nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	no software was used
Data analysis	Graphpad Prism version 8, 9 and 10 were used for statistical analysis. The following software were used for sequencing data quality control: FastQC (v0.11.9), MultiQC (v1.10.1), Trimmomatic (v0.39). For RNA-seq, STAR aligner (v2.7.8a) and Picard Toolkits (v2.23.8) were used for preprocessing and downstream analyses used R (v4.2.1) and the R packages DESeq2 (v1.36.0) and fgsea (v1.22.0). For ATAC-seq and ChIP-seq, BWA aligner (v0.7.17-r1188), Samtools (v1.12), Picard toolkit (v4.2.2.0), Bedtools (v2.29.2), MACS2 (v2.2.7.1) were used for preprocessing and downstream analyses used the Homer suite (v4.11.1), Deeptools suite (v3.5.1), and the R package DiffBind (v3.6.5).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data availability:

Data needed to evaluate the conclusions from this work are available in the main text or the supplementary materials. The RNA-seq, ATAC-seq and Chip-seq data generated in this study have been deposited in the GEO database under the accession number SuperSeries GSE247593 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247593). Source data are provided with this paper.

Code availability:
The code to reproduce the results from the RNA-seq, ATAC-seq and Chip-seq data is available through the project's source code repository (https://gitlab.oit.duke.edu/dcibioinformatics/pubs/mcdonnell-lab-androgens-dose-2023).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For xenograft studies, using the sample size and the power function in JMP statistical software (SAS Institute, Inc, Cary, NC.), it was estimated that a group size of 10-12 per treatment arm would be required to reliably detect with 80% confidence a statistically relevant ($P < 0.05$) change of 30% given the anticipated 15% variability for the tumor models used in these studies ($\alpha = 0.05$, s.d. = 0.15, confidence of 0.8, s/delta of 0.3). These estimates were based on one way ANOVA followed by the Student Newman-Keul's test. This group size is in accordance with current literature in the field.
Data exclusions	For those animals that died prior to completing treatment courses (no more than 1 per group), recorded data was excluded from graphing and statistical analyses.
Replication	Replication was described as indicated in figure legends and methods.
Randomization	For LNCaP and VCaP xenograft studies, ramdomization into treatment groups took place before tumor initiation and all groups considered equivalent. For the LNCaP-AR xenograft study, when tumor volume reached ~0.15-0.2 cm3, mice were randomized (n = 10-12) to receive treatments.
Blinding	The investigator and personnel were not blinded during these studies as user knowledge of groups/tretaments could not have affected outcome.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibody for β-Actin (AC-15, A5441, 1:800,000, lot#000118417) was purchased from Sigma. Antibodies for Phospho-Rb (Ser807/811; D20B12, #8516, 1:50,000, lot#9), Phospho-p70 S6 Kinase (Thr389; 1A5, #9206, 1:2000, lot#27), Phospho-S6 Ribosomal Protein (Ser235/236; D57.2.2E, #4858, 1:4,000, lot#16) and Phospho-4E-BP1 (Ser65; #9451, 1:1,000, lot#16), c-MYC (D84C12, #5605, 1:2,000, lot#16), PSA (D11E1, #2475, 1:20,000, lot#3), NDRG1 (#5196, 1:1,000, lot#1), FoxM1 (D3F2B, #20459, 1:2,000, lot#1) and Vinculin (E1E9V, #13901, 1:160,000, lot#7) were purchased from Cell Signaling. E2F1 antibody (KH95/E2F, #554213, 1:1,000, lot# 1209343) was purchased from BD Biosciences. Antibodies to AR (441, SC-7305, 1:10,000) and FKBP51 (D-4, sc-271547, 1:60,000, lot# K1419) were purchased from Santa Cruz Biotechnology. Anti-Puromycin (MABE343, 12D10, 1:300,000) was purchased from Sigma. AR antibody (ab236225) used in ChIPseq was purchased from Abcam.

Validation

-Phospho-Rb Ser807/811 antibody was validated by the manufacturer in serum-starved WI-38 cells followed by 10% serum treatment for an additional 48hrs using Western Bot (WB). Furthermore, our data provide additional validation showing that mTOR inhibitors decrease RB phosphorylation, while low dose androgens enhance and high dose androgens decrease RB phosphorylation. Reactivity: human, mouse and rabbit.

-Phospho-p70 S6 Kinase. Antibody was validated by the manufacturer in serum starved NIH/3T3 cells followed by 20% serum treatment using WB. Furthermore, our data provide additional validation showing that mTOR inhibitors decrease while androgens enhance Phospho-p70 S6 phosphorylation. Reactivity: human, mouse and rabbit.

-Phospho-S6 Ribosomal Protein antibody was validated by the manufacturer in serum starved MCF-7 cells followed by IGF-1 treatment using WB. Furthermore, our data provide additional validation showing that mTOR inhibitors decrease Phospho-S6 Ribosomal phosphorylation while androgens enhance Phospho-S6 Ribosomal phosphorylation. Reactivity: human, mouse and rabbit.

-Phospho-4E-BP1 Ser65 antibody was validated using WB by the manufacturer in 293T cells that were starved in serum-free media for 24 hours followed by 1-hour amino acid deprivation. Amino acids were then replenished for 1 hour. Furthermore, our data provide additional validation showing that mTOR inhibitors decrease while androgens increase 4E-BP1 phosphorylation. Reactivity: human, mouse and rabbit.

-FKBP51 antibody has been validated by the manufacturer using WB. Furthermore, our data provide additional validation showing that androgens increase FKBP5 mRNA and protein expression. Reactivity: human, mouse and rabbit.

-c-MYC antibody was validated by the manufacturer in HeLa cells transfected with SignalSilence c-Myc siRNA using WB. Reactivity: human, mouse and Rabbit.

-PSA antibody was validated by the manufacturer showing that only AR expressing LNCaP but no DU145 cells express PSA. Furthermore, our data provide additional validation showing that androgens increase the PSA mRNA and protein expression. Reactivity: human.

-NDRG1 antibody was validated using WB by the manufacturer in HeLa cells transfected with SignalSilence NDRG1 siRNA. Furthermore, our data provide additional validation showing that androgens increase NDRG1 mRNA and protein expression. Reactivity: human, mouse and rabbit.

-FoxM1 antibody was validated using WB by the manufacturer in HeLa cells treated with aphidicolin. Furthermore, our data provide additional validation showing, mTOR inhibitors repress FoXM1 protein expression. Low dose androgens enhance while high dose androgens repress FoXM1 protein expression. Reactivity: human.

-E2F1 antibody was validated by the manufacturer using in vitro translated E2F1 protein. Furthermore, our data provide additional validation showing that mTOR inhibitors repress E2F1 mRNA and protein. Low dose androgens enhance while high dose androgens repress E2F1 mRNA and protein expression. Reactivity: human.

-AR antibodies was validated using an AR degrader (PROTAC). Reactivity: human, mouse and rat

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	All cell lines were obtained from ATCC. LNCaP: human male prostate; VCaP: human male prostate; HEK293: human embryonic kidney; CV-1: monkey kidney cells.
Authentication	ATCC uses short tandem repeat (STR) DNA profiles for authentication.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	None of the cell lines used for these studies are listed in the database of commonly misidentified cell lines maintained by ICLAC.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Six-week-old male NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice were obtained from Jackson Laboratories (Bar Harbor, ME)
Wild animals	N/A
Reporting on sex	male mice were used as the study address the role of androgen levels in prostate cancer.
Field-collected samples	N/A
Ethics oversight	All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th ed., and approved by the Duke University Institutional Animal Care and Use Committee (AUP# A220-21-11 and A278-18-12).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	Processed data are available in GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247593 Access token: ergbaqombnwvvaj Raw data are available in SRA (submitted by GEO on our behalf) with accession number PRJNA1093098.
Files in database submission	Processed data: VCaP_VehicleCFS_AR_1.sorted.filtered.deduped.bflt.bw VCaP_VehicleCFS_AR_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.01_AR_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.01_AR_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.03_AR_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.03_AR_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.06_AR_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.06_AR_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-10_AR_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-10_AR_2.sorted.filtered.deduped.bflt.bw VCaP_VehicleCFS_Input_1.sorted.filtered.deduped.bflt.bw VCaP_VehicleCFS_Input_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.01_Input_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.01_Input_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.03_Input_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.03_Input_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.06_Input_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-0.06_Input_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-10_Input_1.sorted.filtered.deduped.bflt.bw VCaP_R1881-10_Input_2.sorted.filtered.deduped.bflt.bw VCaP_R1881-CFS-0p01_AR_Rep1_TR_peaks.narrowPeak VCaP_R1881-CFS-0p01_AR_Rep2_TR_peaks.narrowPeak VCaP_R1881-CFS-0p03_AR_Rep1_TR_peaks.narrowPeak

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Genome browser session
(e.g. UCSC)

UCSC, GRCh38
https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg38&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr7%3A155799529%2D155812871&hgsid=2086639210_AHA9FAZnwYPhezMDr74VYfSwjHR5

Methodology

Replicates

Two biological replicates were used for each experimental group, with the replicate agreement assessed using IDR (Irreproducible Discovery Rate) procedure, and all groups, except for one (LNCaP, R1881, CFS, 0.03, AR), exhibited both rescue ratio and self-consistency ratio values of less than 2, meeting ENCODE standards.

Sequencing depth

cell line, drug, media, dose, antibody, replicate, total number of raw reads, uniquely mapped reads, length of reads, single- or paired-end
VCaP, R1881, CFS, 0.01, AR, Rep1, 81742572, 60750647, 38bp, paired-end
VCaP, R1881, CFS, 0.01, AR, Rep2, 110270800, 81114612, 38bp, paired-end
VCaP, R1881, CFS, 0.01, Input, Rep1, 100801398, 76800282, 38bp, paired-end
VCaP, R1881, CFS, 0.01, Input, Rep2, 137628228, 70966175, 38bp, paired-end
VCaP, R1881, CFS, 0.03, AR, Rep1, 88585142, 65938692, 38bp, paired-end
VCaP, R1881, CFS, 0.03, AR, Rep2, 109355700, 84468803, 38bp, paired-end
VCaP, R1881, CFS, 0.03, Input, Rep1, 95792636, 72913963, 38bp, paired-end
VCaP, R1881, CFS, 0.03, Input, Rep2, 102583728, 79953879, 38bp, paired-end
VCaP, R1881, CFS, 0.06, AR, Rep1, 92006554, 69034004, 38bp, paired-end

VCaP, R1881, CFS, 0.06, AR, Rep2, 117163778, 92590940, 38bp, paired-end
VCaP, R1881, CFS, 0.06, Input, Rep1, 99018692, 74963556, 38bp, paired-end
VCaP, R1881, CFS, 0.06, Input, Rep2, 87949866, 68817160, 38bp, paired-end
VCaP, R1881, CFS, 10, AR, Rep1, 96229398, 72384934, 38bp, paired-end
VCaP, R1881, CFS, 10, AR, Rep2, 133457490, 106046302, 38bp, paired-end
VCaP, R1881, CFS, 10, Input, Rep1, 94987178, 70208985, 38bp, paired-end
VCaP, R1881, CFS, 10, Input, Rep2, 100431344, 78517657, 38bp, paired-end
VCaP, Vehicle, CFS, 0, AR, Rep1, 103970470, 77287864, 38bp, paired-end
VCaP, Vehicle, CFS, 0, AR, Rep2, 121698102, 95099556, 38bp, paired-end
VCaP, Vehicle, CFS, 0, Input, Rep1, 134364250, 100175162, 38bp, paired-end
VCaP, Vehicle, CFS, 0, Input, Rep2, 123525542, 96574797, 38bp, paired-end
LNCaP, R1881, CFS, 0.03, AR, Rep1, 117340850, 87950092, 38bp, paired-end
LNCaP, R1881, CFS, 0.03, AR, Rep2, 108881458, 78366674, 38bp, paired-end
LNCaP, R1881, CFS, 0.03, Input, Rep1, 81680584, 61110469, 38bp, paired-end
LNCaP, R1881, CFS, 0.03, Input, Rep2, 103941278, 76017237, 38bp, paired-end
LNCaP, R1881, CFS, 0.06, AR, Rep1, 120028252, 89643158, 38bp, paired-end
LNCaP, R1881, CFS, 0.06, AR, Rep2, 124138386, 91505520, 38bp, paired-end
LNCaP, R1881, CFS, 0.06, Input, Rep1, 86718726, 64875663, 38bp, paired-end
LNCaP, R1881, CFS, 0.06, Input, Rep2, 91705834, 68461480, 38bp, paired-end
LNCaP, R1881, CFS, 0.1, AR, Rep1, 129262284, 98267981, 38bp, paired-end
LNCaP, R1881, CFS, 0.1, AR, Rep2, 120257676, 88200419, 38bp, paired-end
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LNCaP, R1881, CFS, 10, AR, Rep1, 150316334, 117367291, 38bp, paired-end
LNCaP, R1881, CFS, 10, AR, Rep2, 147283890, 110556005, 38bp, paired-end
LNCaP, R1881, CFS, 10, Input, Rep1, 96712744, 73020982, 38bp, paired-end
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LNCaP, Vehicle, CFS, 0, AR, Rep1, 145117276, 105359781, 38bp, paired-end
LNCaP, Vehicle, CFS, 0, AR, Rep2, 109185438, 76116951, 38bp, paired-end
LNCaP, Vehicle, CFS, 0, Input, Rep1, 130237772, 99193335, 38bp, paired-end
LNCaP, Vehicle, CFS, 0, Input, Rep2, 93492182, 67739230, 38bp, paired-end

Antibodies

Chromatin corresponding to 1 million cells were incubated with 4µg of the AR antibody (AR: Abcam ab236225, lot GR3341807-3) was used.

Peak calling parameters

macs2 callpeak --treatment {CELLLINE}_R1881-CFS-{DOSE}_AR_{REP}.sorted.filtered.deduped.bflt.bam --control {CELLLINE}_Vehicle-CFS-0_Input.sorted.filtered.deduped.bflt.bam --gsize hs --pvalue 1e-2 --keep-dup all --SPMR --bdg --nomodel --shift 0 --extsize 130 --call-summits -f BAMPE

Data quality

Adapters and low-quality bases were initially trimmed from the raw sequencing reads. Reads that were unaligned, multi-mapped, failed platform quality checks or had a mapping quality less than 30 were removed using Samtools. Duplicate reads were identified by MarkDuplicates program from Picard toolkit and were removed using Samtools. Reads falling into curated blacklisted regions were detected and removed using Bedtools.

Contrast, Number of peaks passed the FDR of 5% and above 5-fold enrichment
VCaP_AR_10_vs_0, 12859
VCaP_AR_0.01_vs_0, 0
VCaP_AR_0.03_vs_0, 0
VCaP_AR_0.06_vs_0, 159
LNCaP_AR_10_vs_0, 19968
LNCaP_AR_0.03_vs_0, 0
LNCaP_AR_0.06_vs_0, 294
LNCaP_AR_0.1_vs_0, 3280

Software

FastQC, MultiQC, samtools, bedtools, Picard-MarkDuplicates and IDR were used for quality control.
Trimmomatic was used to trim adapters and low-quality bases.
BWA-aln was used to align reads to the human reference genome.
Macs2 was used to call peaks and output in narrowPeak format.
Deeptools was used for generating coverage files in bigwig format, and creating visualizations.
Homer was used for motif analysis and annotating peaks to genomic features.
R package Diffbind was used for differential binding analysis.
R package Fgsea was used for pathway analysis.
For more details please refer to the Methods section in the manuscript. And the code for QC and analysis has been deposited to https://gitlab.oit.duke.edu/dcibioinformatics/pubs/mcdonnell-lab-androgens-dose-2023.