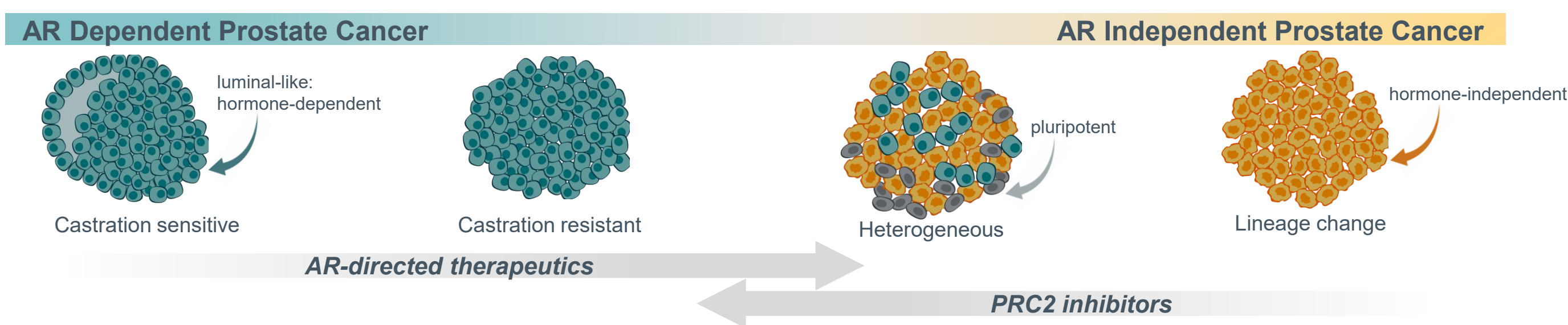
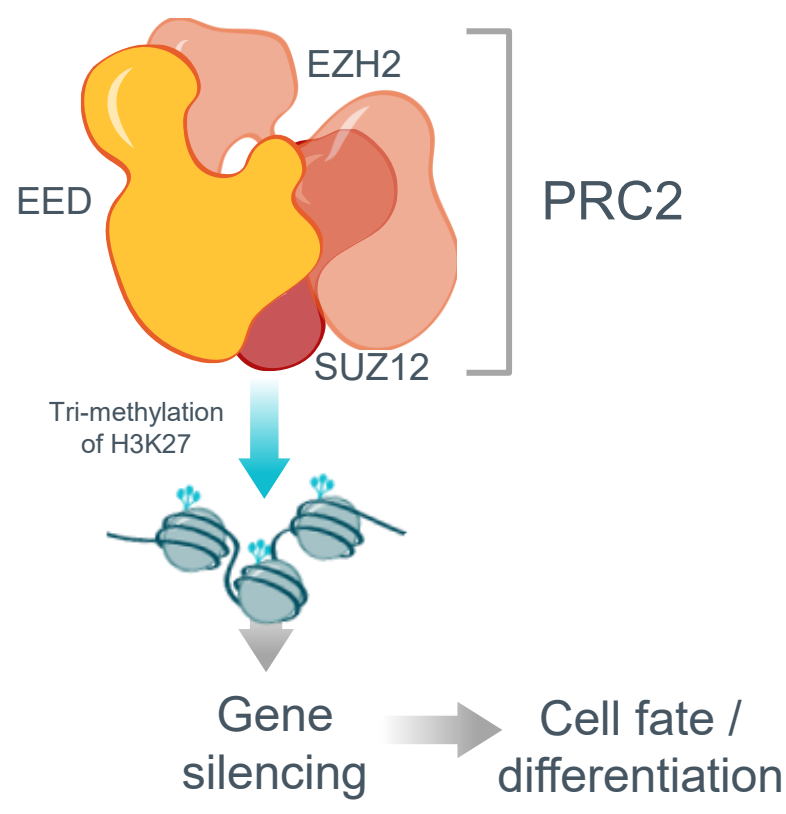


## BACKGROUND

- PRC2:** Polycomb repressive complex 2 (PRC2) silences transcription by trimethylating histone H3 at lysine 27, thereby regulating cell growth and differentiation. Elevated PRC2 activity promotes lineage plasticity and therapeutic resistance and has been associated with poor prognosis in prostate cancer.
- ORIC-944:** ORIC-944 is a next-generation, potent, highly selective, orally bioavailable small molecule inhibitor of PRC2 that allosterically targets the EED subunit, with potential best-in-class drug properties including limited CYP interactions and superior pharmacokinetics (PK) and half-life.
- Prostate Cancer:** Combining androgen receptor pathway inhibitors (ARPIs) with PRC2 inhibitors may overcome or delay resistance, with clinical trials showing promise in metastatic castration-resistant prostate cancer (mCRPC).



- Preclinical synergy has been shown for the combination of ORIC-944 and AR inhibition in CRPC models. In mechanistic studies underlying this combination synergy, we observed PRC2 inhibition increasing AR signaling, bolstering luminal features and restricting lineage adaptation.
- In this study we evaluated the broader applicability of ORIC-944 and AR inhibition combination for castration-sensitive prostate cancer (CSPC).

## 1. ORIC-944 Synergizes with AR Inhibition in Castration-Sensitive Prostate Cancer Cells In Vitro

ORIC-944 and Enzalutamide Synergistically Impair LNCaP Cell Growth In Vitro | Mevrometostat and Enzalutamide Synergistically Impair LNCaP Cell Growth In Vitro



Drug Combination	Loewe Score	BLISS Score	HSA Score
ORIC-944 + Enzalutamide	15.4	10.3	17.4
Mevrometostat + Enzalutamide	14.2	9.9	15.6

Figure 1. Synergistic cell viability effects are achieved when inhibiting PRC2 either with allosteric EED inhibition using ORIC-944 or EZH2 inhibition using mevrometostat. 14-day CellTiter-Glo assay on LNCaP cells in 10% FBS, with dose-ranging concentrations of AR inhibitor enzalutamide and ORIC-944 or mevrometostat, alone or in combination. Synergy was scored in three synergy models (Loewe, BLISS, HSA), with scores of >10 assessed as synergistic, scores of 10 to -10 additive, and < -10 antagonistic [Ianevski et al., NAR (2022)].

## 2. Transcriptional Effects of ORIC-944 + AR Inhibition Are Comparable Regardless of the AR Inhibitor Used

Equivalent Transcriptional Effects of ORIC-944 Combined with Enzalutamide vs. Apalutamide | Equivalent Transcriptional Effects of ORIC-944 Combined with Apalutamide vs. Darolutamide

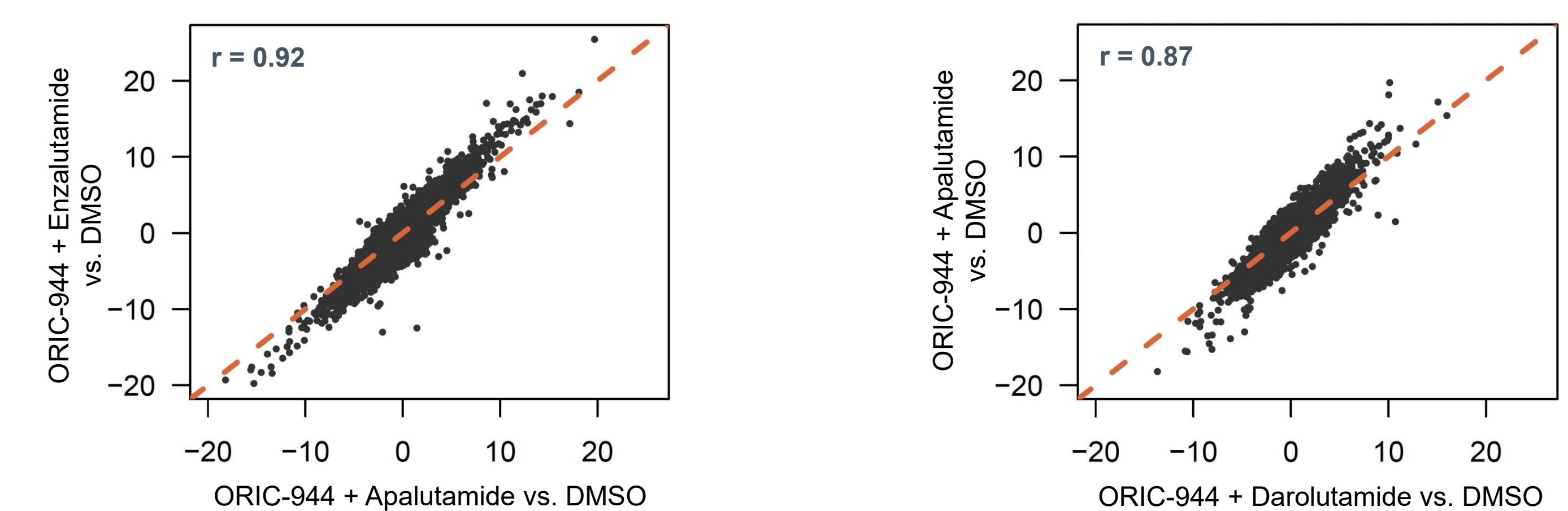
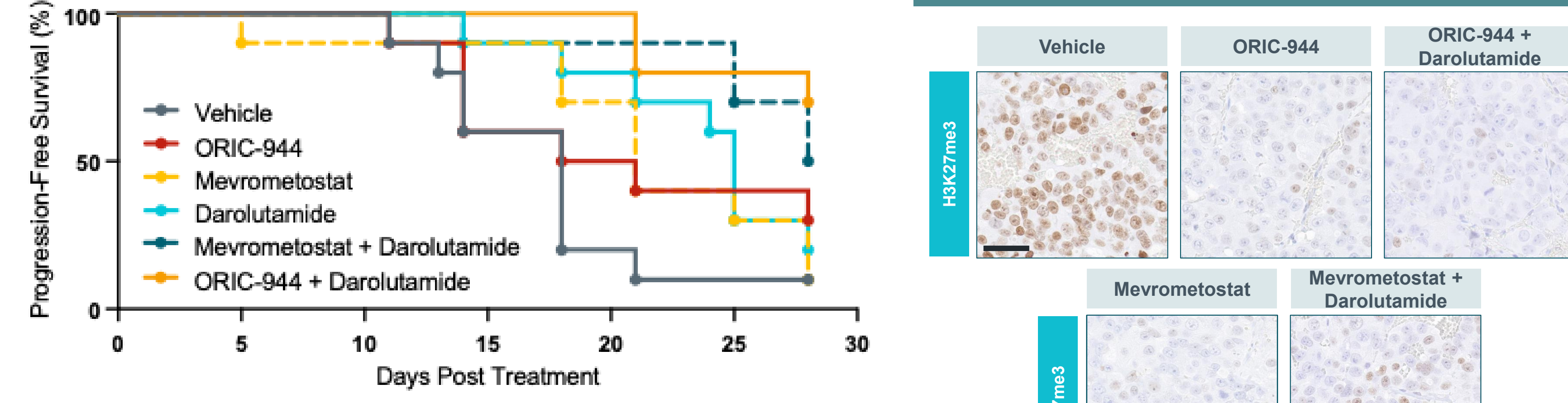


Figure 2. Castration-sensitive LNCaP cells grown in FBS with 7-day treatment of 1µM of ORIC-944 in combination with either 1µM of enzalutamide, apalutamide or darolutamide. RNA sequencing performed, followed by correlation analysis of DESeq2-derived test statistics for the indicated comparisons. N = 2/treatment. r = Pearson correlation.

## 3. ORIC-944 + AR Inhibitor Improves Survival in CSPC Xenografts

Progression-Free Survival of Intact LNCaP Xenograft Model | Robust Reduction in Tumor Levels of H3K27me3 with PRC2 Inhibitors In Vivo

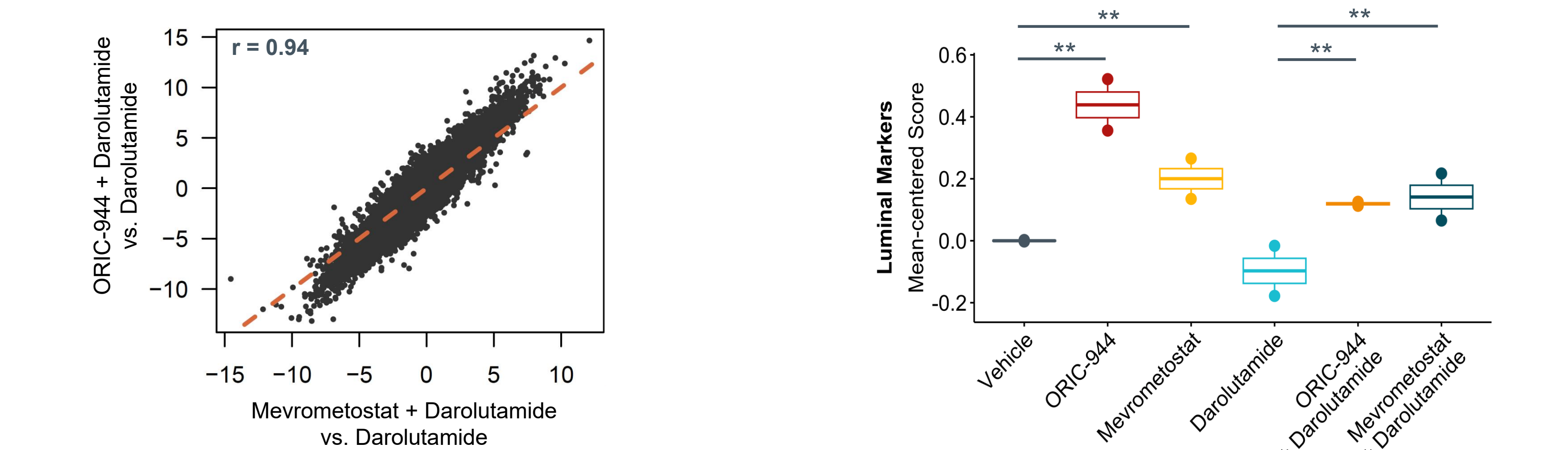


LNCaP	Vehicle	ORIC-944	Mevrometostat	Darolutamide	Mevrometostat + Darolutamide	ORIC-944 + Darolutamide
Median PFS (days)	18	19.5	21	25	28	Not reached

Figure 3. ORIC-944, and mevrometostat, in combination with AR inhibitor darolutamide, prolong survival in CSPC xenografts. Left. LNCaP prostate cancer (fast-growing clone) xenografts grown in intact mice and treated with vehicle, darolutamide 50 mg/kg BID, ORIC-944 100 mg/kg QD, mevrometostat 100 mg/kg BID, or combinations for 28 days. N = 10/cohort. Tumors were measured by caliper and mice weighed twice weekly. Progression event for either tumor volume >800mm<sup>3</sup> or morbidity. No drug-related tolerability issues. Right. H3K27me3 staining of xenograft tumors obtained at end-of-study from the in vivo study on the left. Bar represents 50µm.

## 4. ORIC-944 Reinforces a Luminal Cell State in CSPC Xenografts

Equivalent Transcriptional Effects of Combining ARPI with Either ORIC-944 or Mevrometostat In Vivo | PRC2 Inhibitors Enhance Expression of Luminal Markers in LNCaP Xenograft Model



Confirmed Increase in Protein and mRNA Levels of the Luminal Marker Cytokeratin 8 in LNCaP Xenografts

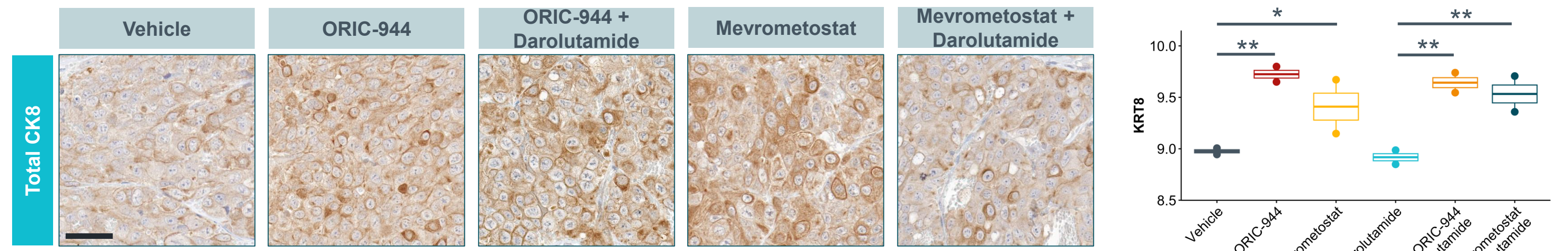


Figure 4. EED and EZH2 targeting in combination with AR inhibition achieve similar transcriptional effects in CSPC xenografts, including enhanced expression of luminal markers. Top-Left. RNA sequencing correlation analysis of DESeq2-derived test statistics for ORIC-944 + darolutamide and mevrometostat + darolutamide vs. darolutamide in LNCaP xenografts, N = 2/treatment. r = Pearson correlation. Top-Right. Luminal marker signature [Liang et al., Prostate Cancer and Prostatic Disease (2022)] expression in LNCaP tumors from the in vivo study in Figure 3. Significance is based on a weighted Stouffer test on DESeq2 results using the inverse of log2 fold change standard errors as weights. Bottom. Total Ck8 protein staining (Left) and Ck8 (KRT8) mRNA levels (Right) from LNCaP tumors. Bar represents 50µm. Significance is based on the DESeq2 test against an absolute fold change threshold of 1.2. \*, p<0.05; \*\*, p<0.01.

## 5. ORIC-944 Downregulates Cell Cycle Genes In Vivo in CSPC Model

PRC2 Inhibitors + ARPI Synergistically Downregulate Cell Cycle Genes in LNCaP Xenograft Tumors

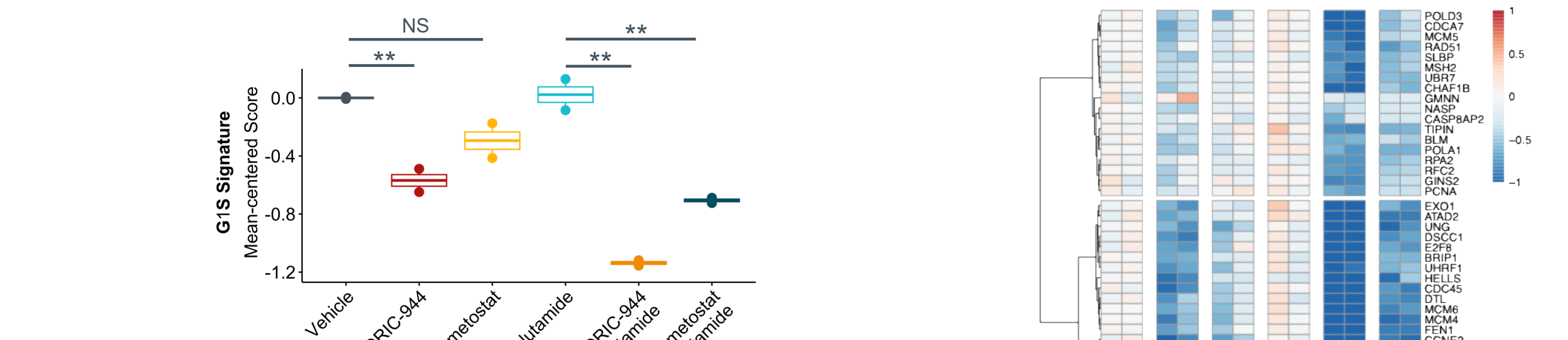


Figure 5. Left. G1/S signature [Tirosch et al., Science (2016)] expression levels in LNCaP tumors from the in vivo study in Figure 3. Same method and p-value cutoffs as in Figure 4 (Top-Right); NS, not significant. ORIC-944 + darolutamide interaction p-value <1e-5; mevrometostat + darolutamide interaction p-value <1e-5. Right. Heatmap with vehicle-centered expression of G1/S signature genes.

## 6. ORIC-944 Restricts Lineage Adaptation in CSPC Xenografts

ORIC-944 + Darolutamide Reduces Accessibility to Lineage Transcription Factors in LNCaP Xenografts

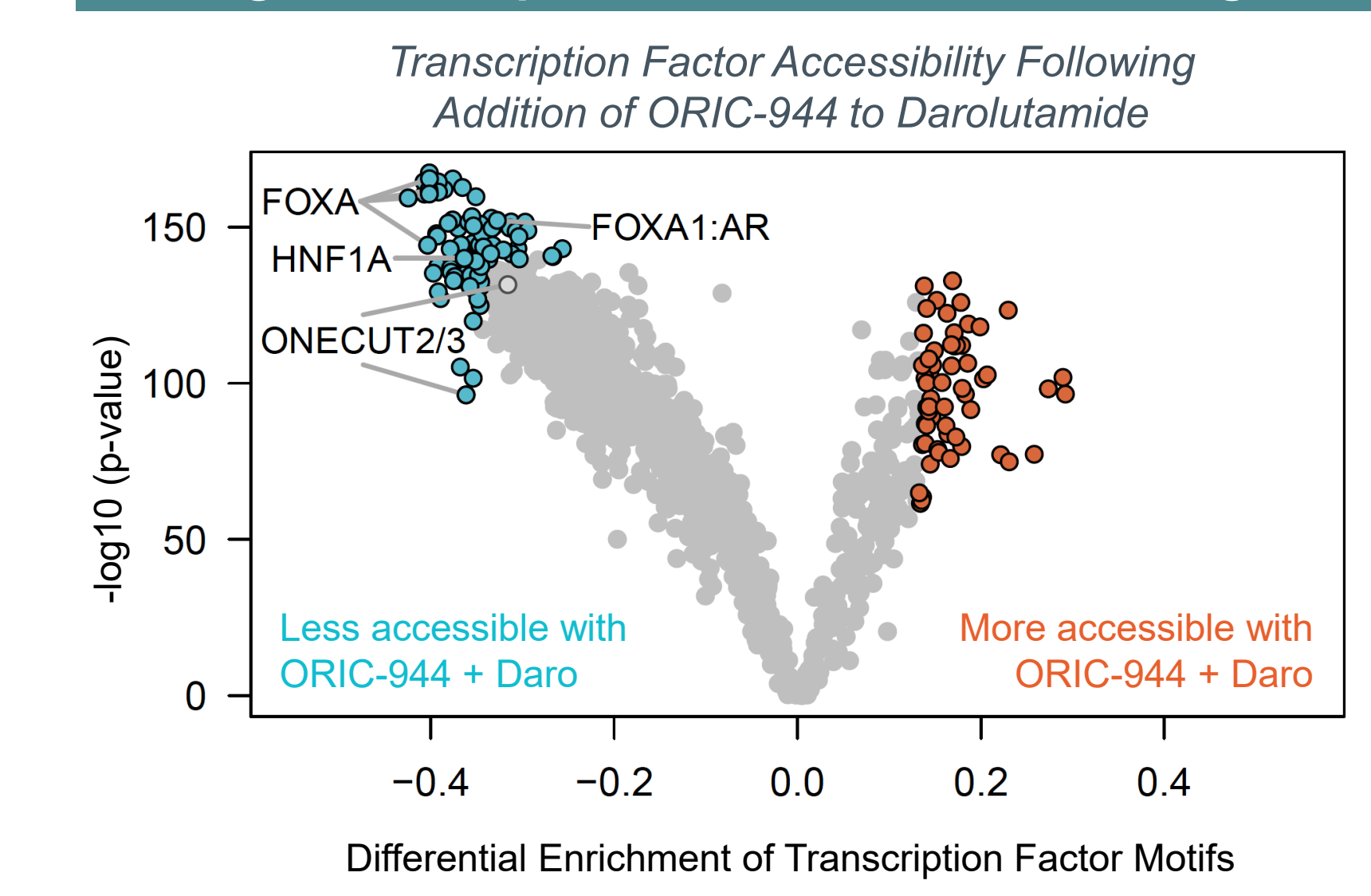
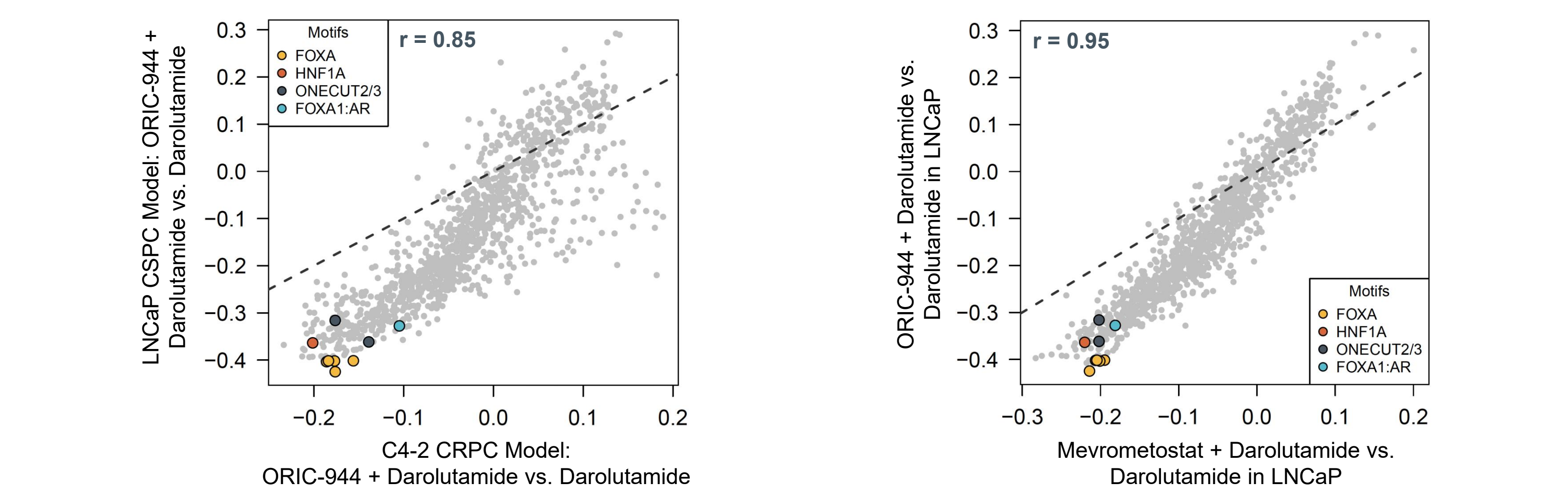


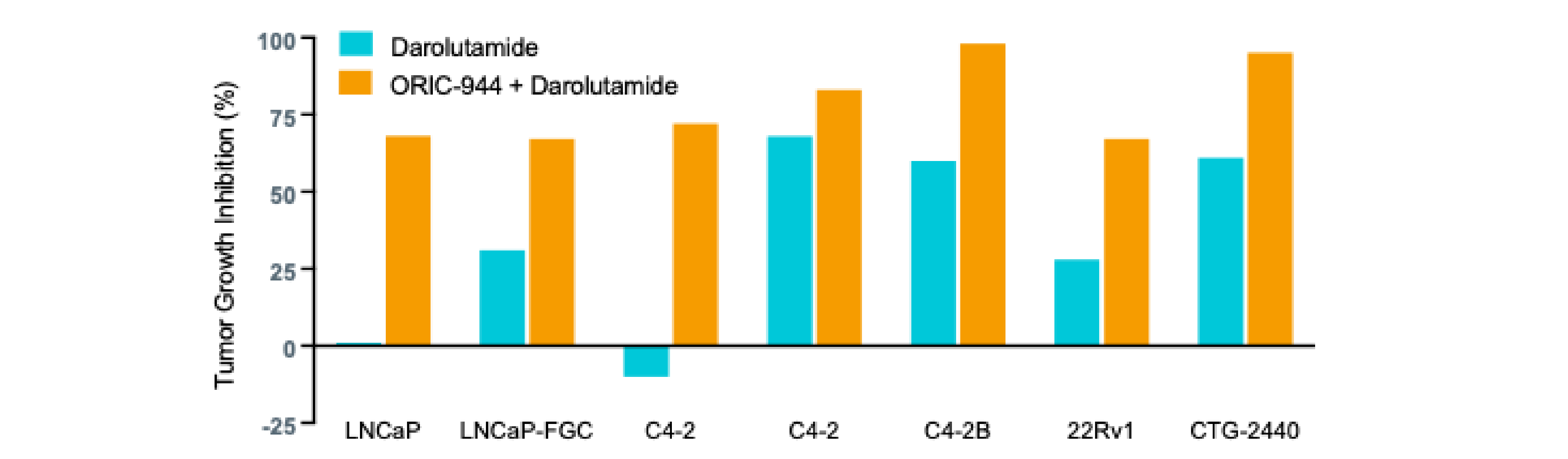
Figure 6. PRC2 activity regulates chromatin accessibility, with PRC2 inhibition blocking access to key factors associated with lineage switch and clinical resistance such as FOXA1/2/3, ONECUT2/3, HNF1A/B and FOXA1:AR. Top-Left. ATAC-seq data comparing transcription factor motif accessibility in LNCaP xenografts grown in intact mice and treated with ORIC-944 + darolutamide vs. darolutamide. The accessibility change for every transcription factor motif and its associated p-value are calculated using TOBIAS [Bentsen et al., Nat Comm (2020)]. Motifs that are significantly more accessible following combination treatment are shown in dark orange; more accessible motifs with darolutamide treatment are shown in blue. FOXA1:AR represents the FOXA1 motif juxtaposed to the AR half motif. Bottom-Left. Comparison of motif accessibility changes induced by the addition of ORIC-944 to darolutamide in LNCaP CSPC xenografts vs. C4-2 CRPC xenografts. Bottom-Right. Addition of ORIC-944 vs. mevrometostat to darolutamide results in accessibility changes to the same key transcription factor binding sites in LNCaP CSPC xenografts. Every point is the TOBIAS-calculated score of motif accessibility change for consolidated motifs. Shown in color are lineage-associated transcription factor motifs with reduced accessibility following treatment with PRC2i. r = Pearson correlation.

ORIC-944 + Darolutamide Equivalently Impacts Transcription Factor Binding Site Accessibility in CSPC and CRPC Models of Prostate Cancer

ORIC-944 + ARPI or Mevrometostat + ARPI Decrease Access for the Same Lineage-associated Transcription Factors



## 7. ORIC-944 Improves Response to AR Inhibitor Darolutamide Across Wide Breadth of Prostate Cancer Models



Context	CSPC		CRPC			
	intact	intact	intact	castrated	intact	intact
Setting						
AR Alteration			AR T878A			
Tissue of Origin	ADT-naïve patient, lymph node biopsy	LNCaP sub-line, selected for faster growth	LNCaP sub-line, aggressive variant grows in castrated or intact	C4-2 mouse bone metastasis	Castration-resistant CWR22R xenograft	Post-abiraterone patient, bone biopsy

Figure 7. Results of in vivo efficacy studies displayed as percent tumor growth inhibition, calculated as  $[1 - (TV_{day 0} / TV_{day 28})] \times 100\%$ ; TV, tumor volume. Treatments were either single agent darolutamide (blue) at 50 mg/kg BID PO, or darolutamide at 50 mg/kg BID in combination with ORIC-944 at 100 mg/kg QD PO (orange). Prostate cancer model annotations include castration-sensitive vs castration-resistant context, whether grown in intact or castrated mice, AR genotype, and the source/biopsy from which each model was derived. WT, wildtype.

## CONCLUSIONS

- ORIC-944 is a potential best-in-class PRC2 inhibitor that induces luminal cell fate and restricts lineage transcription factor accessibility in both CRPC and CSPC preclinical settings
- ORIC-944 plus AR inhibition improves survival and extends the duration of response to AR inhibitors in vivo by restricting cellular plasticity and delaying prostate tumor adaptation in castration-sensitive prostate cancer

ORIC-944 in combination with ARPI is currently in a global Phase 1b trial (NCT05413421)