Supplemental Materials

Computational modeling to identify drugs targeting metastatic castration-resistant prostate cancer characterized by heightened glycolysis

Authors: Mei-Chi Su[1], Adam M. Lee[1], Weijie Zhang[2], Danielle Maeser[2], Robert F. Gruener[1], Yibin Deng[3], R. Stephanie Huang[1,2]*

Affiliations:
1. Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA
2. Bioinformatics and Computational Biology, University of Minnesota, Minneapolis, MN 55455, USA
3. Department of Urology, Masonic Cancer Center, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Addresses for Correspondence:
R. Stephanie Huang, PhD
Experimental and Clinical Pharmacology
University of Minnesota College of Pharmacy
B-138 Phillips-Wangensteen Building
Minneapolis, MN 55455, United States
Phone: 612-625-1372
Email: rshuang@umn.edu

Disclosures: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Conflict of Interest Statement: The authors declare no conflicts of interest.

Gene expression profiles of mCRPC in SU2C/EC study

Inhibitory AUC profiles from CTRPv2 or PRISM

Gene expression profiles of CCLs

OncoPredict R package

GSVA R package

Predicted drug responses of mCRPC in SU2C/EC

Glycolysis scores of mCRPC in SU2C/EC

OXPHOS scores of mCRPC in SU2C/EC

CTRPv2 or PRISM

CTRPv2

PRISM

Regression analysis

Regression analysis

Regression analysis

$\beta_1 < 0$
FDR< 0.05

$\beta_1 < 0$
FDR< 0.05

$\beta_1 < 0$
FDR< 0.05

Drugs for high glycolysis mCRPC

Drugs for high glycolysis mCRPC

Drugs for high OXPHOS mCRPC

Computational validation by using SU2C/WC

MOA that appeared at least twice

Overlapping

Drugs for high glycolysis mCRPC

Computationally validated drugs for high glycolysis mCRPC (Drug_HG)

The top 50% significant drugs

Drugs for high glycolysis/ OXPHOS mCRPC

Computational validation by using the top 50% significant drugs nominated from SU2C/WC

Computational validation by using SU2C/WC

Computationally validated drugs for high glycolysis mCRPC (Drug_HG)

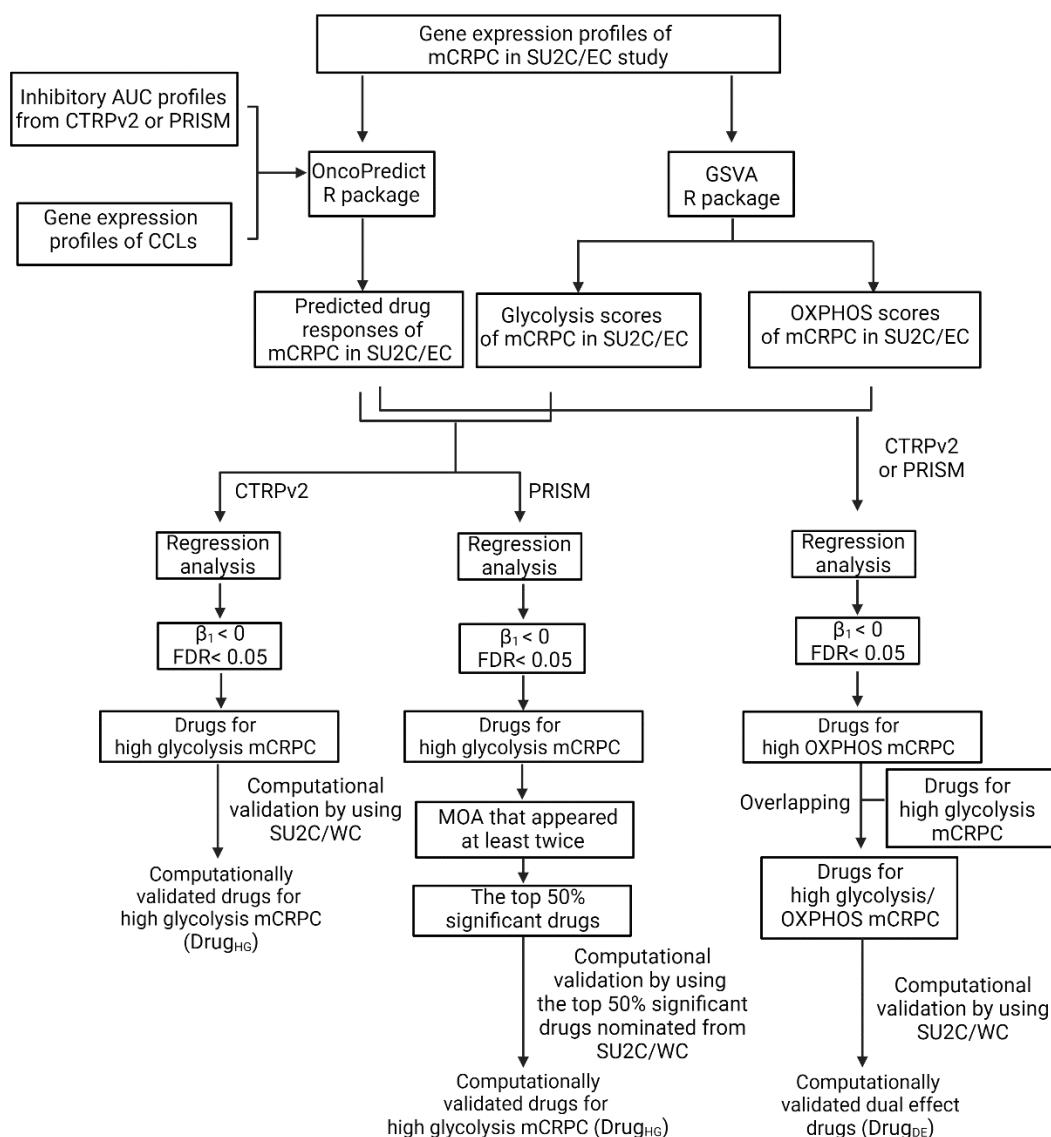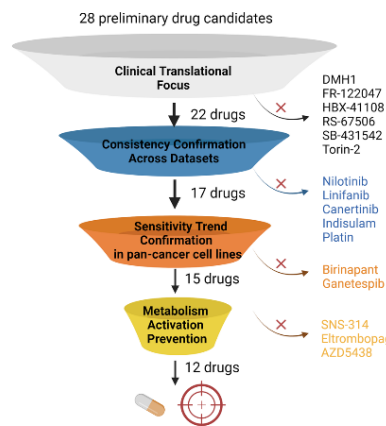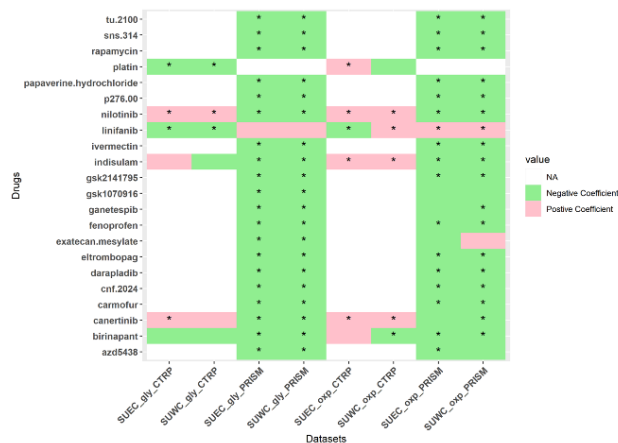Computationally validated dual effect drugs (Drug_DE)

Figure S1. Block diagram of drug candidate nominations.
The nomination of drug candidates began by exploring the relationship between gene expression levels and drug responses using datasets from in vitro cancer cell lines, specifically CTRPv2 and PRISM. This relationship was then leveraged to estimate drug responses in patients with mCRPC based on the gene expression profiles of their tumors. Regression analysis was used to identify potential drug candidates based on the correlation coefficients between predicted drug responses and the glycolysis and OXPHOS scores as well as corresponding statistical significance. Additionally, an independent clinical study, SU2C/WC, was used for validation, enhancing the robustness of selected drug candidates. Abbreviations: CTRPv2: Cancer Therapeutics Response Portal Version 2; PRISM: Profiling Relative Inhibition Simultaneously in Mixtures; CCLs: Cancer Cell Lines; SU2C/EC: Standard Up to Cancer East Coast; SU2C/WC: Standard Up to Cancer West Coast; GSVA: Gene Set Variation Analysis; β_1: a coefficient of predicted drug response of mCRPC tumors; FDR: false discovery rate; mCRPC: metastatic castration-resistant prostate cancer; OXPHOS: oxidative phosphorylation; MOA: mechanism of action.
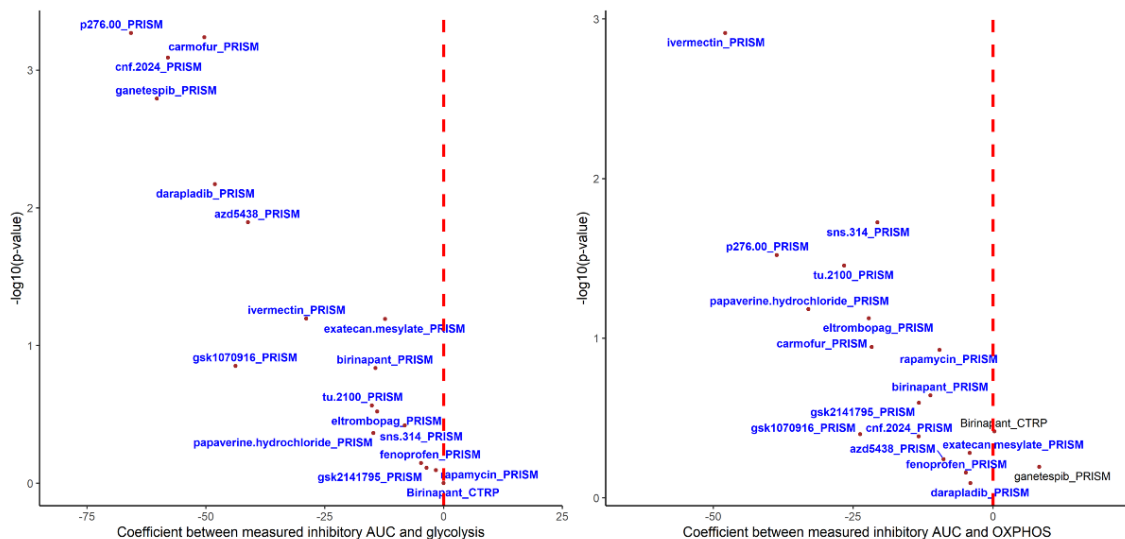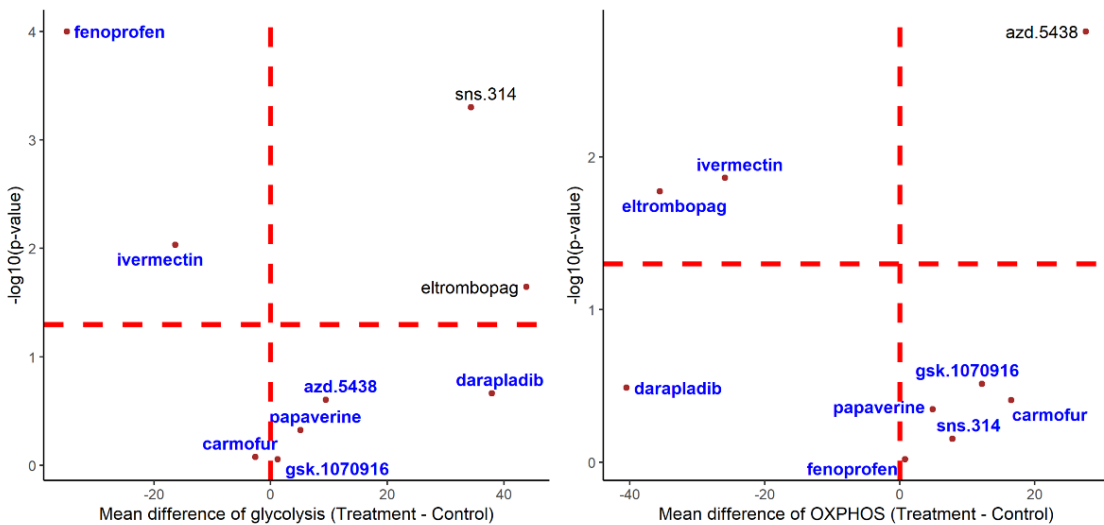
Figure S2. Step-by-step results of the four-step filtering procedure. (A) The flow chart of the number of drug candidates selected after each screening step (B) Heatmap of the coefficient of relationship between imputed drug responses and glycolysis/OXPHOS score across different datasets (CTRPv2 and PRISM). The statistical analysis was performed by Student's t-test. *: FDR <0.05 (C) Dot plot of coefficients between measured inhibitory AUC and glycolysis (left) or OXPHOS (right) in pan-cancer CCLs. The red dashed vertical line indicates a coefficient equal to 0. The statistical analysis was performed by Student's t-test (D) Dot plot of the change of glycolysis (left) and OXPHOS (right) after 10 μM drug treatment for 24 hours in PC3 cells. The red dashed horizontal line indicates a p-value equal to 0.05 and the red dashed vertical line indicates a mean difference equal to 0. The statistical analysis was performed by Welch's t-test. Of note, the perturbation-driven gene expression profiles of CNF2024, Exatecan mesylate, GSK2141795, P276-00, Rapamycin, and TU.2100 are not available in CMAP. Abbreviations: SUEC: Standard Up to Cancer East Coast; SUWC: Standard Up to Cancer East Coast; gly: glycolysis; oxp: oxidative phosphorylation; FDR: false discovery rate.
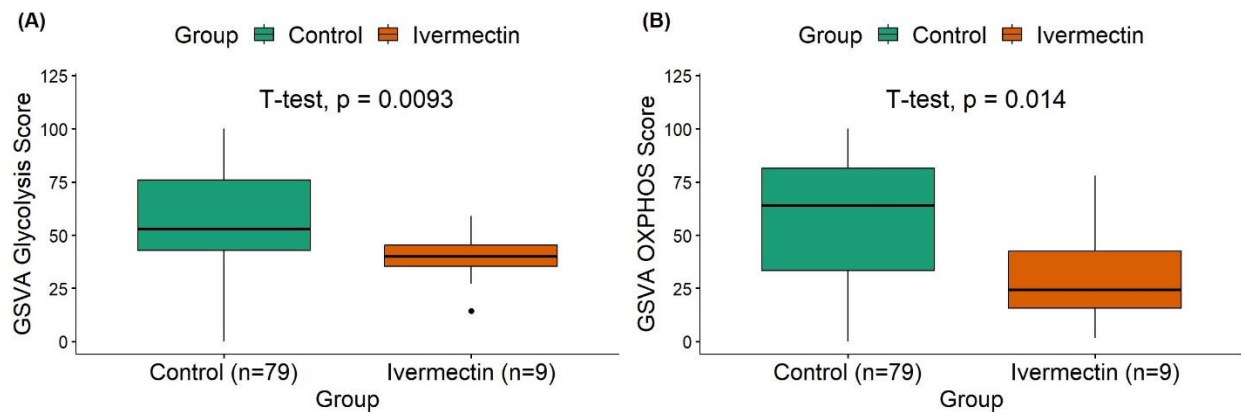

Figure S3. Alternations in cancer metabolism activity after ivermectin treatment. (A) change of glycolysis score after 24 hours of ivermectin treatment in PC3 cells (B) change of OXPHOS score after 24 hours of ivermectin treatment in PC3 cells. The statistical analysis was performed by Welch's t-test. Data sourced from CMAP. Abbreviations: GSVA: Gene set variation analysis; OXPHOS: oxidative phosphorylation.
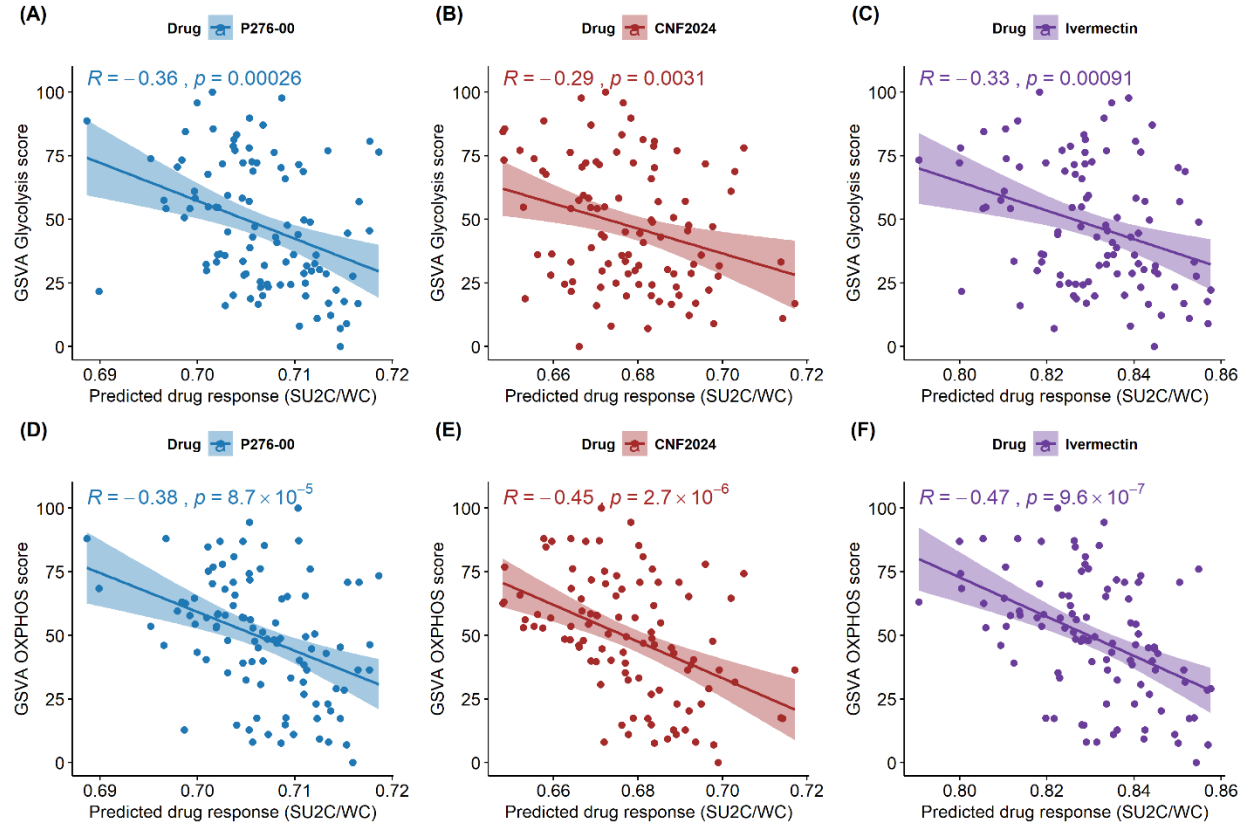
Figure S4. The correlation between glycolysis scores or OXPHOS scores with the predicted drug response in mCRPC patients in SU2C/WC clinical studies. (A-C) Correlations between the glycolysis score and the predicted patient response to P276-00, CNF2024, and ivermectin, respectively (D-F) Correlations between the OXPHOS score and the predicted patient response to P276-00, CNF2024, and ivermectin, respectively. For each correlation plot, the Spearman correlation coefficient and its p-value are given. The statistical analysis was performed by Spearman rank correlation and Student's t-test with a significance level α=0.05.

Docetaxel

Legend:
- ⊖ Low Glucose (1 mM)
- ⊡ Normal Glucose (5.5 mM)

	Low Glucose	Normal Glucose
AUC	202.1	211.7
IC_{50}	0.959 nM	1.012 nM
R^2	0.98	0.98
2-way ANOVA	$p=0.0544$	

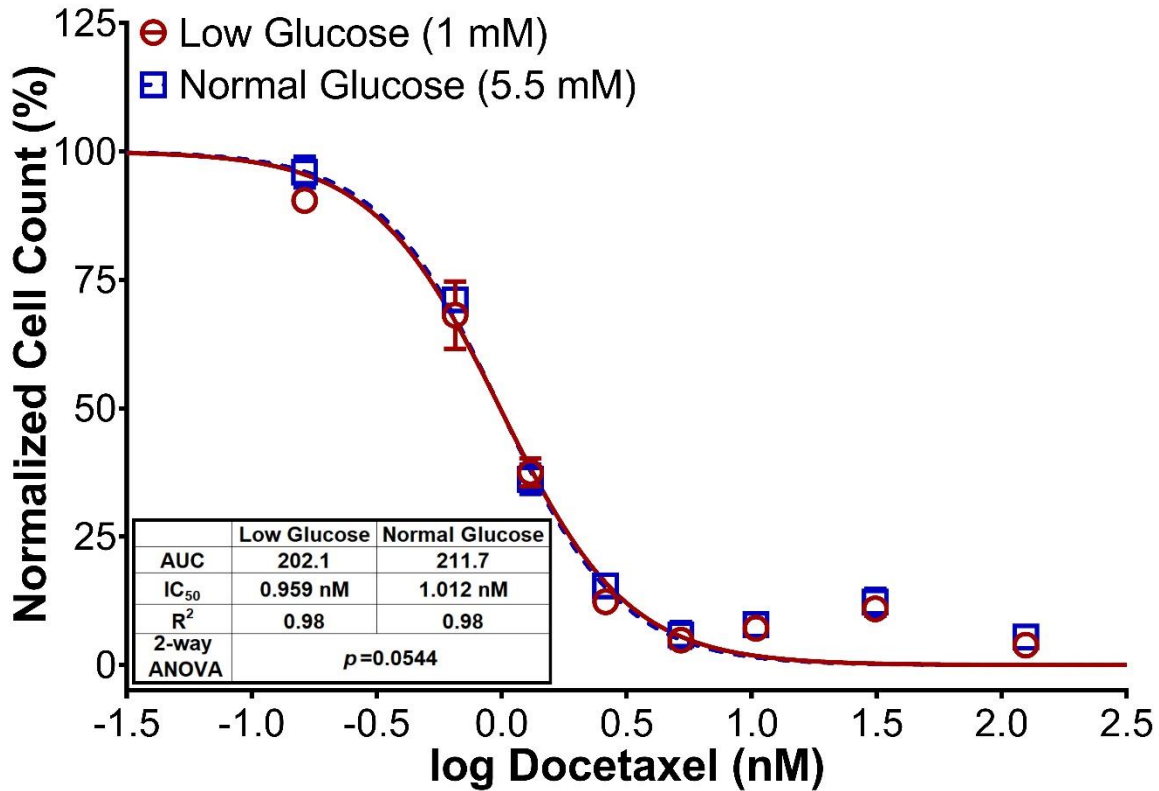Y-axis: Normalized Cell Count (%)
X-axis: log Docetaxel (nM)

Figure S5. The dose-response curve of PC3 cells after docetaxel treatment for 48 hours in either normal glucose or low glucose media. 4 replicates per group and the experiments were repeated 2 times. The statistical analysis was performed by two-way ANOVA.
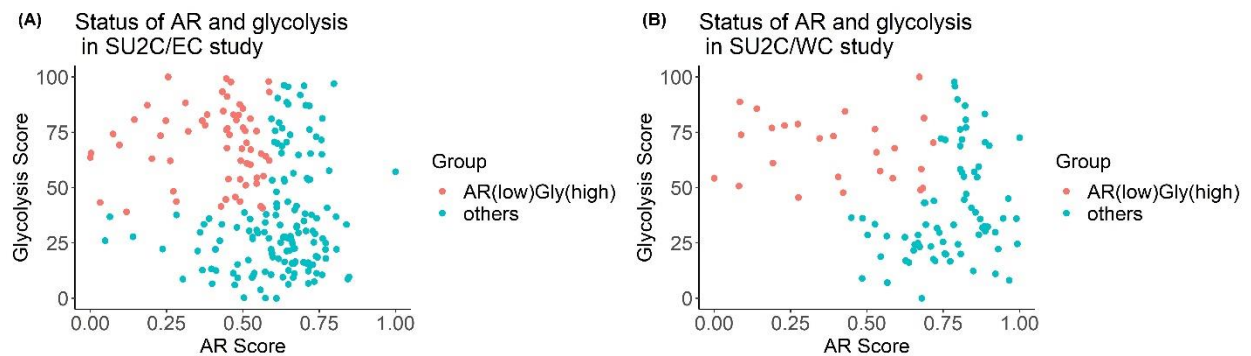

Figure S6. Distribution plots of patients based on AR and glycolysis scores. (A) SU2C/EC study (B) SU2C/WC study. The median is used as a cutoff to classify the low and high groups. Abbreviations: AR: androgen receptor activity; Gly: glycolysis activity; SU2C/EC: Standard Up to Cancer East Coast; SU2C/WC: Standard Up to Cancer West Coast.
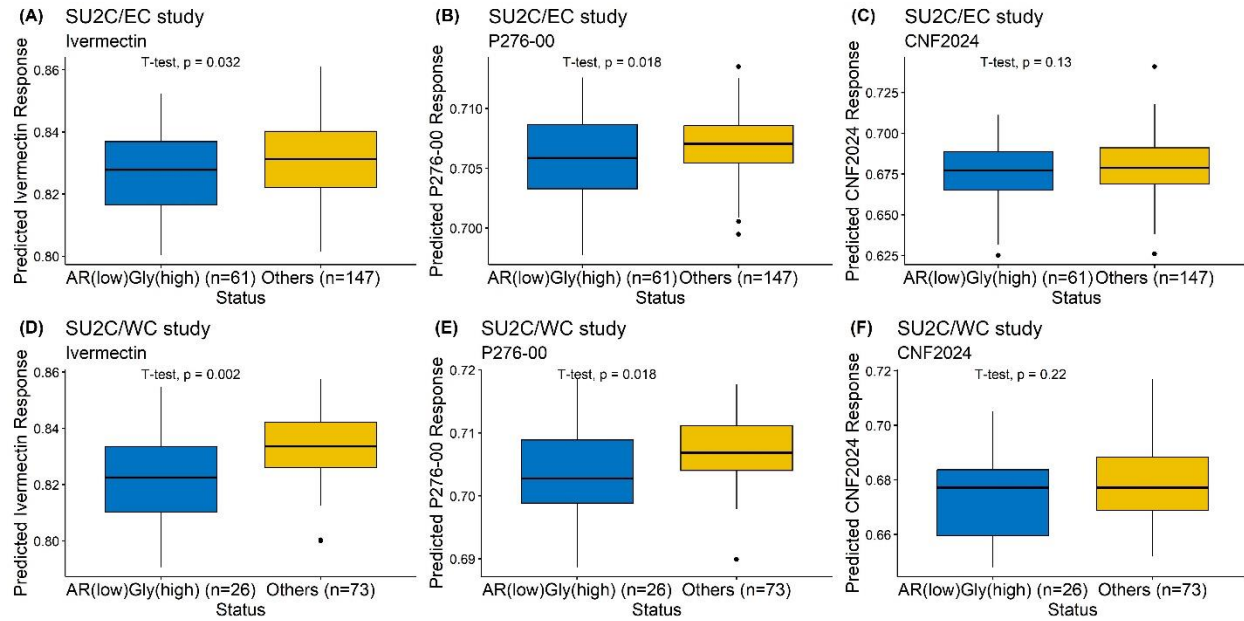
Figure S7. Comparison of predicted drug responses between AR(low) and glycolysis(high) group and the others group. (A-C) Comparison of predicted Ivermectin, P276-00, and CNF2024 responses in the SU2C/EC study. (D-F) Comparison of predicted Ivermectin, P276-00, and CNF2024 responses in the SU2C/WC study. The statistical analysis was performed by Welch's t-test. Abbreviations: AR: androgen receptor activity; Gly: glycolysis activity; SU2C/EC: Standard Up to Cancer East Coast; SU2C/WC: Standard Up to Cancer West Coast.
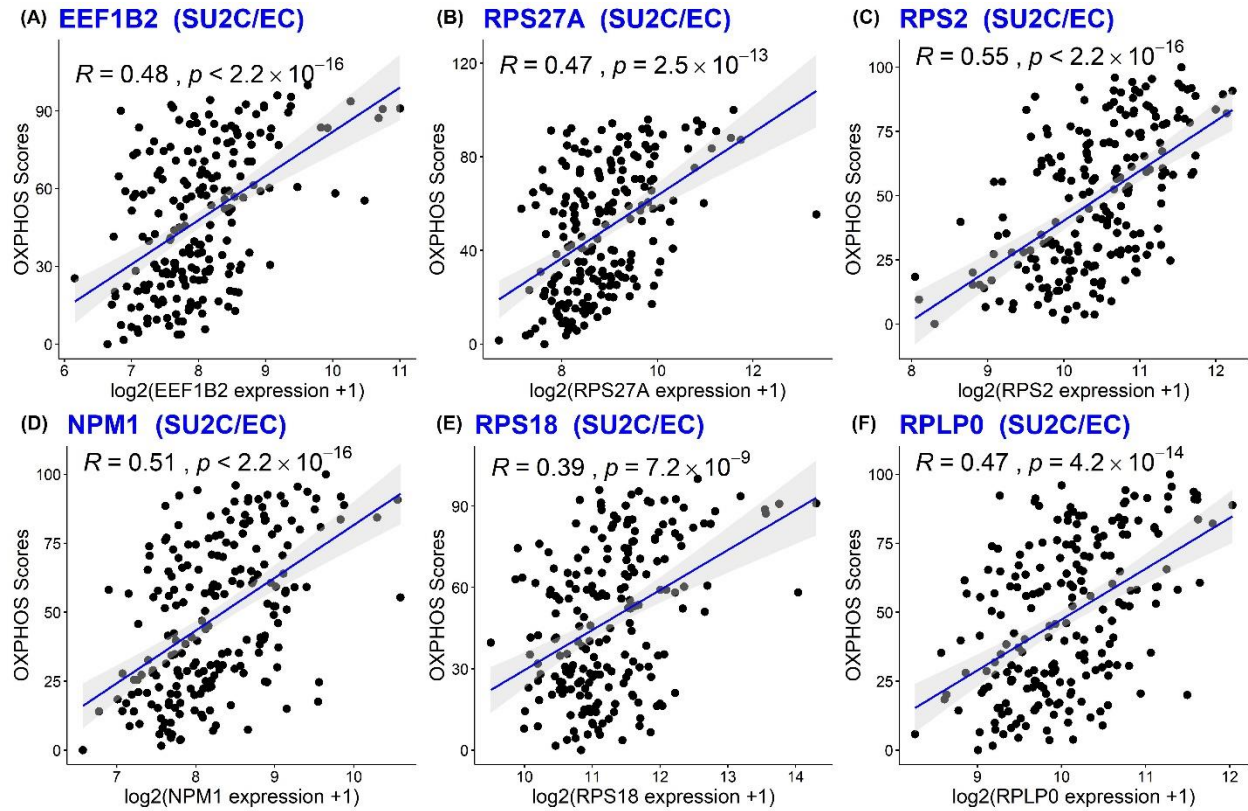
Figure S8. The correlation between gene expression level against OXPHOS score of patients with mCRPC in the SU2C/EC clinical study. (A) *EEF1B2* (B) *RPS27A* (C) *RPS2* (D) *NPM1* (E) *RPS18* and (F) *RPLP0*. For each correlation plot, the Spearman's rho values and its p-value are given. The statistical analysis was performed by Spearman rank correlation and Student's t-test with a significance level α=0.05. Abbreviations: SU2C/EC: Standard Up to Cancer East Coast; SU2C/WC: Standard Up to Cancer West Coast; OXPHOS: oxidative phosphorylation.
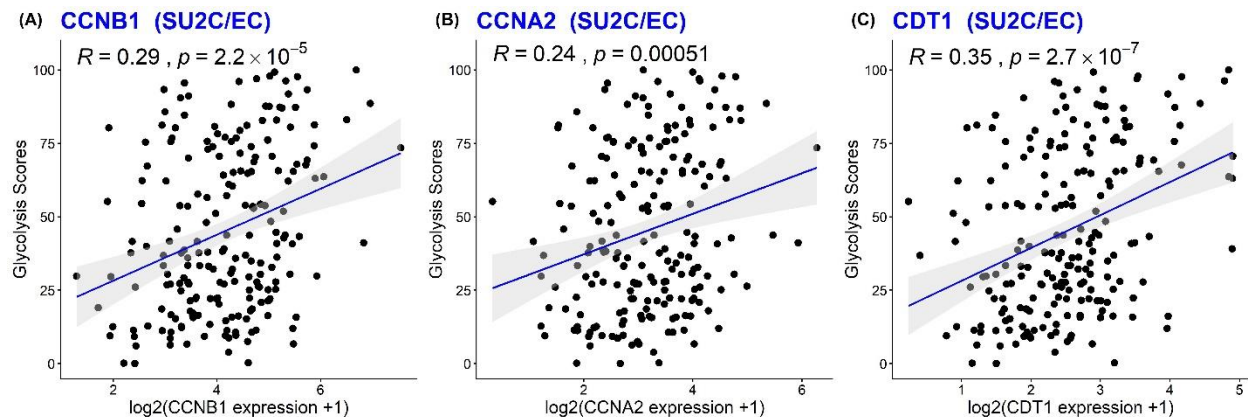

Figure S9. The correlation between gene expression level against glycolysis score of patients with mCRPC in the SU2C/EC clinical study. (A) *CCNB1* (B) *CCNA2* (C) *CDT1*. For each correlation plot, the Spearman's rho values and its p-value are given. The statistical analysis was performed by Spearman rank correlation and Student's t-test with a significance level

α=0.05. Abbreviations: SU2C/EC: Standard Up to Cancer East Coast; SU2C/WC: Standard Up to Cancer West Coast.