

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Flow cytometry data was acquired by BD FACSCorus™ Software (v.5.2.0). qPCR data was acquired Bio-Rad CFX Manager (v. 3.1). Western and northern blot imaging were acquired using a Bio-Rad ChemiDoc Go Imaging System with Image Lab Touch Software (v.4.1). Tissue slides were scanned by SLIDEVIEW VS200 slide scanner with VS200 ASW software (v.4.2). Confocal images were collected using an Andor Dragonfly spinning-disk confocal microscope equipped with Fusion acquisition software (v.2.7). Polysome profiling data was collected by using Gradient Profiler software (v.2.07). For real-time cell confluence monitoring, Sartorius Incucyte Live-Cell Analysis Systems (v.2025A, v.2026A) was used. Bioluminescence signal was measured by LagoX small-animal imaging system with Aura software (v.5.0.0).
Data analysis	For RNA-seq, sequencing reads were mapped to the GRCh38/hg38 human genome using STAR.v2.7.3a. Gene level abundance was quantitated using the GenomicAlignments summarizeOverlaps function using mode=IntersectionStrict, restricting to primary aligned reads, and counting reads mapping to the exonic regions of genes. FPKM (fragments per kilobase per million mapped reads) were calculated using R and used for all downstream analyses. For polysome RNA-seq, raw sequencing reads were assessed for quality using FastQC (v.0.12.1). Libraries passing quality control were trimmed of sequencing adapters and aligned to the GRCh38 human genome using STAR2 (v.2.7.3a) and quantified for gene-level expression using HTSeq (v.0.11.1) against the GENCODE v39 gene annotation database to calculate strand-specific read count for each gene. For msr-seq, barcode demultiplexing and sequence alignment were performed as previously described (Wakins et al., 2022, PMID: 35513407). Briefly, the libraries were aligned to the custom curated GRCh38/hg38 human transcriptome from the nonredundant, high-scoring tRNA gene sequences using bowtie2 (v.2.5.4) and converted to .bam files by SAMtools (v.1.21). Analyses of tRNA expression, fragmentation, modifications were performed with custom scripts developed by Fietze et al (https://github.com/Luke-F1875/MSRseq_data_processing_pipeline).

For CUT&RUN, sequencing reads were aligned to the human reference genome (hg38) using bowtie2, and only uniquely mapped reads were retained for downstream analyses. Peak calling was performed using SEACR (Sparse Enrichment Analysis for CUT&RUN; v.1.3) with matched IgG controls using the relaxed threshold setting. CUT&RUN reads were quantified over annotated tRNA loci extended by 50 bp upstream and downstream using summarizeOverlaps from the Bioconductor GenomicAlignments package (v.1.40.0). Only primary, properly paired alignments with a mapping quality (MAPQ) ≥ 30 were included in the analysis. Raw fragment counts were imported into the edgeR package, where library sizes were normalized using the trimmed mean of M-values (TMM) method. Differential occupancy between conditions was assessed using the quasi-likelihood negative binomial generalized linear model framework implemented in edgeR (4.2.0), and P values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure.

For tissue slide images, signal intensity and size were measured by HALO software (v.3.6).

For confocal imaging analysis, Imaris software (v.10.2) was used for signal measurement and quantification.

For cell confluence imaging analysis, Incucyte software (v.2025A, v.2026A) was used.

Bioluminescence signal was analyzed by Aura software (v.5.0.0).

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](#)

MSR-seq, Polysome RNA-seq, RNA-seq, and CUT&RUN data can be accessed in the Gene Expression Omnibus under accession GSE304469.

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	This study involves tissues from patients with metastatic prostate cancer and only males were included.
Reporting on race, ethnicity, or other socially relevant groupings	No socially constructed/relevant categorization was done.
Population characteristics	Patients include those with metastatic castration resistant prostate cancer from UW/Fred Hutchinson Cancer Center. The human participants were terminal stage prostate cancer patients (47 - 90+ years).
Recruitment	Tissue samples from patients were collected after informed consent at the UW/Fred Hutchinson Cancer Center clinics. As the study used existing clinical specimens, recruitment was not performed specifically for this study. Selection bias related to specimen availability, clinical characteristics, and the source population cannot be excluded and may affect the generalizability of the findings.
Ethics oversight	Collection and use of patient tissue samples were approved by UW IRB (IRB#2341).

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](https://www.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	No formal a priori power calculation or statistical methods were used to determine the sample size. The sample sizes for in vitro studies were chosen based on optimization experiments and previous studies using similar experimental systems/conditions, and the need to obtain reproducible measurements of the indicated biological effects. Each quantitative experiment was performed in at least three independent biological replicates, and the sample size for each experiment is provided in the corresponding figure legend.
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For in vivo studies, no formal sample size calculation was performed. Sample sizes were based on prior studies using similar animal models and experimental endpoints, as well as practical and ethical considerations. The selected sample sizes were considered sufficient to detect reproducible differences in the predefined experimental endpoints while minimizing the number of animals used.

Data exclusions	A standard quality control process was followed for MSR-seq and polysome RNA-seq to trim adapter sequences and low-quality base calls. For MSR-seq and polysome RNA-seq, we excluded replicates that do not meet our QC metrics. The details of these datasets are provided in the Methods section.
Replication	All experiments were performed independently at least three times and each replicate was successful.
Randomization	No randomization was performed in this study. Age-matched mice were assigned to different experimental groups. For experiments other than those involving mice, covariate control was not applicable because the experiments used defined in vitro experimental systems/ conditions without relevant participants or subject-level covariates. Experimental conditions were kept consistent across groups, and independent biological replicates were performed as indicated in the figure legends.
Blinding	No blinding was conducted in this study. All analyses were based on objective quantification of the data generated from in vitro and in vivo experiments.

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	Details of all antibodies used in this study are provided in the Methods section and Supplementary Table 9. For immunoblotting, the following antibodies were used: PSA/CLK3 antibody (Cell Signaling Technology, #2475, 1:1000 dilution), FKBP5 antibody (Cell Signaling Technology, #8245, 1:1000 dilution), NKX3-1 antibody (Abcam, #ab196020, 1:1000 dilution), SMARCC2 antibody (Santa Cruz Biotechnology, #sc-10757, 1:1000 dilution), SMARCB1 antibody (Cell Signaling Technology, #91735, 1:1000 dilution), ASCL1 antibody (Santa Cruz Biotechnology, #sc-374104, 1:1000 dilution), FLAG-tag antibody (Cell Signaling Technology, #2368, 1:1000 dilution), Myc antibody (Abcam, #ab32072, 1:1000 dilution), GAPDH antibody (Santa Cruz Biotechnology, #sc-32233, 1:1000 dilution), beta-actin antibody (Sigma-Aldrich, #A5316, 1:1000 dilution), alpha-tubulin antibody (Sigma-Aldrich, #T8203), Vinculin antibody (Cell Signaling Technology, #13901, 1:1000 dilution), goat anti-rabbit IgG HRP (Fisher Scientific, #PI31460, 1:10000 dilution), goat anti-mouse IgG HRP (Fisher Scientific, #PI31430, 1:10000 dilution). For IHC, the following antibodies were used: Myc antibody (Abcam, #ab32072, 1:100 dilution) For IF, the following antibodies were used: TARDBP antibody (Santa Cruz Biotechnology, #sc-376532, 1:50 dilution), ZSCAN29 antibody (Proteintech, #21630-1-AP, 1:20 dilution), Alexa Fluor 488 conjugated anti-rabbit IgG secondary antibody (Thermo Fisher Scientific, #A-11034, 1:500 dilution), Alexa Fluor 594 conjugated anti-mouse IgG secondary antibody (Thermo Fisher Scientific, #A-11032).
Validation	All the primary antibodies were selected based on manufacturer-validated reactivity for the indicated species and application. Antibodies used for the experiments were validated for the corresponding application according to the manufacturer's specifications.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Human cell lines: LNcaP (LNcaP Clone FGC; ATCC, #CRL-1740), C4-2B (ATCC, #CRL-3315), 22RV1 (ATCC, #CRL-2505), DU145 (ATCC, #HTB-81), PC3 (ATCC, #CRL-1435), VCaP (ATCC, #CRL-2876), LNcaP-abl (RRID:CVCL_4793), HEK293T (ATCC, #CRL-3216), LNcaP AD, LNcaP LP, C4-2B AD, C4-2B LP (from Dr. John Lee) Mouse cell lines: prostate organoid lines derived from Hi-Myc;nTr22+/+ and Hi-Myc;nTr22+/- male mice
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Authentication	The cell lines were authenticated from the manufacturer by STR profiling.
Mycoplasma contamination	All cell lines used are mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used.

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	Hi-Myc mice (Tg(ARR2/Pbsn-MYC)7Key, RRID:MGI:5486199), n-Tr22-/- mice (C57BL6/J 6J.6N-rs46447118, nTr22(-3_90del)), TRAMP mice (C57BL/6-Tg(TRAMP)8247Ng/J, RRID:IMSR_JAX:003135), and n-Tr22OE mice (B6J-Tg(trNA-Arg-TCT-1-1)529Slac) were bred and maintained in specific pathogen-free conditions in the animal facility of Fred Hutchinson Cancer Center. Hi-Myc mice were aged for 9 months before castration and aged 2 additional months after the surgery before prostate harvest. TRAMP mice were aged 5-6 months prior to castration and aged 1 month following surgery before prostate harvest. 8-12 wk-old NSG male mice for in vivo metastasis assay were obtained from the Comparative Medicine Translational Research Model Services Core at the Fred Hutchinson Cancer Center.
Wild animals	No wild animals were used in the study.
Reporting on sex	Only male mice were used as this study focuses on prostate cancer.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The in vivo study was approved by the Fred Hutchinson Cancer Center Institutional Animal Care and Use Committee.

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Mouse prostate organoid establishment: Prostates from 8-12 wk old Hi-Myc;nTr22+/+ and Hi-Myc;nTr22+/- male mice were harvested and dissected in DMEM medium supplemented with 10% FBS, 1% GlutaMax, 1% Penicillin-Streptomycin. Prostate lobes were microdissected using clean blade followed by incubation with collagenase I (Gibco, 17018029) for 1 h at 37 °C and trypsin-EDTA 0.05% for 5 min on a 37 °C rocker. Enzyme reaction was quenched by dissecting media containing 2 mg DNase (Sigma, 101041590010). Enzyme-dissociated prostates were mechanically dissociated 2-3 times using a 3 ml syringe with an 18 G needle and filtered through a 40 µm cell strainer. The dissociated prostates were centrifuged at 350 g for 5 min and resuspended in 1 ml dissecting media for cell count and stained with 3 µl each of CD49f-PE (R&D Systems, FAB13501P, RRID:AB_357017) and EpCAM-APC (Biolegend, 118214, RRID:AB_1134102) antibodies. Isolated prostate cells were enriched for basal stem cell populations (CD49f-positive and EpCAM-positive) by FACS sorting. In vivo metastasis assay: C4-2B shScr and shUCU cell lines were transduced with lentivirus containing the pFUGW-FerH-
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ffLuc2-eGFP plasmid (Addgene, 71393) to express luciferase-eGFP. Following 72-96 h of transduction, selection was done using 1.5 µg ml⁻¹ puromycin for 5-7 days and the transduced cells were grown to ~80% confluence in 100-mm dishes. Cells were harvested, filtered through a 35 µm strainer, and sorted for eYFP+/eGFP+ cell populations.

Instrument

BD FACSDiscover™ S8 Cell Sorter

Software

BD FACSCorus™ (v.5.2.0)

Cell population abundance

Cells positive for both markers (CD49f and EpCAM) were sorted, collected, used for this study.
For luciferase expressing cell lines, C4-2B shScr and shUCU cell lines positive for both eYFP (shRNA) and eGFP (luciferase) were sorted and expanded for in vivo metastasis assay.

Gating strategy

For both mouse prostate cells and C4-2B cell lines, they were first gated on forward scatter (FSC-A) and side scatter (SSC-A). The live cell population was then gated on forward scatter (FSC-A and FSC-H). Single cells from the mouse prostate basal cell population were gated based on their staining with CD49f-PE and EpCAM-APC and those from C4-2B luciferase expressing cell population were gated based on their eYFP+ and eGFP+ intensity.

C4-2B cell lines were first gated on forward scatter (FSC-A) and side scatter (SSC-A). The live cell population was then gated on forward scatter (FSC-A and FSC-H). Single cells were gated based on their staining with CD49f-PE and EpCAM-APC. C4-2B cell lines were Mouse prostate cells were first gated on forward scatter (FSC-A) and side scatter (SSC-A). The live cell population was then gated on forward scatter (FSC-A and FSC-H). Single cells were gated based on their staining with CD49f-PE and EpCAM-APC.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.