#1209

Comprehensive *in vitro* characterization of the mechanism of action of EPI-7386, an androgen receptor N-terminal domain inhibitor

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Background

- As a key driver of prostate cancer, androgen receptor (AR) signaling has been a major target of prostate cancer therapy
- All current anti-androgens function through the ligand-binding domain (LBD) of the AR
- Anti-androgen resistance mechanisms generally develop at the LBD, due to mutations in the LBD itself and the expression of constitutively active splice variants of AR that lack the LBD
- Anitens are small molecules capable of targeting the AR N-terminal domain that can inhibit transcriptional activity of the AR even in the presence of LBD-driven anti-androgen resistance
- EPI-7386 is a new generation of aniten which exhibits high potency, low metabolism, on-target specificity

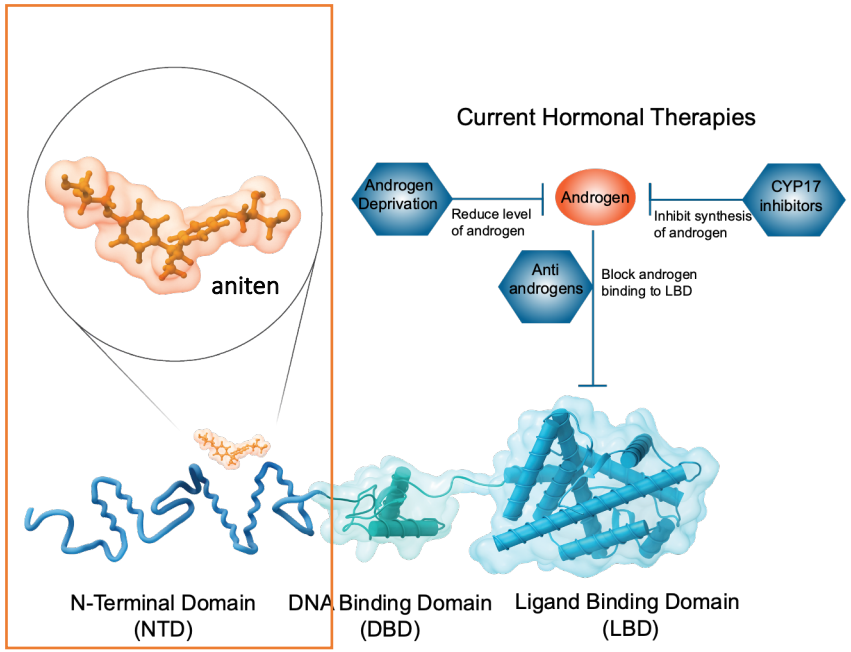

Figure 1. Anitens are first-in-class NTD inhibitors of the androgen receptor. Structure of the androgen receptor (AR) and mechanism of inhibition. The AR comprises three main functional domains: the LBD, involved in binding with androgens, the DBD, and the NTD, which orchestrate the transactivation of the receptor. It was previously demonstrated that the first-generation aniten and its stereoisomers specifically bind to the transactivation unit 5 (Tau5) of AR NTD to block essential protein-protein interactions required for the transcriptional activity of AR[1-3].

[1]*De Mol et al., ACS Chem Biol, 2016,* [2]*Andersen et al., Cancer Cell, 2010,* [3]*Sadar, Cancer Res, 2011*

EPI-7386 interacts with both AR-FL and AR-V7

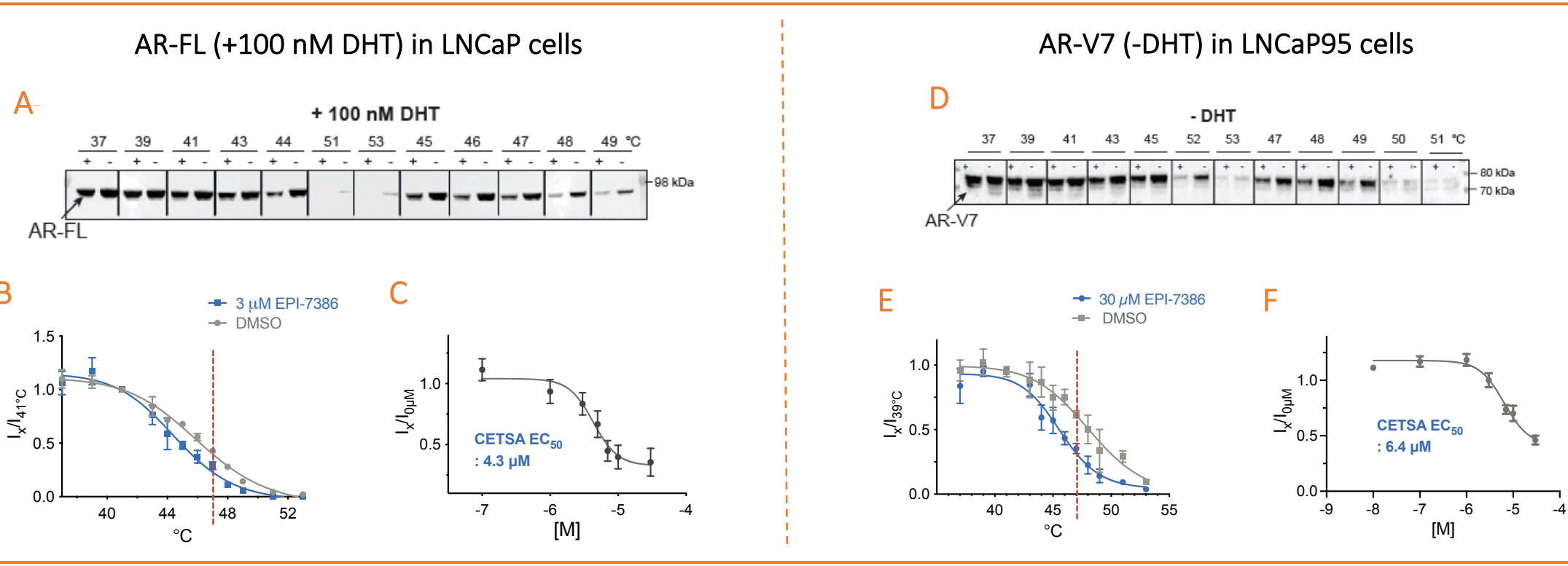

Figure 2. Cellular Thermal Shift Assay (CETSA) of AR target engagement by EPI-7386. AR target engagement by EPI-7386 induced a decrease in AR thermal stability. (A-C) AR-FL target engagement in LNCaP cells was determined in the presence of 100 nM DHT and (D-F) AR-V7 in LNCaP95 cells was determined in the absence of DHT. (A, D) Representative Western Blot showing thermostable AR-FL in LNCaP cells (A) or AR-V7 in LNCaP95 (D) following heat shock in the presence (+) or absence (-) of EPI-7386. (B, E) Melt and shift curve of AR-FL in LNCaP cells (B) or AR-V7 in LNCaP95 cells (E) treated with EPI-7386 (blue) at the indicated concentration and DMSO control (black). (C, F) Isothermal (47°C) dose-response curve of AR-FL in LNCaP cells and AR-V7 in LNCaP95 cells. Potency of target engagement (CETSA EC_{50}) was calculated from multiple independent experiments (n≥9).

** AR-V7 specific antibody (clone RM7; RevMab) was used to detect AR-V7 in LNCaP95.*

*** No AR-V7 thermal shift was induced by DHT unlike AR-FL, confirming assay specificity to AR-V7 lacking LBD*

EPI-7386 is active in the AR splice variant (ARv567es) expressing cell line

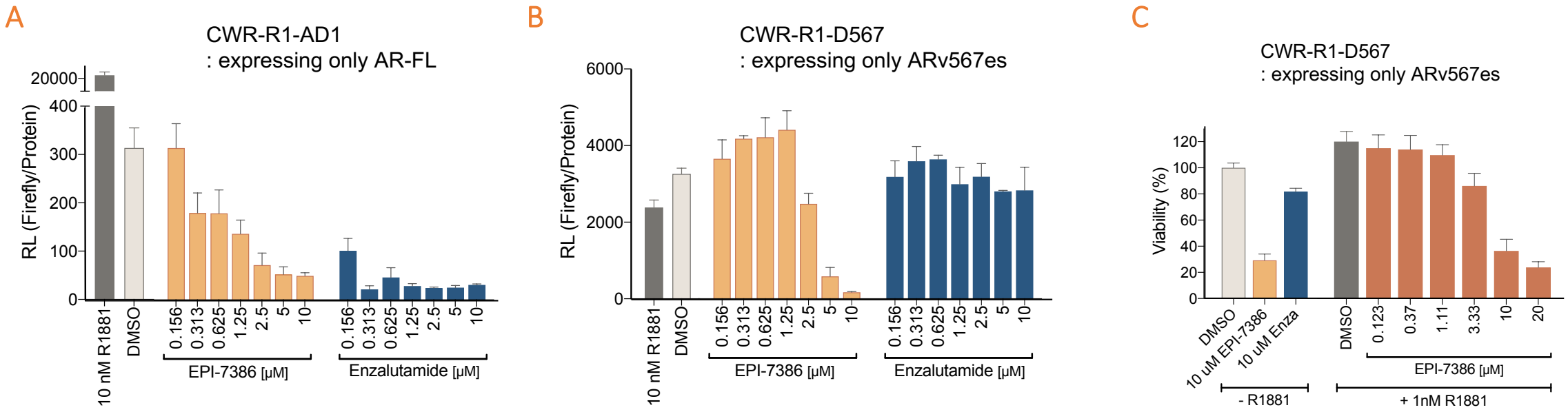

Figure 3. Effect of EPI-7386 against AR splice variant driven transcriptional activity and cell growth.
(A,B) AR-driven transcriptional activity was assessed using a probasin promoter (ARR2PB) driven luciferase reporter in CWR-R1-AD1 cells expressing only AR-FL in the presence of 5%FBS (A) or in CWR-R1-D567 cells expressing only AR-V567es under serum free condition (B). (C) Cell viability analysis of CWR-R1-D567 with MTT assay showing relative viable cell % to DMSO control after 72 hr treatment of EPI-7386 or enzalutamide in the absence or presence of R1881.

* *CWR-R1-D567 cells were derived from the CWR-R1-AD1 cells by TALEN-mediated deletion of AR exons 5-7*[4]

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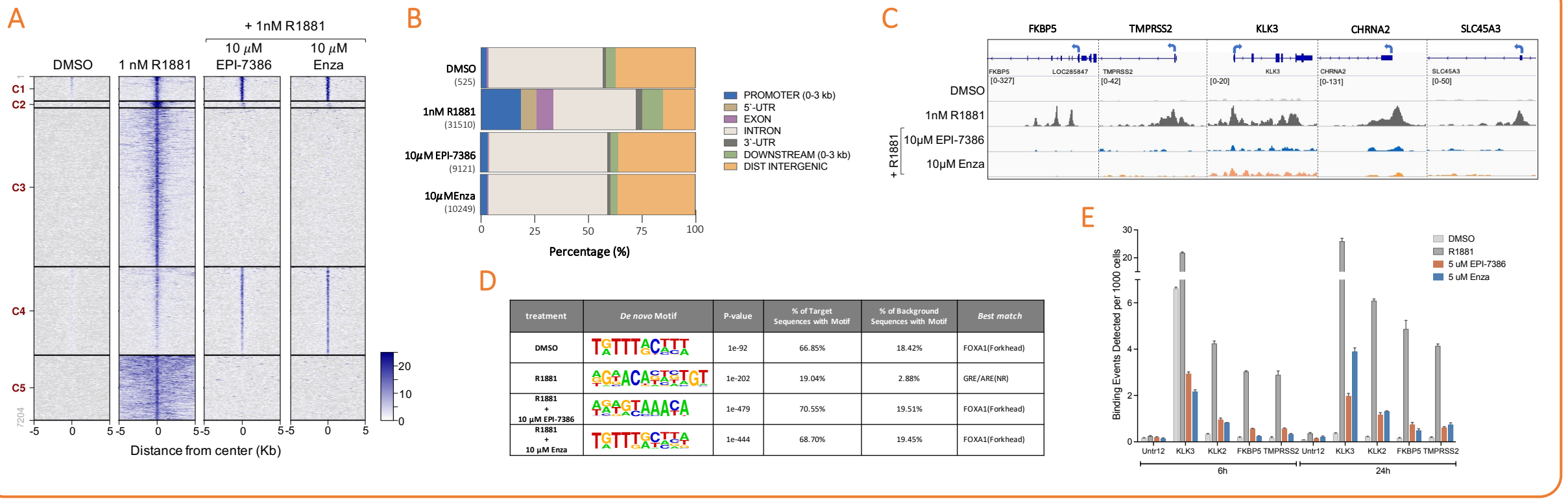
[4]*Nyquist et al., PNAS, 2013*

EPI-7386 inhibits AR genomic binding



treatment	*De novo* Motif	P-value	% of Target Sequences with Motif	% of Background Sequences with Motif	*Best match*
DMSO		1e-92	66.85%	18.42%	FOXA1(Forkhead)
R1881		1e-202	19.04%	2.88%	GRE/ARE(NR)
R1881 + 10 μM EPI-7386		1e-479	70.55%	19.51%	FOXA1(Forkhead)
R1881 + 10 μM Enza		1e-444	68.70%	19.45%	FOXA1(Forkhead)

Figure 4. ChIP-seq analysis of AR genomic distribution. (A-D) Cells were treated with DMSO, R1881, EPI-7386 or enzalutamide(Enza) in the presence of R1881 as indicated for 24 h. (A) Heatmap view of AR ChIP-seq signals around AR peaks (± 5 kb) detected in LNCaP cells with treatments as indicated. (B) Bar chart showing genomic distribution of AR-binding peaks including promoters (withing 3 kb upstream of TSS), 5' UTRs, exons, introns, 3'UTRs, downstream (within 3 kb downstream of the gene), and intergenic regions. Total peak numbers are also noted. (C) Gene track view of AR ChIP-seq data at FKBP5, TMPRSS2, KLK3, CHRNA2, and SLC45A3 loci, visualized by Integrative Genomics Viewer (IGV). (D) The top enriched motifs present in AR ChIP-seq peaks of each condition identified using Homer. (E) ChIP-qPCR validation in LNCaP cells with AR antibody. Cells were treated with DMSO, 1 nM R1881, EPI-7386, or enzalutamide in the presence of 1nM R1881 for 6 or 24 h. Untr12, primer that binds to gene dessert area in chromosome 12, was used for negative control locus.

EPI-7386 shows superior activity than enzalutamide in the AR-V7 expressing LNCaP95 cells by modulating both AR-FL and AR-V7 driven gene expression

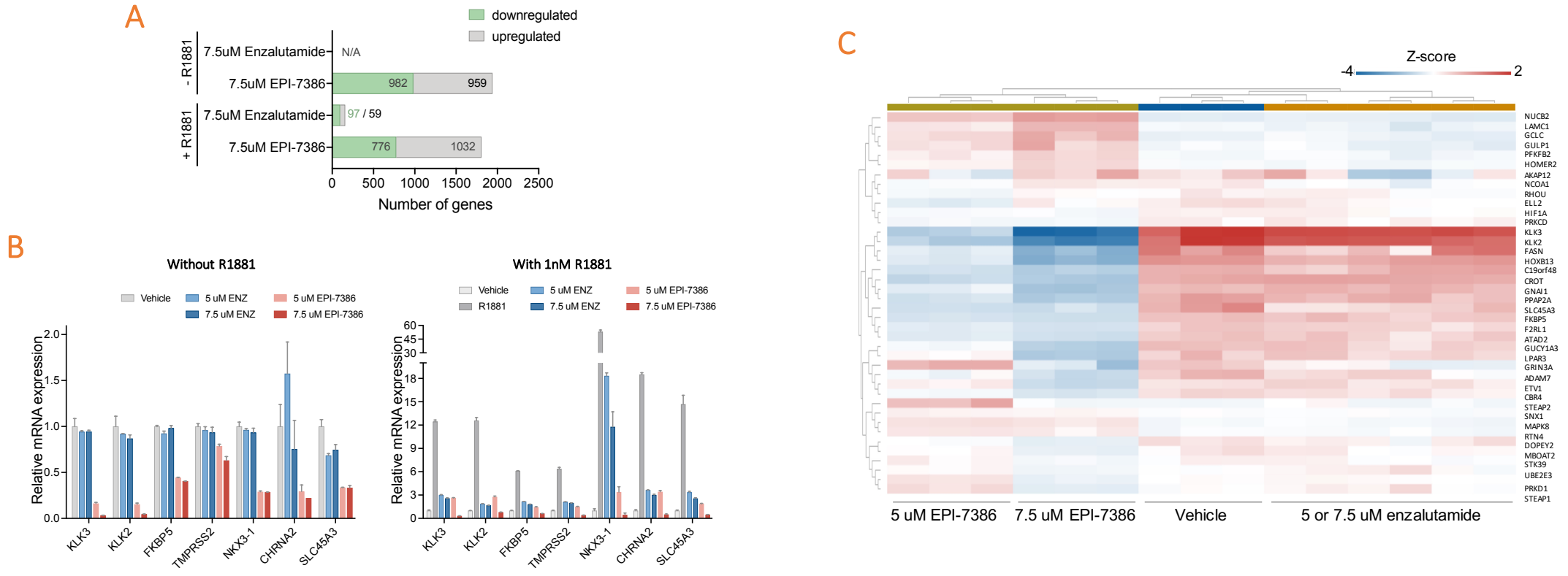


Figure 5. Evaluation of the effect of EPI-7386 on transcriptome in LNCaP95 cells. RNAseq analysis of cells treated with EPI-7386 or enzalutamide (ENZ) for 24 h in the presence or absence of 1 nM R1881. N=3 (A) Number of genes significantly up- or down-regulated following treatments. Fold change (FC) >3, pAdj<0.01. (B) Relative expression levels of representative AR-regulated genes in the absence of R1881 (left) or presence of R1881 (right). (C) Heatmap showing expression of AR-V7 regulated genes following treatments in the absence of R1881.

EPI-7386 inhibits AR transcriptional activity similarly to enzalutamide but with a few notable differences, and combination with enzalutamide exhibits a broader & deeper inhibition of androgen-responsive gene expression

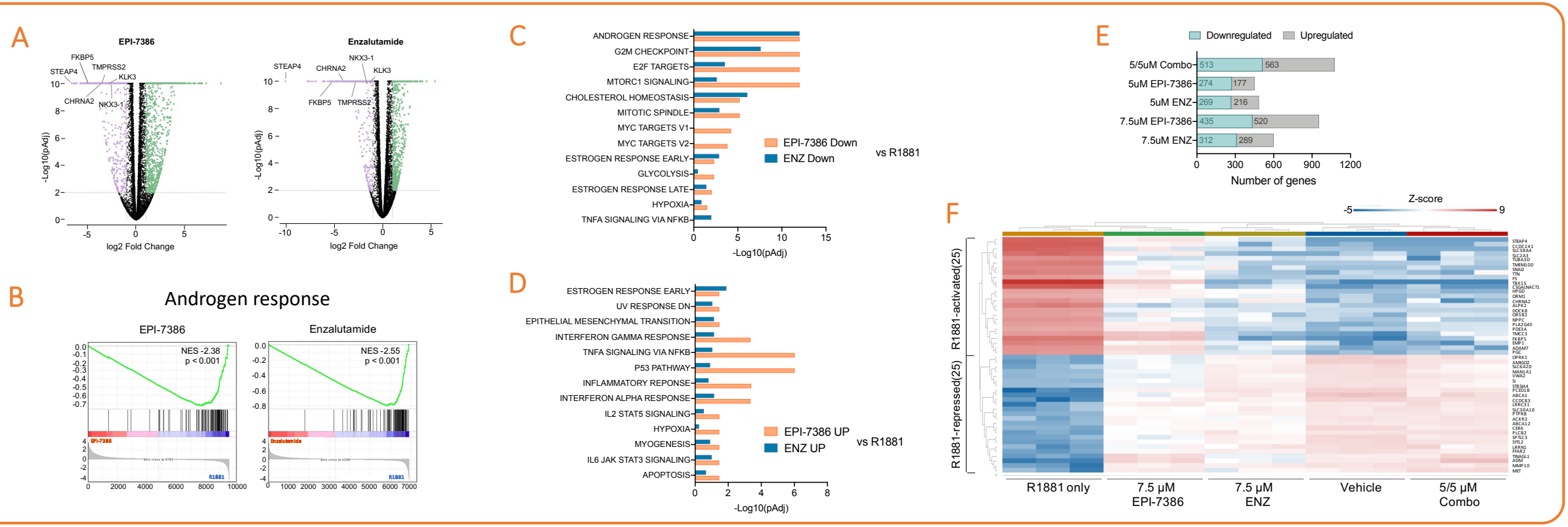


Figure 6. Transcriptomic analysis in LNCaP cells. RNA-seq analysis of LNCaP cells treated with EPI-7386 or enzalutamide (ENZ) as a single agent and in combination for 24 h in the presence of 1 nM R1881. N=3 (A) Volcano plots of the differentially expressed genes between 7.5 µM EPI-7386 or 7.5 µM ENZ treatment vs R1881 only. Significantly down-regulated genes are in purple and significantly up-regulated genes are in green. FC >2, pAdj <0.01. (B) GSEA plots showing enrichment of androgen response gene signature. (C,D) Hallmark pathway enrichment analysis for down- (C) and up-regulated genes (D) following treatments. (E,F) Broader and deeper inhibition of AR pathway by combination treatment of EPI-7386 with ENZ. (E) Number of R1881-responsive genes significantly up- or down-regulated following treatment. FC >3, FDR <0.01. (F) Heatmap showing top 50 R1881-responsive genes that are modulated more than 3-fold by single agent and combination treatment.

*LNCaP cells are resistant to apalutamide and darolutamide due to T878A mutation, and not tested in this model

EPI-7386 combination with ‘lutamides exhibits a broader & deeper inhibition of AR-regulated gene expression in AR amplified VCaP cells

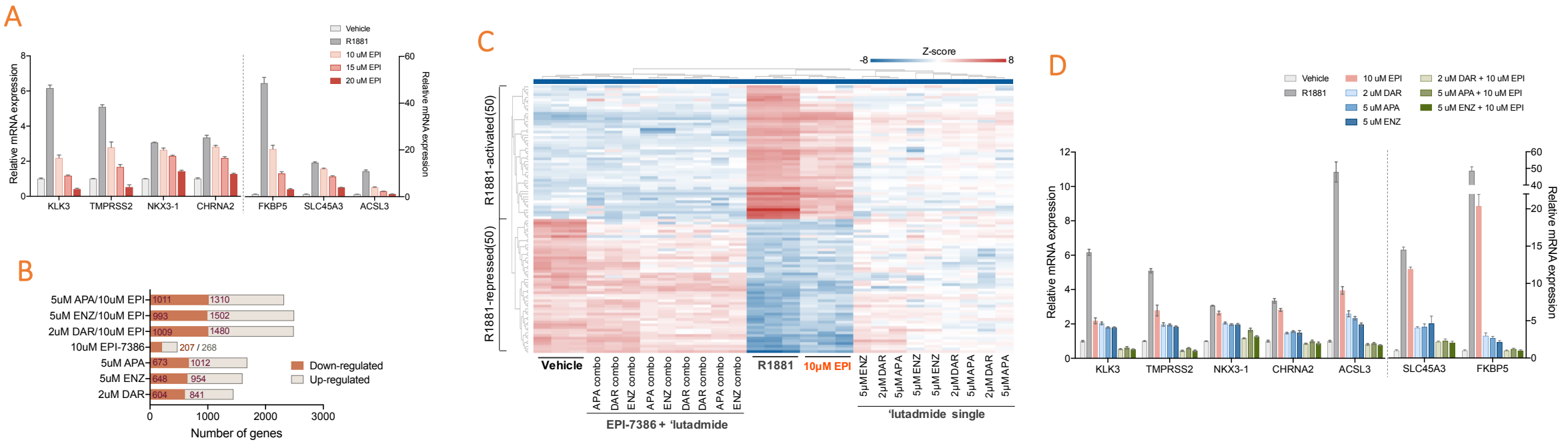


Figure 7. Transcriptomic analysis in VCaP cells. VCaP cells were treated with EPI-7386 (EPI), enzalutamide (ENZ), apalutamide (APA), or darolutamide (DAR) as a single agent and in combination for 24 h in the presence of 1 nM R1881. N=3 (A) Bar graph showing dose response activity of EPI-7386 against androgen-responsive genes from RNAseq. (B) Number of genes significantly up- or down-regulated following treatments compared to R1881 stimulation only condition. FC >2, pAdj <0.01 (C) Heatmap comparing expression level of top 50 R1881-activated or -repressed genes across VCaP cells treated with single agent or combination. (D) Relative expression levels of representative AR-regulated genes in the presence of R1881, showing deeper inhibition by combination treatment.

Summary

EPI-7386 is a second-generation AR NTD inhibitor (aniten) with the following characteristics:

- Engagement with both AR-FL and AR-V7 in cells
- Active against both full-length AR and multiple AR splice variants (AR-V7 and ARv567es)
- Strong displacement of R1881 (androgen)-induced genomic AR binding
- On-target activity against the transcriptional activity of the AR, overall, similarly to enzalutamide but with a few notable qualitative and quantitative differences
- Complementarity with the second generation of 'lutamides in inhibiting the AR-associated transcriptional activity, with broader and deeper inhibition of the AR pathway

- ➢ A Phase 1 dose escalation clinical trial (NCT04421222) of EPI-7386 in men with mCRPC progressing on standard of care therapies including second generation anti-androgens is underway.
- ➢ Early signs of biological activity and declining PSA levels in a multi-refractory patient (01-002) in the initial 200 mg cohort were observed (2021 ASCO-GU, poster #119).
- ➢ Currently, 800 mg cohort is being dosed.