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TITLE: High-Throughput Screen of Advanced Prostate Cancer Organoids and PDX Preclinical Trials to Identify Single and Combination Therapies Correlated with Genotype

PRINCIPAL INVESTIGATOR: Eva Corey

CONTRACTING ORGANIZATION: University of Washington

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Eva Corey

E-Mail: ECOREY@UW.EDU

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University of Washington
4333 Brooklyn Ave
Seattle WA 98195-9472

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective: Our goal is to guide the design of future clinical trials for aggressive prostate cancer and the optimum patient selection for those trials. Our objectives are 1) to establish pre-clinically validated efficacious drugs and drug combinations together with predictive molecular correlates when possible, and 2) analyze and provide to the prostate cancer research community a large data set encompassing CRPC drug responsiveness for genotypically and phenotypically characterized patient-derived samples.

Impact: This innovative proposal is designed to address a major limitation in our knowledge concerning the breadth of therapeutic vulnerabilities for advanced prostate cancer and the molecular properties associated with drug responsiveness. If successful, we expect that novel combinations comprised of clinically translatable agents could proceed directly to biomarker-driven phase II clinical trials, addressing the PCRP Overarching Challenge to develop effective treatments and address mechanisms of resistance for men with high-risk or metastatic prostate cancer, and the PCRP Focus Area of Therapy and Mechanisms of Resistance and Response. Indeed, the NIH Clinical Center is well-poised to conduct such a trial. In addition, the availability of an extensive drug response database will provide to the community a platform that can be further leveraged for preclinical studies, bioinformatics/statistical mining, and mechanistic analysis.

15. SUBJECT TERMS NONE LISTED

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1. INTRODUCTION:

Metastatic castration resistant prostate cancer (mCRPC), which develops in response to suppression of androgen receptor pathway signaling, is responsible for almost all prostate cancer-related deaths. The development of therapeutic approaches for advanced prostate cancers have centered upon androgen receptor (AR) signaling pathway inhibition (ARIs), sometimes followed by taxane or platinum chemotherapeutics. Thus, there are multiple agents for the same target, AR, but few agents for other key vulnerabilities. However, clinical and genomic characterization of mCRPC tumors have revealed substantial heterogeneity with respect to various drivers of disease progression and mechanisms of resistance. Outside of ARI based therapies, *BRCA1* and *BRCA2* deficiencies are the only approved genomic biomarkers for targeted therapies in CRPC. We seek to discover additional effective therapies for mCRPC and to identify phenotypic or genomic properties that guide their use. This project takes advantage of using a large collection of mCRPC patient derived xenografts (the LuCaP PDX cohort) that represent the genomic and phenotypic diversity of patient tumors in combination with newly developed organoid culture techniques that have enabled in vitro growth of the above PDX models. The purpose of the project is to establish novel efficacious drug responses, singly and in combination, and to identify associated molecular markers.

2. KEYWORDS:

Prostate cancer, high throughput screening, organoids, patient-derived xenografts, effective treatment, combination therapy

3. PROGRESS REPORT

SPECIFIC AIM 1: IDENTIFY AGENTS WITH HIGH ANTI-TUMOR SUPPRESSIVE ACTIVITY USING PC ORGANOIDS AND PATIENT-DERIVED XENOGRAFTS

The specific objectives of this aim were to perform the high throughput drug screen using organoids (**Fig. 1**, see appendix) and validate selected agents' efficacy in vivo. A number of novel conclusions have been made, representing the first comprehensive analysis of responsiveness to multiple drug classes in vitro based on the high-through put screen using organoids and validation in vivo using PDX models. We have screened 35 CRPC organoid models (>45 high throughput screens, HTS) and LNCaP and RWE cell lines against more than 110 drugs. Importantly, as this is one of the first comprehensive organoid screens, we have also validated the screening results using independent biological replicates in both robotic high throughput and lab bench formats. After the first 20 screens, we made adjustment in the therapeutics library, eliminating therapeutics that showed no evidence of activity (about 20 drugs) and replacing these with other drugs of interest (**Fig. 2 and 3**, see appendix)

We have selected specific agents that showed activity in HTS in vitro for in vivo testing. The *in vivo* data showed that volasertib treatment demonstrated complete response in 3/12 PDX models, and partial response in 5/12 models while roniciblib, S63845 and carboplatin were generally not efficacious. while the other monotherapies we evaluated (roniciclib, S63845, carboplatin) had generally no to low efficacy B (**Fig. 4**).

SPECIFIC AIM 2: DETERMINE EFFICACY OF COMBINATORIAL TREATMENT STRATEGIES OF SELECTED AGENTS

We have analyzed the HTS results and our analyses identified options for combination therapies. Agents targeting mitotic and replicative processes, apoptosis, cell cycle regulation, and DNA damage repair have significant efficacy overlap. (**Fig. 5**). We have tested selected combination therapies in vivo and showed that the combinations tested exhibited significant anti-tumor activities in adeno-CRPC as well as in NEPC (**Fig. 6A**). In five of the models tested combination of roniciclib and carboplatin provided a complete response while the monotherapies' efficacy was minimal. (**Fig. 6A and B**). In additional in vivo tested

combinations, we used inhibitors of proliferation and apoptosis, Plk1, MCL1 and bcl2 inhibitors, and similarly the other combination treatment exhibited stronger efficacy when compared to monotherapies in some models (**Fig. 6C**).

SPECIFIC AIM 3: INTEGRATE AND ANALYZE PDX MOLECULAR CHARACTERISTICS AGAINST RESPONSE TO THERAPEUTIC REGIMENS AND IDENTIFY MOLECULAR DETERMINANTS OF RESPONSES AND CANDIDATE PREDICTIVE BIOMARKERS.

These major activities were focused on analyzing in vivo responses with transcriptomic signatures of the models.

First, we performed RNA-Seq analyses of the organoids, and the analysis clearly shows separation of the tumors based on their histological classification (**Fig. 7**). We have also identified the response to a cluster of drugs, enriched for activity in the G2/M phases of the cell cycle, across multiple models that include both NEPC and adeno-CRPC. Importantly, these models can be identified by direct or indirect loss of *RB1* activity. *RB1* loss and replication stress initiated by distinct DNA repair mutations are biomarkers of drug responsiveness (**Fig. 8**).

Additionally, we have analyzed the response to docetaxel, a highly clinically relevant drug for CRPC, and determined responder and non-responder biomarker classes. We have identified docetaxel response signature that highly corelates with the response (**Fig. 9).** We have also identified a class of drugs (apoptosis inducers) that appear to synergize with docetaxel. Our data showed that docetaxel responsiveness is correlated with responsiveness to BCLXL inhibitors, suggesting that those models that are responsive to docetaxel have ongoing apoptotic stress, inhibited at least in part by BCLXL. These data suggest that taxanes in combination with BCLXL inhibitors or other appropriate drugs that target apoptosis downstream of BCLXL may convert some models of taxane non-responders to responders. Finally, we made a highly novel and important discovery that docetaxel non-responsiveness in adeno-CRPC is highly correlated with HNF1 expression and with an HNF1-driven transcriptomic signature **(Fig. 10 and 11).**

The interrogation of RNA-Seq of the parental PDX, and showed significant transcriptomic differences between adeno-CRPC and NEPC **(Fig. 12)**. Moreover, when interrogating correlation of responses to roniciclib with transcriptome of the tumors our data showed that responding tumors had lower expression of FGFR and MEK signaling gene sets. **(Fig. 12C).**

In summary, we have accomplished all tasks of our specific aims and our results provide significant novel information about potentially clinically efficacious agents and their combinations as well as a signature of responsiveness to docetaxel, and role of NHN1alpha. The partnering PI, Dr. Kelly is working on additional validation of the docetaxel signature and HNF1 alpha in docetaxel resistance in her additional one year NCE.

How were the results disseminated to communities of interest?

The Kelly lab and Corey lab teams meet frequently via zoom to discuss ongoing experiments and the interpretation of results. The opportunities for trainees to present work has been limited due to COVID restrictions. However, postdoctoral fellows from Dr. Kelly's lab have presented data from this project at the Laboratory of Genitourinary Cancer Pathogenesis and the Data Sciences departmental seminar series as well as to the CCR Prostate Cancer PI Working Group. The results were also presented at the University of Minnesota Cancer Center, the NIH Prostate SPORE annual meeting and Fred Hutchinson Cancer Center/University of Washington Cancer Consortium. The data has also been shared by investigators who request information.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
The HTS data will be widely used throughout the prostate cancer community. We anticipate publishing our generated results in 2023. Under this award we performed the first comprehensive drug screen coupled with molecular markers, allowing generalizations and in-depth correlative analyses. The data will be used by basic researchers investigating mechanisms of drug response as well as translational/clinical investigators designing clinical trials. In particular, we have identified treatments and combination treatments that were highly effective in vivo for Adeno-CRPC as well as NEPC. Moreover, we have identified a docetaxel response signature and a new biomarker (HNF1) that potentially can be used to make treatment decisions for docetaxel, a commonly used drug for castration-resistant prostate cancer. This data contributes to clinical trial design for prospective biomarkers.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report.

Actual problems and delays
The COVID pandemic has slowed the completion of work due to delays in receiving supplies and mice as well as insufficient personnel at NIH. This slowed down obtaining data through technical core-supported work as well as animal studies that rely on specialized veterinary technical contributions. Similarly, at the University of Washington, there were delays due to receiving supplies as well as limitations on personnel being allowed in vivarium and present at the work site. We were experiencing problems that were occurring throughout the scientific community. Work slowdowns were sporadic and outside of our control, but we did our best to adjust and with the one year no cost extension we accomplish the major tasks.

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
Nothing to report.

Journal publications:
Nothing to report.

Books or other non-periodical, one-time publications:
Nothing to report.

Other publications, conference papers, and presentations:
Chung Lee Lectureship at Lurie Cancer Center, Northwestern University, Chicago
Sidney Kimmel Cancer Center seminar series, Thomas Jefferson University, Philadelphia
FDA Neuroendocrine Prostate Cancer Mini-symposium, NIH SPORE Annual meeting, FHCC/UW cancer consortium.

Website(s) or other Internet site(s):
Nothing to report.

Technologies or techniques:
We have developed techniques for high throughput screening of organoids and for improved castration-resistant prostate cancer organoid growth. We have also identified effective dosing regimens with no/low negative side effects in the in vivo experiments with the selected agents.
We have shared our protocols with several laboratories upon request.

Inventions, patent applications, and/or licenses:
Nothing to report.

Other Products:
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Kathleen Kelly- NO CHANGE
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	Eva Corey- NO CHANGE
Project Role:	
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	Craig Thomas- NO CHANGE
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
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?
Nothing to Report.

8. APPENDICES:

SUPPORTING FIGURES

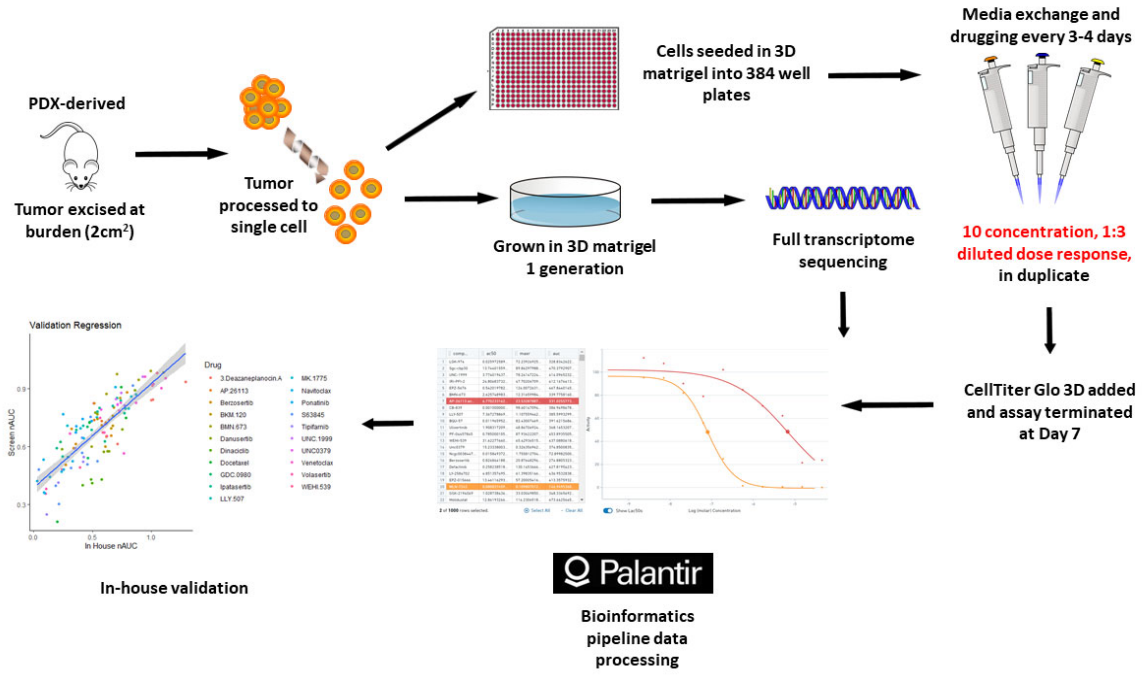

Fig. 1. Scheme of HTS.

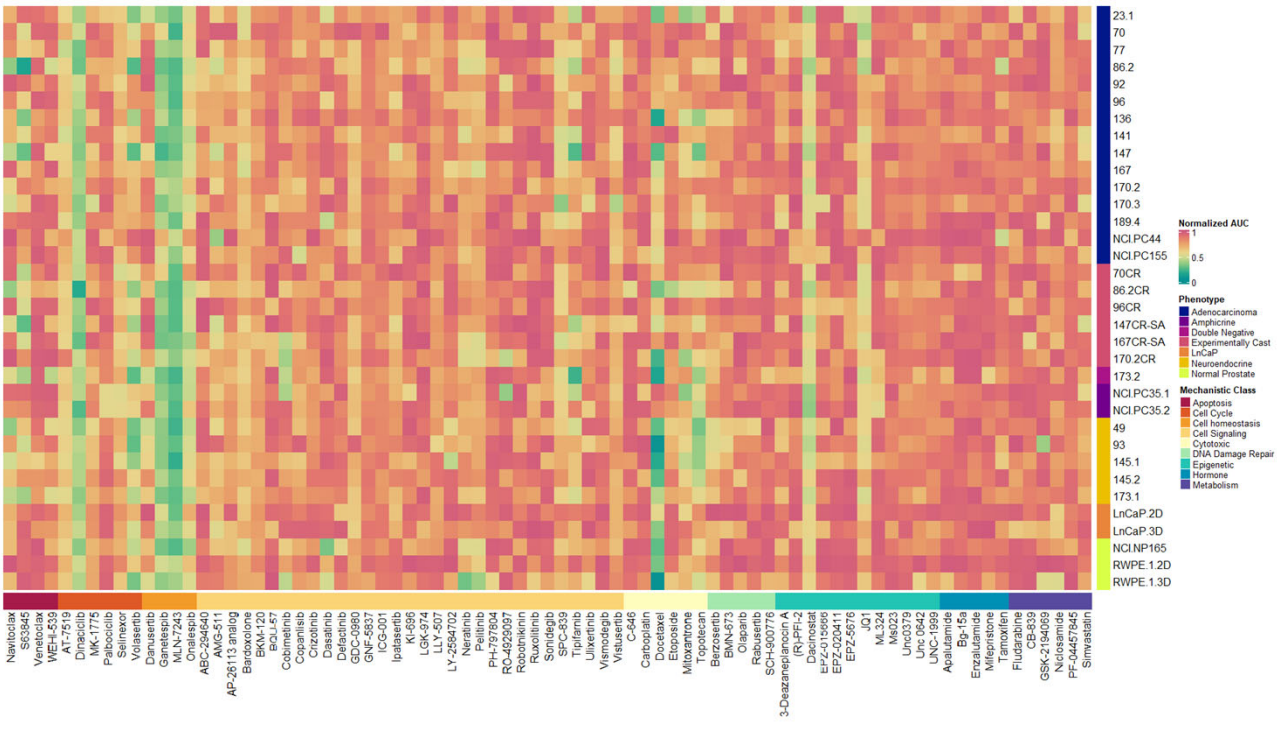

Fig 2. Heatmap of normalized area under curve (nAUC) as a single continuous variable summarizing response across 10 doses of every compound tested against every organoid. Organoids are annotated by phenotype and drugs are annotated by mechanistic class.

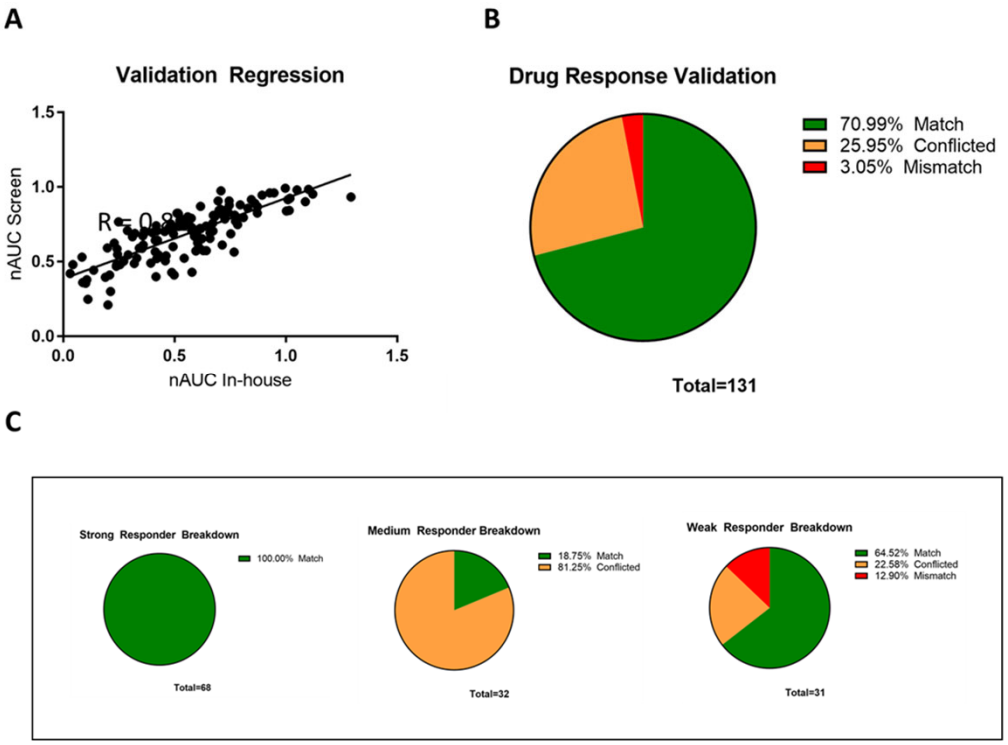

Fig. 3. (A) linear Regression of nAUC derived from the screen matched with the same model-drug pair tested as biological replicates in house. (B) Compound-specific validation using screen-wide nAUC average with "strong responders" classified as organoid responses failing in the top 33%, "weak responder" as bottom 33% and all others as "medium responders". Match conditions require preserved response class from screen to in-house, "conflicted" are medium responders in the screen which are classified as weak or strong in-house and mismatch requires a screened strong responder to perform as a weak responder in-house or a vice versa. (C) For each responder class, percentages of compounds matching conflicting, and mismatching.

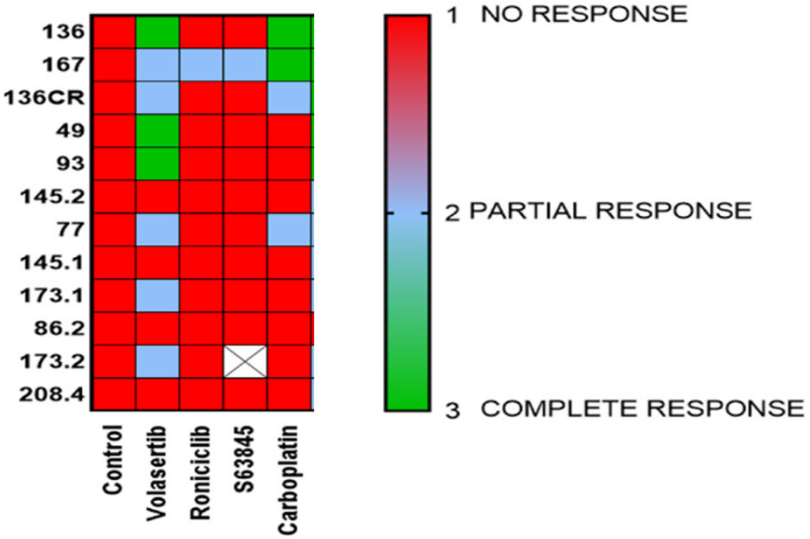

Fig.4. In vivo responses to monotherapies

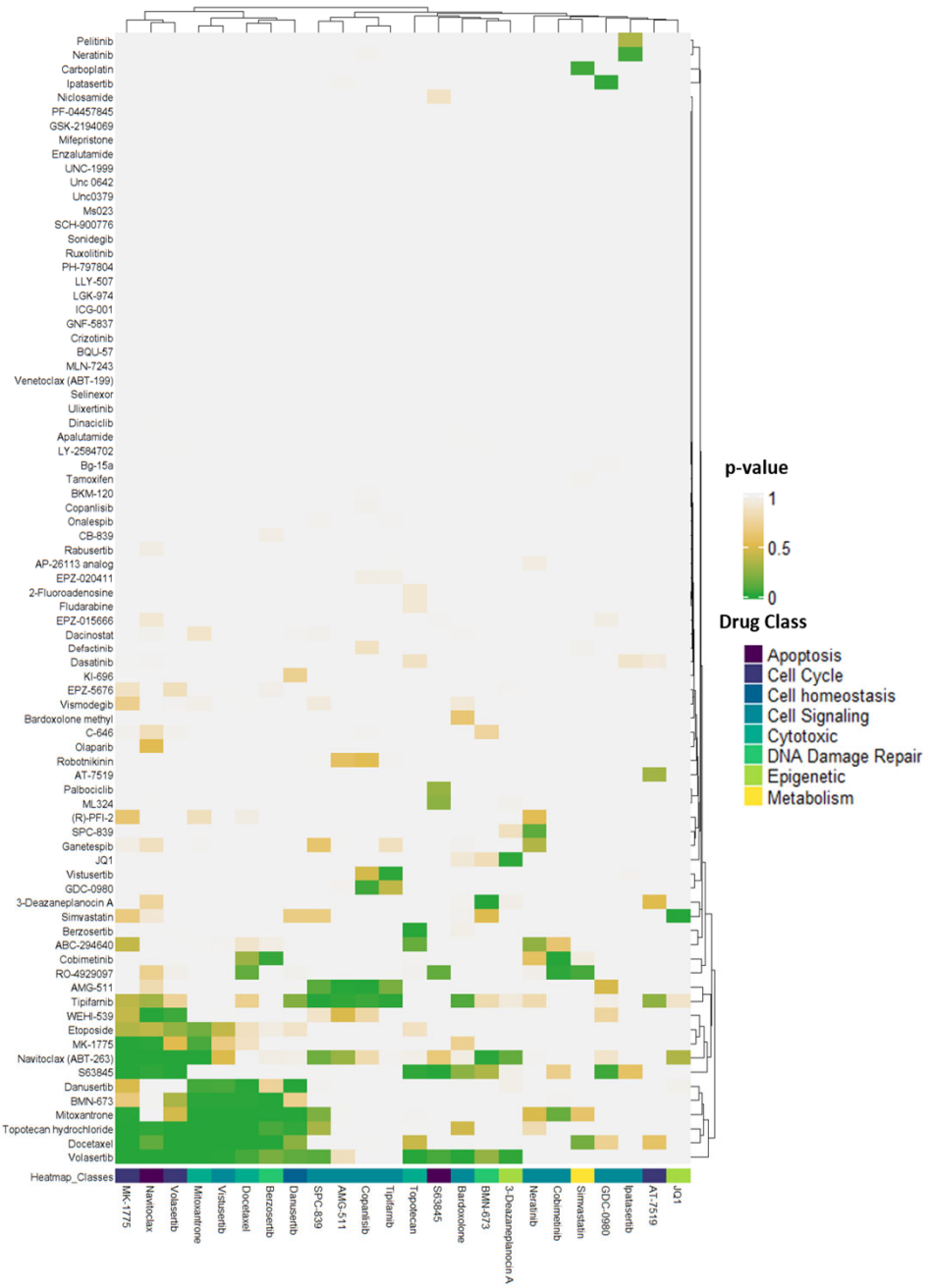

Fig. 5. Heatmap representing p values showing statistically co-enriched positive responses across screened models based on individual compounds compared to other compounds in the screen to suggest options for combination therapy. Agents targeting mitotic and replicative processes, apoptosis, cell cycle regulation , and DNA damage repair have significant efficacy overlap.

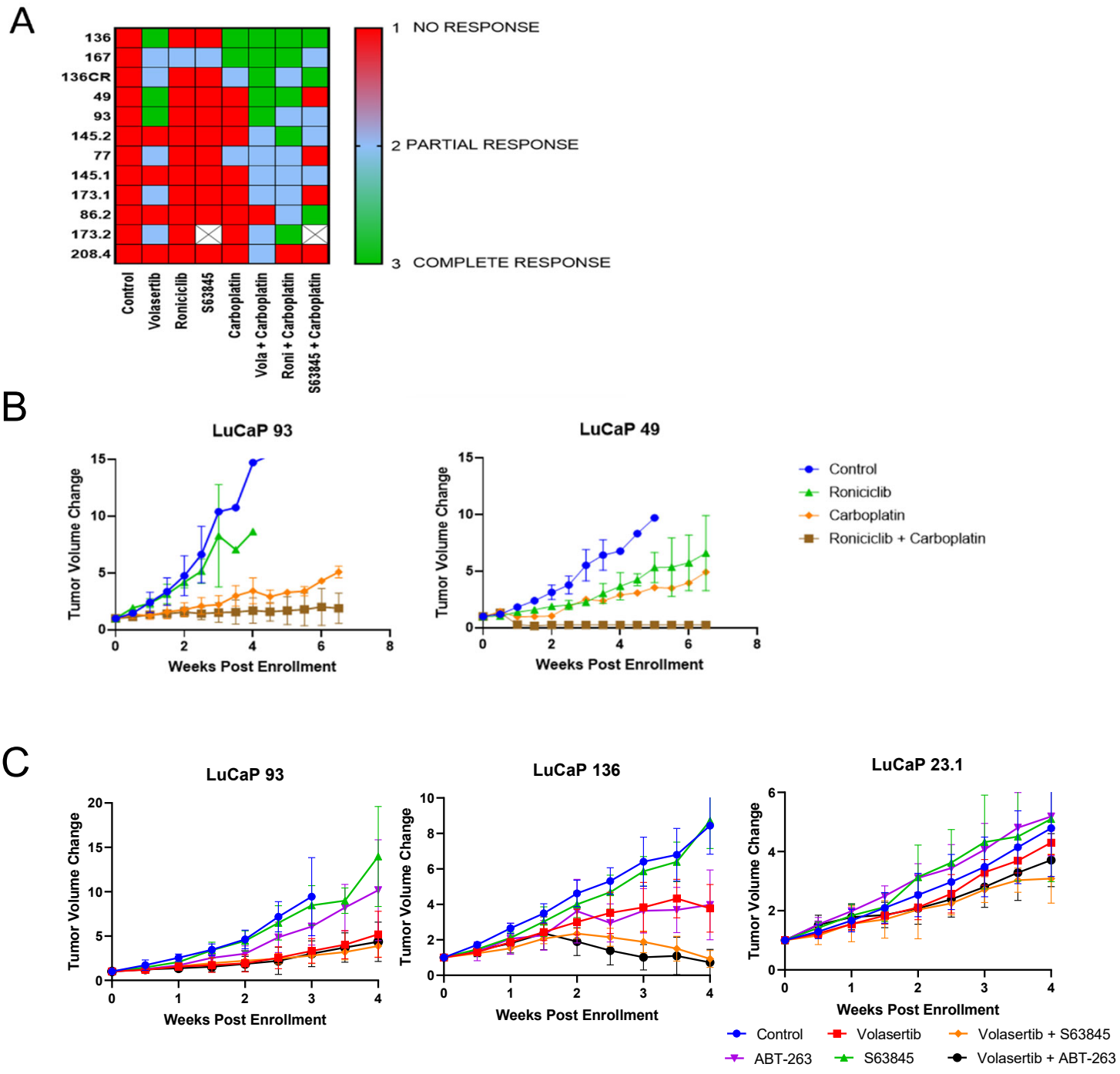

Fig. 6. (A) Categorical responses *in vivo* demonstrate high efficacy of combination therapy with carboplatin.(B) Example tumor vs time response curves showing data behind categorical heatmap. (C) In vivo responses to combination of Plk1 with BCl2 or MCL1 inhibitors

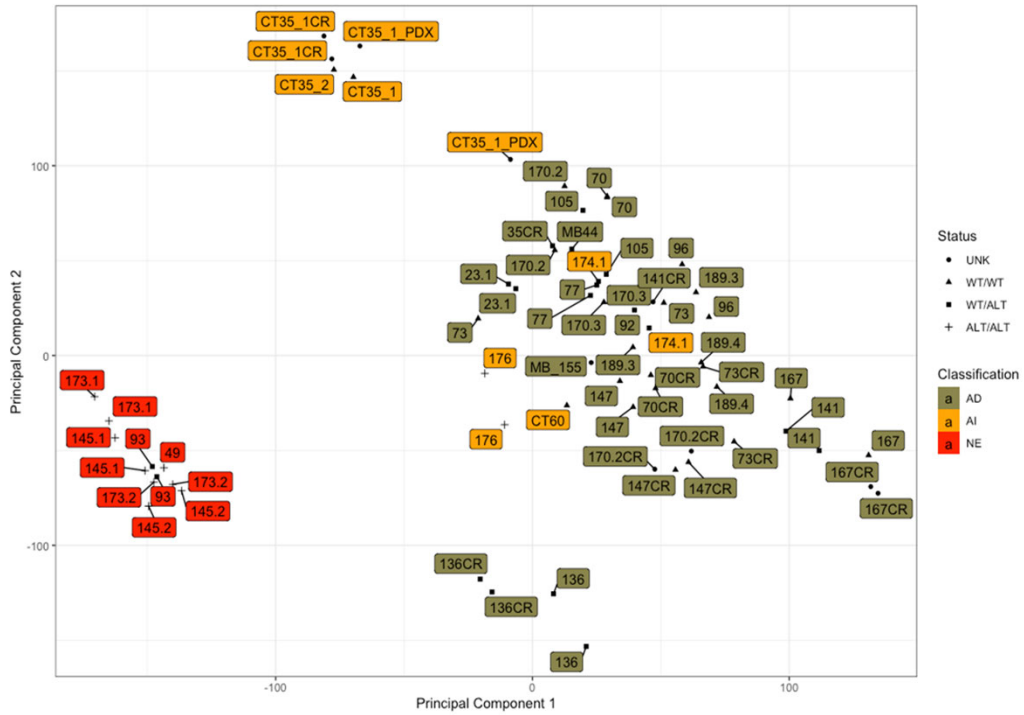

Fig. 7. Principal component analysis of organoid RNA-Seq data. Individual models are labelled with colors by their histological classifications; adenocarcinoma (AD), amphicrine (AI), and neuroendocrine)NE). Mutational status of RB and TP53 is encoded using shape with status recorded as unknown at both loci (UNK) or wild type (WT) or alternative (ALT) at both loci for RB and TP53 respectively.

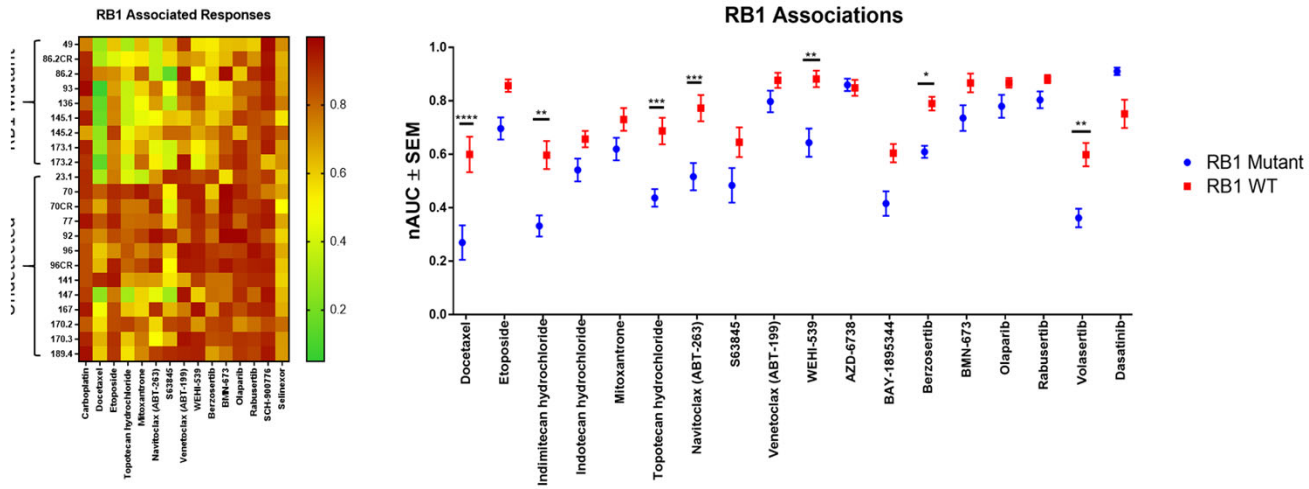

Fig. 8. RB1 status segregates models in terms of drug efficacy for a variety of compounds spanning cytotoxic, apoptosis, DNA damage response, and cell cycle regulatory mechanisms. ****denotes p<0.001, *** p<0.005, * P,0.05

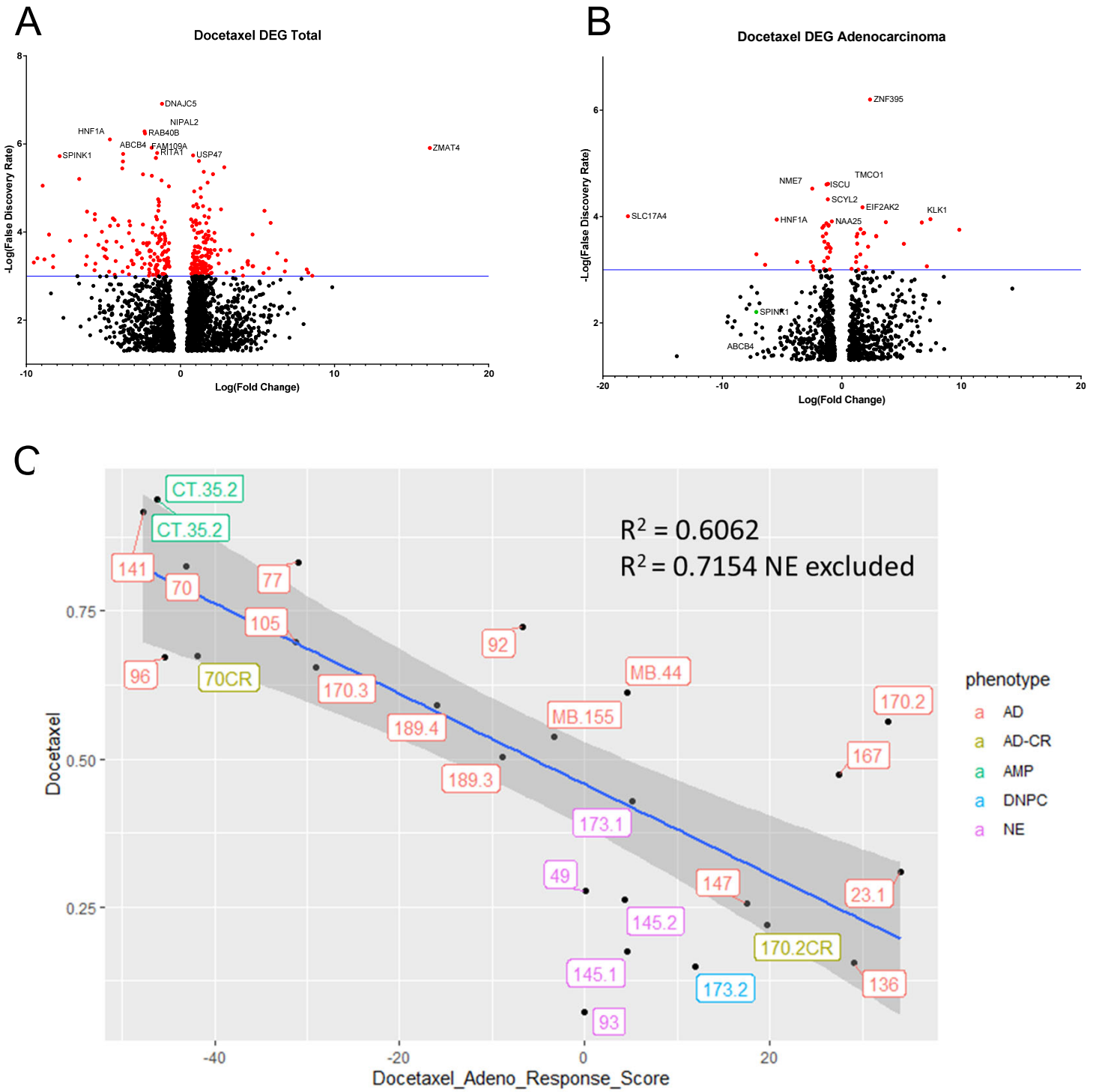

Fig. 9. Determination of Biological Characteristics of Docetaxel Resistant vs Sensitive Phenotypes. (A) all models. (B) adeno CRPC. (C) Docetaxel response- signature in PDXs

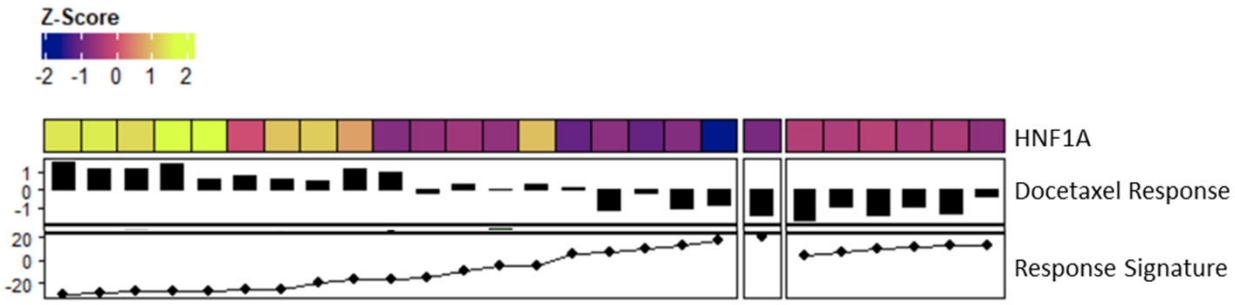

Fig. 10. Composite summary showing HNF1A is almost exclusively expressed in docetaxel resistant models, while docetaxel response and HNF1A expression correlates strongly with Response Signature.

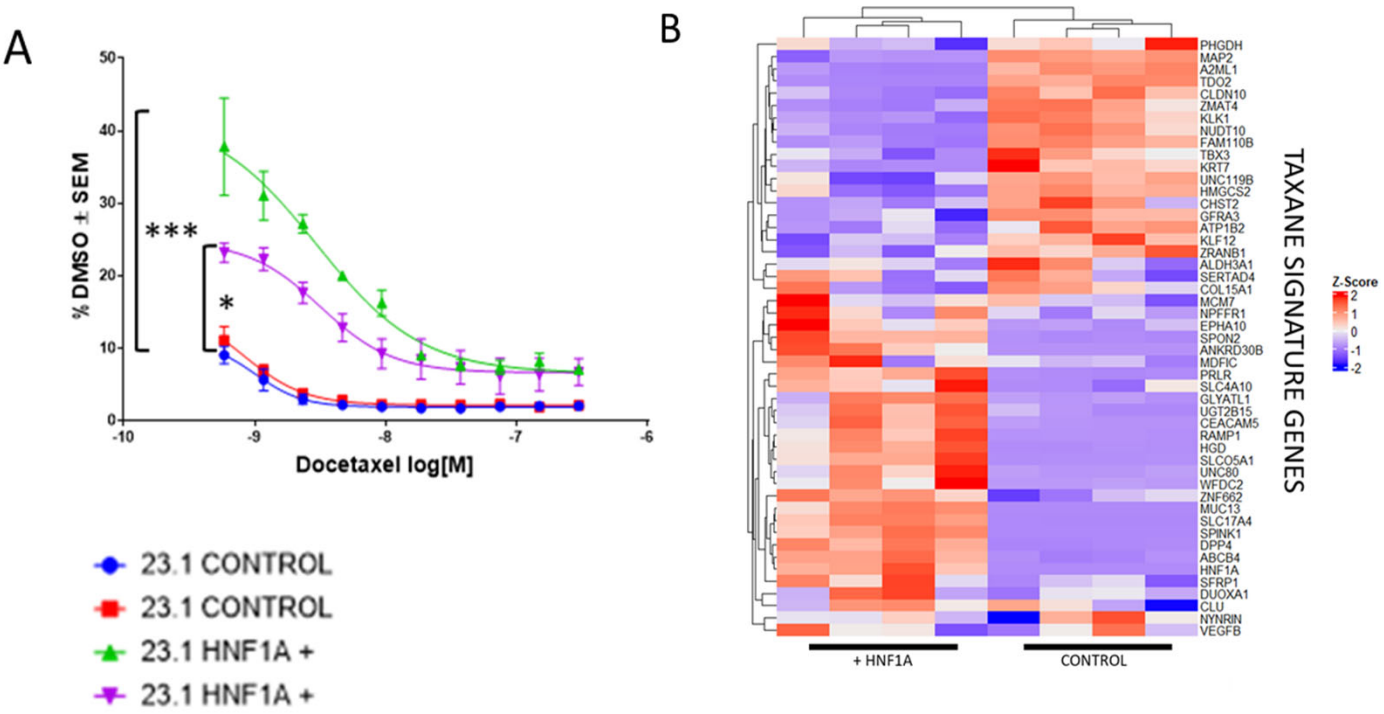

Fig.11: (A) Drug response curves comparing 2 biological replicates of LuCaP 23.1 ectopically expressing HNF1A vs. control vector. *** denotes $p < 0.001$, * denotes $p < 0.05$. (B) Transcriptomic heatmap comparing 4 biological HNF1A+ 23.1 replicates with their corresponding native controls showing directional change in nearly every gene present in the response signature upon expression of HNF1A confirming its transcriptional role in signature score.

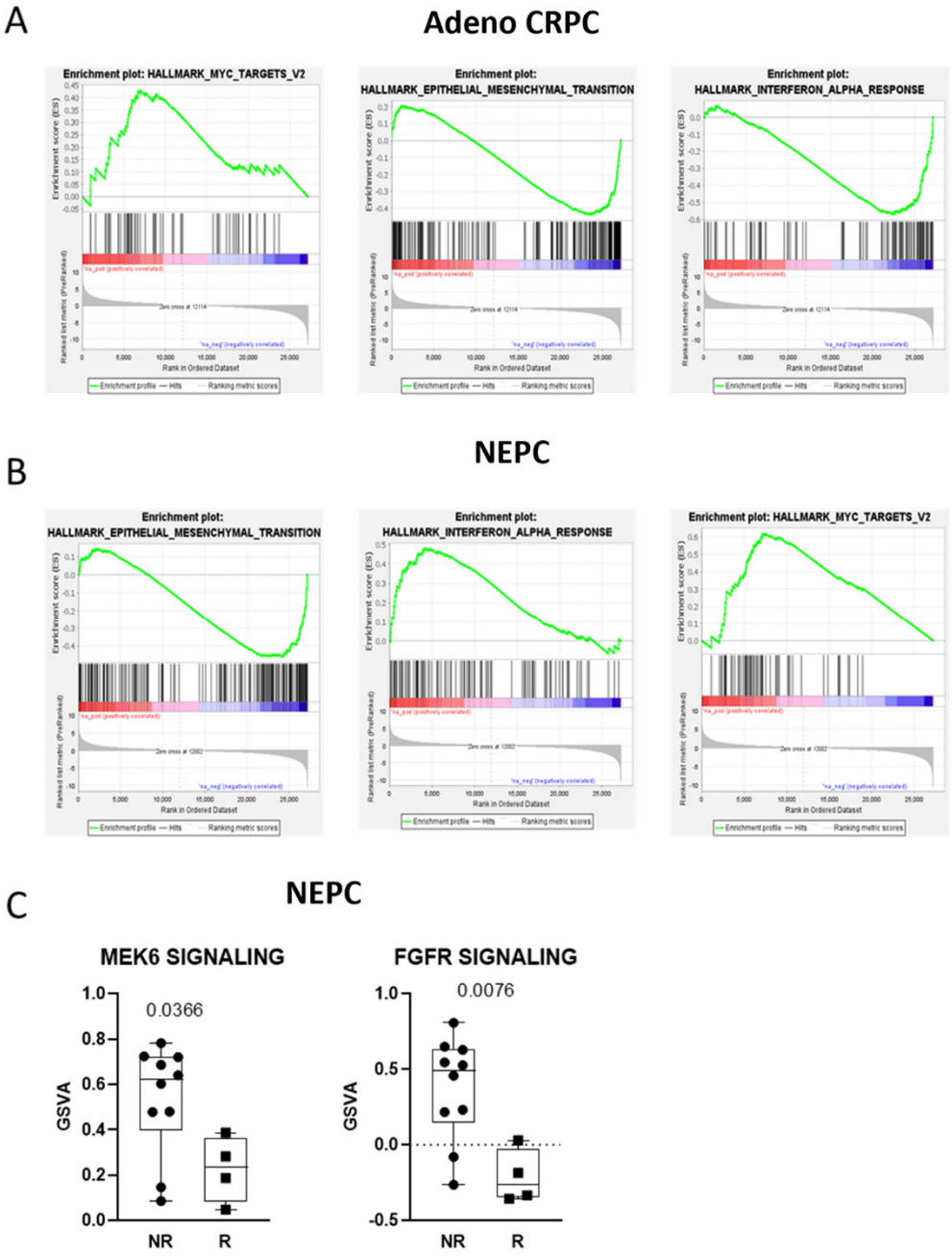

Fig.12: GSEA analysis of parental tumors. (A) Examples of Hallmark gene sets enriched in adenocarcinoma castrate-resistance prostate cancer (CRPC). (B) Examples of Hallmark gene sets enriched in neuroendocrine prostate cancer (NEPC). (C) GSVA detects differential MEK and FGFR signaling in NEPC non-responders (NR) vs responders (R).