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	Flow cytometry data were collected using FACSDiva version 7.0. Immunofluorescent images were obtained using Aperio ImageScope version 12.4.
Data analysis	Statistical analyses were conducted using GraphPad Prism version 9.5. Flow cytometry data were analyzed using FlowJo version 10.8. Immunofluorescent images were analyzed using Aperio ImageScope version 12.4. For the scRNA-seq analysis, FastX-Toolkit v. 0.0.13.2 quality trimmer was used to trim reads based on quality score and FASTX-Toolkit quality chart was used to make read per-base quality plots. The htseq-count script in HTSeq v.0.6.1 and Biopython v.2.7.3 were used to generate a counts matrix. R version R-3.4.1 and the package Seurat v. 1.4 was used to cluster cells into subpopulations using the first 4 principal components, a resolution of 0.5, and k.param = 4. Seurat was used to identify differentially expressed genes. Ingenuity Pathway Analysis (IPA, Qiagen) was used to perform pathway analyses and enrichment analyses. For the ATAC-seq analysis, the quality control for ATAC-seq data was performed using FastQC v0.11.6. Raw sequencing reads were aligned to mm10 using BWA mem v0.7.17-r1188 with "-M" with other parameters kept as default. Duplicate reads were removed using PicardTools v2.12.2 (http://broadinstitute.github.io/picard/). Properly mapped pairs were extracted by SAMtools. Reads were offset by using the publicly available script ATAC_BAM_shifter_gappedAlign.pl and those with TLEN < 30bp were removed. Required tag directories were generated by makeTagDirectory from HOMER v4.8. Differential chromatin accessible regions/peaks between naïve and inflamed bPSC were obtained using getDifferentialPeaksReplicates.pl from HOMER v4.8 with "-f 1.5 -edgeR -balanced". pos2bed.pl was used to generate merged peak files from differential peaks for each sample. makeUCSCfile was used to generate bedGraph files from tag directories. bedGraphToBigWig was then used to generate bigwig files from bedGraph files with the mm10 chromosome size file. For the CUT&Tag analysis, CUT&Tag reads were aligned to the mm10 genome build using Bowtie2 (v2.5.1) with the '--local --very-sensitive --no-mixed --no-discordant --phred33 -I 10 -X 700' parameters. The resulting BAM files were sorted with SAMtools (v1.17) and duplicate reads were marked using Picard MarkDuplicates (v3.1.1) (http://broadinstitute.github.io/picard/). BigWig files were generated from the aligned

reads using DeepTools (v3.5.1) with the '--normalizeUsing CPM --minMappingQuality 30' parameters. The differential peaks from ATAC-seq were grouped into three clusters using the computeMatrix and plotHeatmap functions from Deeptools. These peak clusters were further analyzed by subclustering CUT&Tag AR samples to identify ATAC-seq differential peaks that contain AR binding. findMotifsGenome.pl from HOMER v4.11 was used to find motifs enriched in the peaks of different clusters and annotatePeaks.pl was used to annotate the peaks and identify adjacent genes. Metascape web tool was used to assess enrichment in KEGG and Hallmark pathways.

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

The scRNA-seq, ATAC-seq and CUT&Tag data acquired in this study have been deposited in the Gene Expression Omnibus database under the accession codes GSE271146, GSE269582 and GSE269548, respectively.

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	Sample sizes were not pre-calculated. For each study from which the data were presented, we used statistical analyses consistent with the sample sizes and all the sample sizes were large enough to perform statistical analysis. We also made every effort to avoid excessive use of animals.
Data exclusions	No animal data were excluded from analyses.
Replication	All attempts at replication were successful. The data presented were from at least three independent experiments.
Randomization	Age-matched transgenic mice with the same genotypes were randomized into different experimental groups, e.g. naive and inflamed, sham and castrated.
Blinding	Investigators were not blinded. Blinding was not relevant in this study. 1) Mice were previously genotyped to be assigned into different experimental and control groups; 2) The experiment procedures, e.g. prostate inflammation and castration, generated distinct outcomes, e.g. infiltration of inflammatory cells and dramatically reduced prostate size.

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

Antibodies used in flow cytometry and sorting:
Mouse:
CD45-FITC (#103108, Clone 30-F11, Biolegend)
CD31-FITC (#102506, Clone MEC13.3, Biolegend)
Sca-1-APC (#122512, Clone E13-161.7, Biolegend)
CD49f-PE (#313612, Clone GoH3, Biolegend)
CD45-PerCP (#103130, Clone 30-F11, Biolegend)
Sca-1-APC (#122512, Clone E13-161.7, Biolegend)
EpCAM-PE/Cyanine7 (#118215, Clone G8.8, Biolegend)
CD49f-BV421(#313624, Clone GoH3, Biolegend)
AR (#ab52615, Clone EP670Y, Abcam)

Human:
CD45-FITC (#304006, Clone HI30, Biolegend)
EpCAM-PE (#324206, Clone 9C4, Biolegend)
CD26-APC (#302710, Clone BA5b, Biolegend)
CD49f-BV421 (#313624, Clone GoH3, Biolegend)

Antibodies used in organoid treatments:
IL1-RA (#AF-480-NA, R&D Systems)
IL-1a (#AF-400-NA, R&D Systems)

Antibodies used in immunoblots:
AR (N-20) (#sc-816, Santa Cruz Biotechnology)

Antibodies used in immunohistochemistry and immunofluorescence:
AR (#ab133273, Clone EPR1535(2), Abcam)
AR (#MA1-150, Clone AN1-15, Invitrogen)
AR (#PA5-16363, Invitrogen)
GFP (#A-11122, Invitrogen)
CK5 (#905501, Clone Poly19055, Biolegend)
CK8 (#904804, Clone 1E8, Biolegend)

Antibodies used in CUT&Tag:
Primary antibodies: AR (#ab133273, Abcam), H3K27ac (#ab4729, Abcam), and IgG (#31235, Invitrogen)
Secondary antibody (#ABIN101961, Antibodies-online)

Validation

Antibodies used in this study were all commercially available and validated by the manufacturers we purchased them from. Citations for these antibodies were listed on manufacturers' websites for each antibody.

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

Genetically modified mice with C57BL/6 background were used in this study. Male mice aged 8-12 weeks were utilized. All animals were housed and maintained under pathogen-free conditions with 12 hour-light/dark cycles.

Wild animals	The study did not involve wild animals.
Reporting on sex	Only male mice were used as the study was focused on prostate inflammation.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal procedures were performed in accordance with protocols approved by Purdue University Animal Care and Use Committee (PACUC).

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.*	The CUT&Tag data acquired in this study have been deposited in the Gene Expression Omnibus database under the accession codes GSE269548.
Files in database submission	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE269548
Genome browser session (e.g. UCSC)	No longer applicable.

Methodology

Replicates	Each replicate is from 350-550k bPSC (naïve or inflamed) collected from 4-9 animals.
Sequencing depth	Minimum of 20 million reads per sample
Antibodies	Primary antibodies (dilution 1:50): AR (#ab133273, Abcam), H3K27ac (#ab4729, Abcam), and IgG (#31235, Invitrogen). Secondary antibody (#ABIN101961, Antibodies-online) (dilution 1:100).
Peak calling parameters	Differential chromatin accessible regions/peaks between naïve and inflamed bPSC were obtained using getDifferentialPeaksReplicates.pl from HOMER v4.859 with "-f 1.5 -edgeR -balanced". pos2bed.pl was used to generate merged peak files from differential peaks for each sample.
Data quality	Read mapping rates were above 80%, and the qualities were appropriate according to FastQC evaluations.
Software	CUT&Tag reads were aligned to the mm10 genome build using Bowtie2 (v2.5.1) with the '--local --very-sensitive --no-mixed --no-discordant --phred33 -I 10 -X 700' parameters. The resulting BAM files were sorted with SAMtools (v1.17) and duplicate reads were marked using Picard MarkDuplicates (v3.1.1) (http://broadinstitute.github.io/picard/). BigWig files were generated from the aligned reads using DeepTools (v3.5.1) with the '--normalizeUsing CPM --minMappingQuality 30' parameters. The differential peaks from ATAC-seq were grouped into three clusters using the computeMatrix and plotHeatmap functions from Deeptools. These peak clusters were further analyzed by subclustering CUT&Tag AR samples to identify ATAC-seq differential peaks that contain AR binding. findMotifsGenome.pl from HOMER v4.11 was used to find motifs enriched in the peaks of different clusters and annotatePeaks.pl was used to annotate the peaks and identify adjacent genes. Metascape web tool was used to assess enrichment in KEGG and Hallmark pathways.

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	Minced prostate tissues were digested in 1 mg/mL collagenase (Sigma-Aldrich) in RPMI-1640 (Gibco) media containing 10% FBS (Corning) with shaking at 37ºC for 2 hours, followed by trypsinization. Dissociated cells were passed through 20G needles and 40 µm cell strainers to eliminate aggregates, followed by removal of red blood cells by ACK buffer.
Instrument	Celll sorting was performed on the BD FACSAria under sterile conditions. Flow cytometry data were collected using BD LSRFortessa.
Software	FACSDiva was used to collect and FlowJo was used to analyze the flow cytometry data.
Cell population abundance	The purpose of sorting in our study was to enrich basal prostate stem cells and to compare the same sorted populations using the same gating strategy between different experimental groups. We did not determine the purity of the samples after sorting.
Gating strategy	Preliminary FSC-A/SSC-A gating were performed on the starting cell population to remove cell debris, followed by singlets. Additional gating strategies used for relevant experiments were presented in Figure 1 and Supplemental Figure 3.

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