP Gene Deletion as a Novel Driver of Cancer Heterogeneity and Antiandrogen Resistance. Presented at ***Nature Conference: The Tumour Cell: Plasticity, Progression and Therapy***, New York, NEW YORK.

6.1.3 Invited speaker presentation:

(1) May 2019, ***Invited Speaker***, Biochemistry Molecular Biology (BMB) Seminar Series, Mayo Clinic, Rochester, MN, USA

(2) Nov 2019, ***Invited Speaker,*** Feist-Weiller Cancer Center 2019 Seminar Series, Louisiana State University, Shreveport, LA, USA

6.2 Other product: Nothing to Report this period

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.1 What individuals have worked on the project?

Name	Ping Mu
Project Role	PI
Researcher Identifier	0000-0003-0955-0896
Nearest person month worked	12
Contribution to project	Mr. Mu is leading the project, including experiment design, data analysis and manuscript writing
Funding Support	CPRIT recruiting award, NIH K99/R00

Name	Su Deng
Project Role	Assistant Instructor
Researcher Identifier	NA
Nearest person month worked	12
Contribution to project	Ms Deng has performed all the experiments related to investigating Sox2 regulated lineage plasticity
Funding Support	Partially by CPRIT award

Name	Yaru Xu
Project Role	Postdoc researcher
Researcher Identifier	NA
Nearest person month worked	12
Contribution to project	Ms Xu has performed all experiments related to lncRNA library screening
Funding Support	

Name	Choushi Wang
Project Role	Graduate student
Researcher Identifier	NA
Nearest person month worked	6
Contribution to project	Mr Wang assistant Ms Deng to conduct experiments related to Sox2 driven lineage plastidcity
Funding Support	Partially by NIH K99/R00

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

Yes, there are two more funding support obtained in the last period of this award, which is listed below.

I-2005-20190330 (PI: MU) 06/1/2019-05/31/2022 0.4 calendar
The Welch Foundation
$ 65,000
Small Molecule Inhibitors Targeting Lineage Plasticity and Neuroendocrine Differentiation in Advanced Prostate Cancer
This study aims to develop a high throughput screen to identify novel small molecule inhibitors that reverse the lineage plasticity, neuroendocrine differentiation and subsequent enzalutamide resistance. We will then examine the efficacy of lead compounds from the screen in various human and mouse PCa models, which will provide the first step towards a therapeutic agent with a novel mode of action specifically for treating CRPC-NE.
Agency Contact: Norbert Dittrich, President, Phone: 713.961.9884
Overlap: None

Translational Research Pilot Study (PI: MU & Tambar) 11/1/2018-10/31/2020 0.0 calendar
UT Southwestern Medical Center
$ 25,000
Selective Inhibitors of Sox2 for Targeting Lineage Plasticity Driven Antiandrogen Resistance
In this pilot study, we propose to develop novel selective inhibitors for Sox2 and examine their efficacy in various human and mouse PCa models. We hypothesize that a selective Sox2 inhibitor could counteract the Sox2 mediated lineage plasticity in driving antiandrogen resistance.
Agency Contact: Jordan Brainerd, Grant Administrator, Email: Jordan.Brainerd@UTSouthwestern.edu
Overlap: None

7.3 What other organizations were involved as partners?
Nothing to Report this period

8. SPECIAL REPORTING REQUIREMENTS
Nothing to Report this period

9. APPENDICES:
Nothing to Report this period