

SUPPLEMENTARY MATERIALS AND METHODS

Immunocytochemistry

LNCaP cells seeded on glass coverslips were allowed to adhere overnight before plating medium was replaced with starvation medium (phenol red-free RPMI-1640 supplemented with 5% charcoal-stripped FCS and 2mM L-glutamine). Cells were starved of androgens for 48 hours before treatment with DMSO or 10 μ M CT7001 in the absence or presence of androgen (10nM mibolerone) for 4 hours. Treated cells were washed with PBS, fixed with 4% paraformaldehyde in PBS for 15 minutes and permeabilized with 0.1% Triton-X diluted in PBS for 10 minutes at room temperature. Non-specific sites were blocked with 10% goat serum diluted in PBS for 1 hour at room temperature. Cells were stained with mouse anti-AR antibody (441) (sc-7305, Santa Cruz Biotechnology, 1:200) diluted in 1% BSA in PBS in a humidified chamber at 4°C overnight. The coverslips were washed 3 times in PBS for 5 minutes followed by staining with Alexa Fluor-594 secondary antibody (Invitrogen, 1:1000) diluted in 1% BSA in PBS for 1 hour at room temperature. Following three PBS washes, the coverslips were mounted on slides using DAPI-fluorescence mounting medium (Abcam). Fluorescence images were captured on a Nikon Eclipse E400 fluorescence upright microscope. For each treatment condition, 3 images were taken from 2 coverslips (6 images per condition). The experiment was repeated twice (n=2 biological repeats). Images were analysed using ImageJ v1.52a. A DAPI staining mask was used to define the nuclear regions of interest (ROI) for measuring mean nuclear AR fluorescence intensity.

Chromatin immunoprecipitation (ChIP)

LNCaP cells were grown in androgen depleted media for 72 hours before treatment for 4 hours with vehicle (DMSO) or 10 μ M CT7001 in the presence of 10 nM mibolerone or equivalent vehicle (ethanol). Cells were fixed for 20 minutes with 1% formaldehyde, lysed, and sonicated to produce 300-1500 bp fragments. Chromatin immunoprecipitation was performed as described previously [1] using anti-AR (sc-7305, Santa Cruz Biotechnology) or rabbit mouse IgG at 10 μ g per sample and 100 μ L of Protein A Dynabeads (Invitrogen). DNA was isolated from samples using phenol:chloroform:isoamyl alcohol, washed, dried, and resuspended in nuclease-free water. Enrichment across three androgen-responsive elements (AREs) in the human PSA promoter, and PSA and FKBP5 enhancers was quantified by qPCR, with the primers listed in **Supplementary Table 2**. Data was calculated as percent input and normalized to non-specific control.

SUPPLEMENTARY TABLES

Supplementary Table 1. Primer sequences for RT-qPCR.

Gene	Primer	Sequence (5'-3')
CDKN1A	Forward	CGATGGAACTTCGACTTTGTCA
CDKN1A	Reverse	GCACAAGGGTACAAGACAGTG
GADD45A	Forward	GAGAGCAGAAGACCGAAAGGA
GADD45A	Reverse	CACAACACCACGTTATCGGG
MDM2	Forward	GAATCATCGGACTCAGGTACATC
MDM2	Reverse	TCTGTCTCACTAATTGCTCTCCT
RRM2B	Forward	AGAGGCTCGCTGTTTCTATGG
RRM2B	Reverse	GCAAGGCCCAATCTGCTTTTT
BACTIN	Forward	GGCATCCTCACCTGAAGTA
BACTIN	Reverse	GGTCATCTTCTCGCGGTTG
GAPDH	Forward	ATGGGGAAGGTGAAGGTCC
GAPDH	Reverse	GGGGTCATTGATGGCAACAATA
RPL19	Forward	GCGGAAGGGTACAGCCAAT
RPL19	Reverse	AGCAGCCGGCGCAAA
PSA	Forward	TTGTCTTCCTCACCTGTCC
PSA	Reverse	AGCTGTGGCTGACCTGAAAT

Supplementary Table 2. TaqMan low-density array qPCR assays

Detector	Description	Response to androgens
18S-Hs99999901_s1	Eukaryotic 18S rRNA	Control
ABCC4-Hs00988717_m1	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	Upregulated
ABHD2-Hs00199684_m1	abhydrolase domain containing 2	Upregulated
ACSL3-Hs00244853_m1	acyl-CoA synthetase long-chain family member 3	Upregulated
ALDH1A3-Hs00167476_m1	aldehyde dehydrogenase 1 family, member A3	Upregulated
ANKH-Hs00219798_m1	ANKH inorganic pyrophosphate transport regulator	Upregulated
DBI-Hs01554584_m1	diazepam binding inhibitor	Upregulated
DHCR24-Hs00207388_m1	24-dehydrocholesterol reductase	Upregulated
DNAJB9-Hs01052402_m1	DnaJ (Hsp40) homolog, subfamily B, member 9	Upregulated
ELL2-Hs01023022_m1	elongation factor, RNA polymerase II, 2	Upregulated
FKBP5-Hs01561006_m1	FK506 binding protein 5	Upregulated
GAPDH-Hs99999905_m1	glyceraldehyde-3-phosphate dehydrogenase	Control
GNMT-Hs00219089_m1	glycine N-methyltransferase	Upregulated
HMGCS1-Hs00940429_m1	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)	Upregulated
HPGD-Hs00960587_m1	hydroxyprostaglandin dehydrogenase 15-(NAD)	Upregulated
IQGAP2-Hs00183606_m1	IQ motif containing GTPase activating protein 2	Upregulated
KLK2-Hs00428383_m1	kallikrein-related peptidase 2	Upregulated
KLK3-Hs02576345_m1	kallikrein-related peptidase 3	Upregulated
LIFR-Hs01123581_m1	leukemia inhibitory factor receptor alpha	Upregulated
MAF-Hs00193519_m1	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog	Upregulated
MTMR9-Hs00209995_m1	myotubularin related protein 9	Upregulated

NDRG1- Hs00608387_m1	N-myc downstream regulated 1	Upregulated
NKX3-1- Hs00171834_m1	NK3 homeobox 1	Upregulated
ORM1-Hs01590791_m1	orosomuroid 1	Upregulated
PDIA5-Hs00895698_m1	protein disulfide isomerase family A, member 5	Upregulated
PGM3-Hs00985101_m1	phosphoglucomutase 3	Upregulated
PMEPA1- Hs00375306_m1	prostate transmembrane protein, androgen induced 1	Upregulated
PTPRM- Hs00267809_m1	protein tyrosine phosphatase, receptor type, M	Upregulated
RAB27A- Hs00608302_m1	RAB27A, member RAS oncogene family	Upregulated
RPLP0- Hs99999902_m1	ribosomal protein, large, P0	Control
SEPP1- Hs01032845_m1	selenoprotein P, plasma, 1	Upregulated
SLC35F2- Hs00213850_m1	solute carrier family 35, member F2	Upregulated
SORD-Hs00162091_m1	sorbitol dehydrogenase	Upregulated
TBP-Hs99999910_m1	TATA box binding protein	Control
TMPRSS2- Hs01120965_m1	transmembrane protease, serine 2	Upregulated
TPD52- Hs00893105_m1	tumor protein D52	Upregulated

Supplementary Table 3. Primer sequences for ChIP-qPCR.

Gene	Primer	Sequence (5'-3')
PSA _{prom}	Forward	GTGCATCCAGGGTGATCTAGTAATT
PSA _{prom}	Reverse	CACACCCAGAGCTGTGGAA
PSA _{enh}	Forward	ACAGACCTACTCTGGAGGAAC
PSA _{enh}	Reverse	AAGACAGCAACACCTTTTT
FKBP5 _{enh}	Forward	GCTCCCTCACACCAGATGACCA
FKBP5 _{enh}	Reverse	CAAATCCAACCCGAGACAGGTGTA

SUPPLEMENTARY REFERENCES

1. Leach DA, Mohr A, Giotis ES, Cil E, Isac AM, Yates LL, et al. The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS-CoV-2 in human lung cells. *Nat Commun.* 2021;12. doi:10.1038/S41467-021-24342-Y.
2. Jafari R, Almqvist H, Axelsson H, Ignatushchenko M, Lundbäck T, Nordlund P, et al. The cellular thermal shift assay for evaluating drug target interactions in cells. *Nat Protoc.* 2014;9:2100–22. doi:10.1038/NPROT.2014.138.