RESEARCH ARTICLE

Androgen-dependent alternative mRNA isoform expression in prostate cancer cells [version 1; peer review: 3 approved]

Jennifer Munkley [1], Teresa M. Maia[2,3], Nekane Ibarluzea[1,4,5], Karen E. Livermore[1], Daniel Vodak[6], Ingrid Ehrmann[1], Katherine James[7,8], Prabhakar Rajan[9], Nuno L. Barbosa-Morais[2], David J. Elliott[1]

[1]Institute of Genetic Medicine, University of Newcastle, Newcastle upon Tyne, Newcastle, NE1 3BZ, UK
[2]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, 1649-028, Portugal
[3]VIB Proteomics Core, Albert Baertsoenkaai 3, Ghent, 9000, Belgium
[4]Biocruces Bizkaia Health Research Institute, Cruces University Hospital, Barakaldo, 48903, Spain
[5]Centre for Biomedical Research on Rare Diseases (CIBERER), ISCIII, Valencia, 46010, Spain
[6]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway
[7]Interdisciplinary Computing and Complex BioSystems Research Group, Newcastle University, Newcastle upon Tyne, NE4 5TG, UK
[8]Life and Earth Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK
[9]Barts Cancer Institute, Queen Mary University of London, John Vane Science Centre, London, EC1M 6BQ, UK

v1 **First published:** 03 Aug 2018, **7**:1189 (https://doi.org/10.12688/f1000research.15604.1)
Latest published: 03 Aug 2018, **7**:1189 (https://doi.org/10.12688/f1000research.15604.1)

Abstract

Background: Androgen steroid hormones are key drivers of prostate cancer. Previous work has shown that androgens can drive the expression of alternative mRNA isoforms as well as transcriptional changes in prostate cancer cells. Yet to what extent androgens control alternative mRNA isoforms and how these are expressed and differentially regulated in prostate tumours is unknown.
Methods: Here we have used RNA-Seq data to globally identify alternative mRNA isoform expression under androgen control in prostate cancer cells, and profiled the expression of these mRNA isoforms in clinical tissue.
Results: Our data indicate androgens primarily switch mRNA isoforms through alternative promoter selection. We detected 73 androgen regulated alternative transcription events, including utilisation of 56 androgen-dependent alternative promoters, 13 androgen-regulated alternative splicing events, and selection of 4 androgen-regulated alternative 3′ mRNA ends. 64 of these events are novel to this study, and 26 involve previously unannotated isoforms. We validated androgen dependent regulation of 17 alternative isoforms by quantitative PCR in an independent sample set. Some of the identified mRNA isoforms are in genes already implicated in prostate cancer (including *LIG4*, *FDFT1* and *RELAXIN*), or in genes important in other cancers (e.g. *NUP93* and *MAT2A*). Importantly, analysis of transcriptome data from 497 tumour samples in the TGCA prostate adenocarcinoma (PRAD) cohort identified 13 mRNA isoforms (including *TPD52*, *TACC2* and *NDUFV3*) that are differentially regulated in localised prostate cancer relative to normal tissue, and 3 (*OSBPL1A*, *CLK3* and *TSC22D3*) which change significantly with Gleason

Open Peer Review

Reviewer Status ✓ ✓ ✓

	Invited Reviewers		
	1	**2**	**3**
version 1 published 03 Aug 2018	✓ report	✓ report	✓ report

1 **Sebastian Oltean**, University of Exeter, Exeter, UK

2 **Cyril F. Bourgeois**, University of Lyon, Lyon, France

3 **Jennifer Byrne**, The Children's Hospital at Westmead, Westmead, Australia

Any reports and responses or comments on the article can be found at the end of the article.

grade and tumour stage.
Conclusions: Our findings dramatically increase the number of known androgen regulated isoforms in prostate cancer, and indicate a highly complex response to androgens in prostate cancer cells that could be clinically important.

Keywords
Androgens, AR, prostate cancer, alternative splicing, alternative promoters, alternative 3' ends, transcription, mRNA isoforms

Corresponding author: Jennifer Munkley (jennifer.munkley@ncl.ac.uk)

Author roles: Munkley J: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Maia TM**: Data Curation, Formal Analysis, Investigation, Writing – Review & Editing; **Ibarluzea N**: Data Curation, Formal Analysis, Methodology, Validation, Writing – Review & Editing; **Livermore KE**: Investigation, Methodology; **Vodak D**: Formal Analysis, Investigation; **Ehrmann I**: Investigation, Methodology; **James K**: Formal Analysis; **Rajan P**: Conceptualization, Funding Acquisition, Writing – Review & Editing; **Barbosa-Morais NL**: Formal Analysis, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Elliott DJ**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was funded by Prostate Cancer UK [PG12-34, S13-020 and RIA16-ST2-011].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Munkley J, Maia TM, Ibarluzea N *et al.* **Androgen-dependent alternative mRNA isoform expression in prostate cancer cells [version 1; peer review: 3 approved]** F1000Research 2018, **7**:1189 (https://doi.org/10.12688/f1000research.15604.1)

First published: 03 Aug 2018, **7**:1189 (https://doi.org/10.12688/f1000research.15604.1)

Introduction

A single human gene can potentially yield a diverse array of alternative mRNA isoforms, thereby expanding both the repertoire of gene products and subsequently the number of alternative proteins produced. mRNAs with different exon combinations are transcribed from most (up to 90%) human genes, and can generate variants that differ in regulatory untranslated regions, or encode proteins with different sub-cellular localisations and functions[1–5]. Altered splicing patterns have been suggested as a new hallmark of cancer cells[6–8], and in prostate cancer there is emerging evidence that expression of specific mRNA isoforms derived from cancer-relevant genes may contribute to disease progression[9–11].

Androgen steroid hormones and the androgen receptor (AR) play a key role in the development and progression of prostate cancer, with alternative splicing enabling cancer cells to produce constitutively active ARs[11–13]. The AR belongs to the nuclear receptor superfamily of transcription factors, and is essential for prostate cancer cell survival, proliferation and invasion[14–16]. Classically, androgen binding promotes AR dimerization and its translocation to the nucleus, where it acts as either a transcriptional activator or a transcriptional repressor to dictate prostate specific gene expression patterns[17–23]. The major focus for prostate cancer therapeutics has been to reduce androgen levels through androgen deprivation therapy (ADT), either with inhibitors of androgen synthesis (for example, abiraterone) or with antagonists that prevent androgen binding to the AR (such as bicalutamide or enzalutamide)[24]. Although ADT is usually initially effective, most patients ultimately develop lethal castrate resistant disease for which there are limited treatment options[11,12].

Androgens and other steroid hormones have also been associated with alternative splicing. Recent RNA-sequencing-based analysis of the androgen response of prostate cancer cells grown *in vitro* and within patients following ADT identified a set of 700 genes whose transcription is regulated by the AR in prostate cancer cells[25]. However, in addition to regulating transcriptional levels, steroid hormone receptors can control exon content of mRNA[10,26–29]. In prostate cancer androgens can modulate the expression of mRNA isoforms via pre-mRNA processing and promoter selection[9,10,18,30]. The AR can recruit the RNA binding proteins Sam68 and p68 as cofactors to influence alternative splicing of specific genes, and studies using minigenes driven from steroid responsive promoters indicate that the AR can affect both the transcriptional activity and alternative splicing of a subset of target genes[11,31,32]. Other steroid hormones also coordinate both transcription and splicing decisions[29]. The thyroid hormone receptor (TR) is known to play a role in coordinating the regulation of transcription and alternative splicing[27], and the oestrogen receptor (ER) can both regulate alternative promoter selection and induce alternative splicing of specific gene sets that can influence breast cancer cell behaviour[28,33–35].

In previous work we used exon level microarray analysis to identify 7 androgen dependent changes in mRNA isoform expression[10]. However, to what extent androgen-regulated mRNA isoforms are expressed in clinical prostate cancer is unclear. To address this, here we have used RNA-Sequencing data to globally profile alternative isoform expression in prostate cancer cells exposed to androgens, and correlated the results with transcriptomic data from clinical tissue. Our findings increase the number of known AR regulated mRNA isoforms by 10 fold and imply that pre-mRNA processing is an important mechanism through which androgens regulate gene expression in prostate cancer.

Methods

Cell culture

Cell culture was as described previously[25,36]. All cells were grown at 37°C in 5% CO_2. LNCaP cells (CRL-1740, ATCC) were maintained in RPMI-1640 with L-Glutamine (PAA Laboratories, R15-802) supplemented with 10% Fetal Bovine Serum (FBS) (PAA Laboratories, A15-101). For androgen treatment of cells, medium was supplemented with 10% dextran charcoal stripped FBS (PAA Laboratories, A15-119) to produce a steroid-deplete medium. Following culture for 72 hours, 10 nM synthetic androgen analogue methyltrienolone (R1881) (Perkin-Elmer, NLP005005MG) was either added (Androgen +) or absent (Steroid deplete) for the times indicated.

RNA-Seq analysis

RNA-seq transcript expression analysis of previously generated data[25] was performed according to the Tuxedo protocol[37]. All reads were first mapped to human transcriptome/genome (build hg19) with TopHat[38]/Bowtie[39], followed by per-sample transcript assembly with Cufflinks[40]. The mapped data was processed with Cuffmerge, Cuffdiff and Cuffcompare, followed by extraction of significantly differentially expressed genes/isoforms; expression changes between cells grown with androgen and cells grown without androgens were assessed. Reference files for the human genome (UCSC build hg19) were downloaded from the Cufflinks pages: (UCSC-hg19 package from June 2012 was used.). The software versions used for the analysis were: TopHat v1.4.1, SAM tools Version: 0.1.18 (r982:295), bowtie version 0.12.8 (64-bit) and cufflinks v1.3.0 (linked against Boost version 104000). The Tuxedo protocol[37] was carried out as follows: For steps 1–5, no parameters (except for paths to input/output files) were altered. In step 15, additional switches -s, -R, and -C were used when running cuffcompare. Steps 16–18 (extraction of significant results) were performed on the command line.

RNA extraction, RT–PCR and real-time PCR

Cells were harvested and total RNA extracted using TRIzol (Invitrogen, 15596-026) according to manufacturer's instructions. RNA was treated with DNase 1 (Ambion, AM2222) and cDNA was generated by reverse transcription of 500ng of total RNA using the Superscript VILO cDNA synthesis kit (Invitrogen, 11754-050). Alternative events were analysed by either reverse transcriptase PCR or real-time PCR. Exon profiles were monitored and quantified using the Qiaxcel capillary electrophoresis system (Qiagen) and percentage inclusion was calculated as described previously[10]. Real time PCR was performed in triplicate on cDNA using SYBR® Green PCR Master Mix

(Invitrogen, 4309155) and the QuantStudio 7 Flex Real-Time PCR System (Thermo Fisher Scientific). Samples were normalised using the average of three reference genes, GAPDH, β -tubulin and actin. Ct values for each sample were calculated using SDS 2.4 software (Applied Biosystems) and relative mRNA expression was calculated using the 2-ΔΔCt method. All primer sequences are listed in Supplementary Table 1. Raw Ct values are given in Dataset 1[41].

Antibodies

The following commercial antibodies were used in the study: anti-RLN2 rabbit monoclonal (Abcam, ab183505 1:1000 dilution), anti-TACC2 rabbit polyclonal antibody (11407-1-AP, Proteintech 1:500 dilution), anti-NDUFV3 rabbit polyclonal antibody (13430-1-AP, Proteintech 1:500 dilution), anti-actin rabbit polyclonal (A2668, Sigma 1:2000 dilution), anti-α-Tubulin mouse monoclonal (Sigma, T5168 1:2000 dilution), normal rabbit IgG (711-035-152, Jackson labs 1:2000 dilution) and normal mouse IgG (715-036-150, Jackson labs 1:2000 dilution).

Gene ontology analysis

Gene ontology (GO) analysis of RNA-Seq data was carried out as described previously[42]. Enrichment of GO terms (with b500 annotations) was calculated using the goseq R package (version 1.18.0). Genes were considered significant at a p-value threshold of 0.05 after adjustment using the Benjamini-Hochberg false discovery rate.

Bioinformatic analysis of patient transcriptome data

Available clinical and processed RNA-Seq data from The Cancer Genome Atlas (TCGA) prostate adenocarcinoma (PRAD) cohort, comprising 497 tumour samples from as many patients with different stages / Gleason grades and 52 matched samples taken from normal prostate tissue (were downloaded from the Broad Institute TCGA Genome Analysis Center (Firehose 16/01/28 run https://doi.org/10.7908/C11G0KM9[43]). Transcriptome data from the TCGA PRAD cohort were analysed for alternative isoform expression, with transcript models relying on TCGA GAF2.1, corresponding to the University of California, Santa Cruz (UCSC) genome annotation from June 2011 (hg19 assembly). This annotation encompassed 42 of the 73 androgen-regulated alternative mRNA isoform pairs identified. These were studied using two types of analysis: 1) differential transcript expression between tumour and normal prostate tissue and 2) correlation between isoform expression in tumour samples and Gleason score or tumour stage.

Differential isoform and gene expression analysis was performed on estimated read counts using the *limma* software R package (version 3.7) following its RNA-Seq analysis workflow[44]. This workflow was also used for differential isoform ratio analysis, relying on logit-transformed ratio (see below). An FDR-adjusted p-value of 0.05 for the moderated t-statistics was used as threshold for significance of differential expression. Individual isoform expression was estimated in TPM (transcripts per million mapped reads). The expression ratio, henceforth called PSI (percent spliced-in), of each annotated androgen-regulated isoform pair in each TCGA sample was calculated as the ratio between the expression of isoform 1 and the total expression of isoforms 1 and 2 combined, i.e. the sum of their expressions. For each isoform pair, ΔPSI is the difference of median PSI between the tumour and the normal groups of samples.

Two-tailed Spearman's rank correlation tests were used to study the association between isoform expression and both Gleason score and tumour stage (these were used herein as numeric variables). An FDR-adjusted p-value of 0.05 was used as threshold for significance. Isoform expression differences between tumour and normal samples were considered equivalent to those detected in LNCaP cells under androgen stimulation when there was a statistically significant consistent change in the levels of the expected induced or repressed isoform (1 or 2), concomitant with no contradictory change in the PSI. Isoform "switches" were considered equivalent when there was a minimum ($\Delta PSI > 2.5\%$) and statistically significant consistent change in the PSI. Equivalent criteria were used to evaluate the equivalence between androgen-dependence and the associations with Gleason score and tumour stage.

Statistical analysis

Statistical analyses were conducted using the GraphPad Prism software (version 5.04/d). PCR quantification of mRNA isoforms was assessed using the unpaired student's t-test.

Data is presented as the mean of three independent samples ± standard error of the mean (SEM). Statistical significance is denoted as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$.

Results

Global identification of androgen-dependent mRNA isoform production in prostate cancer cells predicts a major role for alternative promoter utilisation

We analysed previously published RNAseq data from LNCaP cells[25] to globally profile how frequently androgens drive production of alternative mRNA isoforms in prostate cancer cells. This analysis identified a group of 73 androgen regulated alternative mRNA isoforms, which could be validated by visualisation on the UCSC Genome Browser[45] (Table 1). 64 AR regulated mRNA isoforms were novel to this study. Experimental validation in an independent RNA sample set using RT-PCR confirmed 17/17 of these alternative events at the mRNA level (Supplementary Figure 1). 73% of genes (53/73) with identified alternative androgen regulated mRNA isoforms also changed their overall expression levels in response to androgens (Table 2). Some of the androgen regulated alternative events are in genes are already implicated in in either prostate cancer or other cancer types (summarised in Table 3). However, Gene Ontology analysis of these 73 genes did not identify any significantly enriched biological processes.

The 73 identified mRNA isoforms were generated via androgen-regulated utilisation of 56 alternative promoters, 4 alternative 3' ends and 13 alternative splicing events (Figure 1A).

Table 1. Details of the 73 androgen regulated mRNA isoforms identified in prostate cancer cells.

		Isoform 1		Isoform 2					TCGA PRAD		
Gene	**Event type**	**Position (hg19)**	**RefSeq**	**Position (hg19)**	**RefSeq**	**Change with androgens**	**PCR Validation**	**Predicted to change protein?**	**Isoform 1 ID**	**Isoform 2 ID**	**Comparable?**
LIG4	Alternative promoter	chr13:108859792-108870716	NM_001098268.1	chr13:108859792-108867130	NM_002312.3	Induction of promoter 2	Yes (Qiaxel)	No (5' UTR)	uc001vqp.2	uc001vqn.2	Yes
TACC2	Alternative promoter	chr10:123748689-124014060	NM_206862.3	chr10:123872554-124014060	NM_001291879.1	Repression of promoter 1	Yes (Qiaxel)	Yes	uc001lfv.2	uc001lfx.2	Yes
TPD52	Alternative promoter	chr8:80947103-81083894	NM_001287144.1	chr8:80947103-80993066	NM_001025252.2	Induction of promoter 2	Yes (Qiaxel)	Yes	uc003ybs.1	uc003ybr.1	Yes
NUP93	Alternative promoter	chr16:56764017-56878861	NM_014669.4	chr16:56815704-56878861	NM_001242795.1	Induction of promoter 1	Yes (SYBR)	Yes	uc002eka.2	uc002ekb.2	Yes
RLN1	Alternative promoter	chr9:5334932-5339873	NM_006911.3	chr9:5335270-5339396	Not annotated	Repression of promoter 2	Yes (Qiaxel)	Yes (change from non-coding)	uc003zjb.1	Not annotated	No
AP2S1	Alternative promoter	chr19:47341415-47354252	NM_001301078.1	chr19:47341415-47353547	NM_001301076.1	Induction of promoter 2	Yes (SYBR)	Yes	uc002pft.1	Not annotated	No
RLN2	Alternative promoter	chr9:5299866-5304611	NM_005059.3	chr9:5299890-5304222	Not annotated	Induction of promoter 1	Yes (Qiaxel)	Yes (change from non-coding)	uc003ziz.1	Not annotated	No
PIK3R1	Alternative promoter	chr5:67511584-67597649	NM_181523.2	chr5:67584252-67597649	NM_181524.1	Repression of promoter 2	Yes (SYBR)	Yes	uc003jva.2	uc003jvc.2	Yes
MAPRE2	Alternative promoter	chr18:32556892-32723432	NM_001143826.2	chr18:32621324-32723432	NM_014268.3	Switch to promoter 2	Yes (Qiaxel)	Yes	uc010xcb.1	uc002kyf.2	Yes
NDUFAF4	Alternative promoter	chr6:97337187-97345767	NM_014165.3	chr6:97337227-97345368	Not annotated	Repression of promoter 2	Yes (Qiaxel)	Yes (change from non-coding)	uc003pov.2	Not annotated	No
DCXR	Alternative promoter	chr17:79993757-79995573	NM_016286.3	chr17:79993765-79995217	Not annotated	Repression of promoter 2	Yes (Qiaxel)	Yes	uc002kdg.2	Not annotated	No
PEX10	Alternative promoter	chr1:2336241-2344010	NM_002617.3	Not annotated		Switch to promoter 2	Yes (Qiaxel)	Yes	uc001ajh.2	Not annotated	No
SNAPC2	Alternative promoter	chr19:7985194-7988136	NM_003083.3	chr19:7985867-7988136	NR_030717.1	Switch to promoter 2	Yes (SYBR)	Yes (change to non-coding)	uc002miw.1	uc002mix.1	Yes
ATP6V0D1	Alternative promoter	chr16:67471917-67515089	NM_004691.4	chr16:67471931-67475338	Not annotated	Repression of promoter 2		Yes	uc002ete.1	Not annotated	No
ARRDC1	Alternative promoter	chr9:140500092-140509812	NM_001317968.1	chr9:140506874-140509793	Not annotated	Induction of promoter 2		Yes (change to non-coding)	uc004cnp.1	Not annotated	No
DENND1A	Alternative promoter	chr9:126141933-126692417	NM_020946.1	chr9:126143408-126586780	Not annotated	Repression of promoter 2		Yes	uc004bnz.1	Not annotated	No

F1000Research 2018, 7:1189 Last updated: 17 MAY 2019

		Isoform 1		Isoform 2					TCGA PRAD		
Gene	Event type	Position (hg19)	RefSeq	Position (hg19)	RefSeq	Change with androgens	PCR Validation	Predicted to change protein?	Isoform 1 ID	Isoform 2 ID	Comparable?
KLHL36	Alternative promoter	chr16:84682117-84701292	NM_024731.3	chr16:84684274-84701134	Not annotated	Induction of promoter 2		Yes	uc002fig.2	Not annotated	No
RAB3IL1	Alternative promoter	chr11:61664768-61687741	NM_001271686.1	chr11:61664768-61685081	NM_013401.3	Repression of promoter 2		Yes	uc001nsp.2	uc001nso.2	Yes
ACER3	Alternative promoter	chr11:76571917-76737841	NM_018367.6	chr11:76631206-76737818	Not annotated	Repression of promoter 2		Yes	uc009yum.1	Not annotated	No
OSBPL1A	Alternative promoter	chr18:21742011-21977833	NM_080597.3	chr18:21742011-21852196	NM_018030.4	Induction of promoter 2		Yes	uc002kve.2	uc002kvd.2	Yes
TRIM16	Alternative promoter	chr17:15531280-15586193	NM_006470.3	chr17:15530970-15555735	Not annotated	Induction of promoter 2		Yes	uc002gow.2	Not annotated	No
VSIG10L	Alternative promoter	chr19:51834795-51845378	NM_001163922.1	chr19:51834795-51843009	Not annotated	Induction of promoter 1		Yes	uc002pwf.2	Not annotated	No
SEPT5	Alternative promoter	chr22:19701987-19710845	NM_002688.5	chr22:19705958-19710845	NM_001009939.2	Repression of promoter 2		Yes	uc002zpv.1	uc002zpw.1	Yes
HMGCR	Alternative promoter	chr5:74632154-74657926	NM_000859	chr5:74632993-74657926	NM_000859.2	Repression of promoter 1		Yes	uc011cst.1	uc003kdp.2	Yes
RDH13	Alternative promoter	chr19:55555692-55580914	NM_138412.3	chr19:55555692-55574585	NM_001145971.1	Induction of promoter 1		Yes	uc002qip.2	uc010esr.1	Yes
GPRIN2	Alternative promoter	chr10:46993001-47000677	Not annotated	chr10:46993546-47000568	NM_014696.3	Repression of promoter 2		No (5' UTR)	Not annotated	uc001jec.2	No
CLK3	Alternative promoter	chr15:74900713-74922542	NM_003992.4	chr15:74,908,246-74,922,542	NM_003992	Repression of promoter 1		Yes	uc002ayg.3	uc002ayj.3	Yes
RNH1	Alternative promoter	chr11:494512-507283	NM_203387.2	chr11:494512-506821	NM_002939.3	Induction of promoter 1		No (5' UTR)	uc001lpp.1	uc001lpl.1	Yes
ZFAND6	Alternative promoter	chr15:80351910-80430735	NM_001242911.1	chr15:80364903-80430735	NM_001242916.1	Repression of promoter 2		No (5' UTR)	uc002bff.1	uc002bfh.1	Yes
CDIP1	Alternative promoter	chr16:4560677-4588816	NM_013399.2	chr16:4560677-4588471	NM_001199054.1	Repression of promoter 2		No (5' UTR)	uc002cwu.2	uc002cwv.2	Yes
YIF1B	Alternative promoter	chr19:38794200-38806606	NM_001039672.2	chr19:38794200-38806445	NM_001145461.1	Switch to promoter 2		Yes	uc002ohz.2	uc002ohx.2	Yes
LIMK2	Alternative promoter	chr22:31608250-31676066	NM_005569.3	chr22:31644348-31676066	NM_016733.2	Switch to promoter 2		Yes	uc003akh.2	uc003aki.2	Yes
TSC22D3	Alternative promoter	chrX:106956452-106959711	NM_001015881.1	chrX:106956452-106960291	NM_004089.3	Repression of promoter 1		Yes	uc004enf.2	uc004eng.2	Yes
ALDH1A3	Alternative promoter	chr15:101419897-101456830	NM_000693.3	chr15:101438281-101457072	Not annotated	Repression of promoter 1		Yes	uc002bwn.3	Not annotated	No
TRABD	Alternative promoter	chr22:50624341-50638028	NM_001320485.1	chr22:50628979-50638028	NM_001320487.1	Switch to promoter 2		No (5' UTR)	uc003bjq.1	uc003bjs.1	Yes
LIMCH1	Alternative promoter	chr4:41361624-41702061	NM_001289124.1	chr4:41362648-41702061	NM_001289122.2	Repression of promoter 2		Yes	uc003gvu.3	Not annotated	No

F1000Research 2018, 7:1189 Last updated: 17 MAY 2019

		Isoform 1		Isoform 2					TCGA PRAD		
Gene	Event type	Position (hg19)	RefSeq	Position (hg19)	RefSeq	Change with androgens	PCR Validation	Predicted to change protein?	Isoform 1 ID	Isoform 2 ID	Comparable?
GMFB	Alternative promoter	chr14:54941209-54955744	NM_004124.2	chr14:54941314-54955637	Not annotated	Induction of promoter 2		Yes (change to non-coding)	uc010tqz.1	Not annotated	No
MLST8	Alternative promoter	chr16:2255178-2259418	NM_022372.4	chr16:2255732-2259418	NM_001199174.1	Switch to promoter 1		No (5' UTR)	uc010uvy.1	uc002cpf.2	Yes
TLE3	Alternative promoter	chr15:70340130-70390256	NM_020908.2	chr15:70340130-70387124	NM_001282982.1	Induction of promoter 2		Yes	uc002asn.2	uc002ask.2	Yes
UBA1	Alternative promoter	chrX:47050199-47074527	NM_153280.2	chrX:47053201-47074527	NM_003334.3	Repression of promoter 1		No (5' UTR)	uc004dhj.3	uc004dhk.3	Yes
TNRC6B	Alternative promoter	chr22:40440821-40731812	NM_001024843.1	chr22:40573929-40731812	NM_001162501.1	Repression of promoter 2		Yes	uc003aym.2	uc011aor.1	Yes
FDFT1	Alternative promoter	chr8:11660120-11696818	NM_004462.4	chr8:11665926-11696818	NM_001287750.1	Repression of promoter 2		Yes	uc003wui.2	uc010lsb.2	Yes
GREB1	Alternative promoter	chr2:11674242-11782912	NM_014668.3	chr2:11680080-11728355	NM_148903.2	Induction of promoter 2		Yes	uc002rbo.1	uc002rbl.2	Yes
NCAPD3	Alternative promoter	chr11:134022337-134094426	NM_015261.2	chr11:134022772-134093593	Not annotated	Induction of promoter 2		Yes	uc001qhd.1	Not annotated	No
SLC36A4	Alternative promoter	chr11:92877337-92931141	NM_152313.3	chr11:92877337-92930621	NM_001286139.1	Induction of promoter 2		Yes	uc001pdn.2	Not annotated	No
KLC2	Alternative promoter	chr11:66024765-66035331	NM_001134775.1	chr11:66025174-66035331	NM_022822.2	Repression of promoter 1		No (5' UTR)	uc010rov.1	uc001ohb.2	Yes
RAP1GAP	Alternative promoter	chr1:21922708-21978348	NM_001145658.1	chr1:21922533-21946950	Not annotated	Repression of promoter 1		Yes	uc001bez.1	Not annotated	No
TMEM79	Alternative promoter	chr1:156252704-156262234	NR_026678.1	chr1:156254070-156262234	NM_032323.2	Repression of promoter 1		No (5' UTR)	uc001fod.2	uc010phi.1	Yes
NR4A1	Alternative promoter	chr12:52416616-52453291	NM_001202233.1	chr12:52445186-52453291	NM_173157.2	Induction of promoter 2		Yes	uc010sno.1	uc001rzr.2	Yes
ZNF32	Alternative promoter	chr10:44139307-44144326	NM_001324166.1	chr10:44139307-44144326	NM_001324167.1	Repression of promoter 2		No (5' UTR)	uc001jbc.2	uc001jbb.2	Yes
C1QTNF3	Alternative promoter	chr5:34017963-34043371	NM_181435.5	chr5:34018571-34035881	Not annotated	Induction of promoter 1		Yes	uc003jio.2	Not annotated	No
UBE2D3	Alternative promoter	chr4:103715540-103748710	NM_181887.2	chr4:103715540-103749105	NM_181886.3	Switch to promoter 2		No (5' UTR)	uc003hwk.2	uc011cet.1	Yes
KRT8	Alternative promoter	chr12:53290971-53343650	NM_001256293.1	chr12:53,290,971-53,298,868	NM_002273	Repression of promoter 1		No (5' UTR)	uc009zml.1	uc001sbd.2	Yes
ELOVL1	Alternative promoter	chr1:43829068-43833745	NM_022821.3	chr1:43829093-43832057	Not annotated	Induction of promoter 2		Yes (change to non-coding)	uc001cjb.2	Not annotated	No
RCAN1	Alternative promoter	chr21:35888740-35987441	NM_004414.6	chr21:35888740-35899308	NM_203418.2	Induction of promoter 2		Yes	uc002yue.2	uc002yub.2	Yes
SORBS3	Alternative promoter	chr8:22409251-22433008	NM_005775.4	chr8:22422332-22433100	Not annotated	Induction of promoter 2		Yes	uc003xbv.2	Not annotated	No

		Isoform 1		Isoform 2					TCGA PRAD		
Gene	**Event type**	**Position (hg19)**	**RefSeq**	**Position (hg19)**	**RefSeq**	**Change with androgens**	**PCR Validation**	**Predicted to change protein?**	**Isoform 1 ID**	**Isoform 2 ID**	**Comparable?**
MAT2A	Alternative 3' end	chr2:85766101-85772403	NM_005911.5	chr2:85,766,101-85,770,775	NM_005911	Repression of isoform 2	Yes (Qiaxel)	Yes	uc002spr.2	uc010ysr.1	Yes
CNNM2	Alternative 3' end	chr10:104678075-104687375	NM_199077.2	chr10:104678075-104838344	NM_017649.4	Induction of isoform 1	Yes (SYBR)	Yes	uc001kwl.2	uc001kwm.2	Yes
TMEM125	Alternative 3' end	chr1:43735698-43736343	Not annotated	chr1:43735665-43739673	NM_144626.2	Induction of isoform 1		Yes (change to non-coding)	Not annotated	uc001cir.2	No
CBWD2	Alternative 3' end	chr2:114195268-114253781	NM_172003.3	chr2:114195169-114199073	Not annotated	Induction of isoform 2		Yes	uc002tju.2	Not annotated	No
NDUFV3	Alternative exon	chr21:44313378-44329773	NM_021075.3	chr21:44313378-44329773	NM_001001503.1	Switch to isoform 2 (exon excluded)		Yes	uc002zcm.2	uc002zcn.2	Yes
ZNF678	Alternative exon	chr1:227751220-227850164	NM_178549.3	Not annotated		Switch to isoform 2 (exon excluded)		Yes (change to non-coding)	uc009xet.1	Not annotated	No
ZNF121	Alternative exon	chr19:9676404-9695209	NM_001308269.1	chr19:9676404-9695209	NM_001008727.3	Switch to isoform 2 (exon excluded)		Yes	uc010xkq.1	uc010xkp.1	Yes
SPATC1L	Alternative exon	chr21:47581062-47604373	NM_032261.4	Not annotated		Induction of isoform 2 (exon included)		Yes	uc002zii.2	Not annotated	No
MOCOS	Alternative exon	chr18:33767480-33848685	NM_017947.2	Not annotated		Switch to isoform 2 (exon excluded)		Yes	uc002kzq.3	Not annotated	No
RBM45	Alternative exon	chr2:178977151-178994382	NM_152945.3	Not annotated		Switch to isoform 2 (exon included)		Yes	uc002ulv.2	Not annotated	No
MIPEP	Alternative exon	chr13:24304328-24463587	NM_005932.3	Not annotated		Repression of isoform 2 (exon excluded)		Yes	uc001uox.3	Not annotated	No
BBS4	Alternative exon	chr15:72978520-73030817	NM_001320665.1	Not annotated		Induction of isoform 2 (exon included)		Yes	uc002avb.2	Not annotated	No
FAM195A	Alternative exon	chr16:691804-698474	NM_138418.3	chr16:691804-698474	NR_138607.1	Switch to isoform 1 (exon exluded)		Yes (change from non-coding)	uc002cic.1	uc002cie.2	Yes
LINC01133	Alternative exon	chr1:159931008-159948851	ENST00000443364.6	chr1:159931014-159948876	NR_038849.1	Induction of isoform 1 (exon excluded)		Both non-coding	Not annotated	uc001fuu.2	No
SS18	Alternative exon	chr18:23596217-23670611	NM_001007559.2	chr18:23596217-23670611	NM_005637.3	Switch to isoform 2 (exon excluded)		Yes	uc002kvm.2	uc002kvn.2	Yes
RHOC	Alternative exon	chr1:113243897-113249757	ENST00000369638.6	chr1:113243947-113249742	ENST00000369636.6	Switch to isoform 2 (exon excluded)		No (5' UTR)	uc009wgk.1	uc001ecr.1	Yes
ZNF226	Retained intron	chr19:44669215-44681838	NM_001319088.1	chr19:44669249-44679582	NM_015919.3	Switch to isoform 1 (intron included)		Yes	uc002oyo.2	uc002oyn.2	Yes

Table 2. Quantitative changes in gene expression in response to androgens for the 73 genes with AR regulated alternative mRNA isoforms.

LNCaP RNA-Seq (+/- androgens for 24 hours)			Reciprocal RNA-Seq (also change in 7 patients following ADT)		
No change	**Upregulated**	**Downregulated**	**No change**	**Upregulated**	**Downregulated**
RLN2	LIG4	NUP93	LIG4	TPD52	None
DENND1A	TACC2	PIK3R1	TACC2	AP2S1	
RAB3IL1	RLN1	MAPRE2	NUP93	DCXR	
OSBPL1A	AP2S1	NDUFAF4	RLN1	PEX10	
TRIM16	DCXR	ACER3	RLN2	HMGCR	
Sep-05	PEX10	GPRIN2	PIK3R1	ALDH1A3	
RDH13	SNAPC2	TLE3	MAPRE2	FDFT1	
ZFAND6	ATP6V0D1	TNRC6B	NDUFAF4	GREB1	
CDIP1	ARRDC1	SORBS3	SNAPC2	NCAPD3	
LIMK2	KLHL36	ZNF121	ATP6V0D1	RAP1GAP	
TSC22D3	VSIG10L	LINC01133	ARRDC1	TMEM79	
GMFB	HMGCR		DENND1A	KRT8	
MLST8	CLK3		KLHL36	ELOVL1	
znf32	RNH1		RAB3IL1	TMEM125	
C1QTNF3	YIF1B		ACER3		
UBE2D3	PAK1IP1		OSBPL1A		
MAT2A	ALDH1A3		TRIM16		
CBWD2	TRABD		VSIG10L		
ZNF678	LIMCH1		SEPT5		
MOCOS	UBA1		RDH13		
	FDFT1		GPRIN2		
	GREB1		CLK3		
	NCAPD3		RNH1		
	SLC36A4		ZFAND6		
	KLC2		CDIP1		
	RAP1GAP		YIF1B		
	TMEM79		LIMK2		
	NR4A1		TSC22D3		
	KRT8		TRABD		
	ELOVL1		LIMCH1		
	RCAN1		GMFB		
	CNNM2		MLST8		
	TMEM125		TLE3		
	NDUFV3		UBA1		
	SPATC1L		TNRC6B		
	RBM45		SLC36A4		
	MIPEP		KLC2		
	BBS4		NR4A1		
	FAM195A		znf32		
	SS18		C1QTNF3		

LNCaP RNA-Seq (+/- androgens for 24 hours)			Reciprocal RNA-Seq (also change in 7 patients following ADT)		
No change	**Upregulated**	**Downregulated**	**No change**	**Upregulated**	**Downregulated**
	RHOC		UBE2D3		
	ZNF226		RCAN1		
	TPD52		SORBS3		
			MAT2A		
			CNNM2		
			CBWD2		
			NDUFV3		
			ZNF678		
			ZNF121		
			SPATC1L		
			MOCOS		
			RBM45		
			MIPEP		
			BBS4		
			FAM195A		
			LINC01133		
			SS18		
			RHOC		
			ICAM3		
			ZNF226		

Of the 56 androgen regulated alternative promoters that were identified, 23 alternative promoters were induced by androgens (including *LIG4*, Figure 1B), 26 promoters were repressed by androgens, and for 7 genes there was a switch in usage from one promoter to another (Table 1). The alternative splicing events that were under androgen control included 12 alternative exons and one androgen-regulated intron retention (Table 1). 10 of these are novel to this study, including exclusion of an alternative exon in *ZNF678* (Figure 1C). Of the alternative exons, six genes contained switches in previously unannotated protein-coding exons in response to androgen-exposure. We also identified four androgen regulated alternative mRNA 3' end isoform switches, including a switch in the 3' end of the mRNA transcript for the *MAT2A* gene (Figure 1D).

Androgen regulated events control the production of alternative protein isoforms, non-coding RNAs and alternative 5' UTRs

48/73 (66%) of the androgen regulated alternative events detected in response to androgen stimulation are predicted to change the amino acid sequence of the resulting protein (Table 1). Some of these are already known to have a well characterised role in prostate cancer progression, including an alternative promoter in the oncogene *TPD52* that produces a protein isoform called PrLZ (Figure 2A)[46–49]. Others are not so well characterised. Using western blotting we could detect a novel shorter protein isoform corresponding to androgen-driven selection of an alternative promoter in the *TACC2* gene (Figure 2B); and exclusion of a cassette exon in the *NDUFV3* gene, which we show also produces a novel shorter protein isoform (Figure 2C). We also detected a switch in the 3' end of the mRNA transcript for the *MAT2A* gene, which is predicted to produce a protein isoform with a shorter C-terminal domain (Figure 1D); and induction of an alternative 3' isoform of *CNNM2,* which is predicted to be missing a conserved CBS domain (Table 1 and Supplementary Figure 1).

11 of the remaining identified androgen-regulated alternative events change the expression of mRNAs from coding to non-coding or untranslated (not predicted to produce a protein) (Table 1). These included promoter switches for the *RLN1* and *RLN2* genes which encode peptide hormones that may be important in prostate cancer[5,50–55]. Androgens drive a promoter switch in both *RLN1* and *RLN2* to produce predicted non-coding or untranslated mRNA isoforms, reducing expression of protein-coding *RLN1* and *RLN2* mRNA isoforms. To

Table 3. Alternative events in genes previously linked to cancer.

Gene name	Function	Clinical importance and roles in other cancer types	Clinical importance and roles in prostate cancer
TACC2 Transforming Acidic Coiled-Coil Containing Protein 2	centrosome- and microtubule-interacting protein	Growth and prognosis of breast cancer[56]	castration-resistant growth of prostate cancer[57]
LIG4	DNA ligase with role in DNA repair	Prognostic marker in nasopharyngeal cancer[58] Upregulated in colorectal cancer with role in wnt signalling[59]	Predictor of poor prognosis[60]
RLN1 and RLN2 (Relaxin1 and 2)	Endocrine hormones (part of insulin gene superfamily)	Breast cancer invasiveness[61,62] metastasis of human osteosarcoma[63] Thyroid cancer oncogenesis[64,65]	Well characterised role in the development and progression of prostate cancer[5,50–55].
TPD52 (Tumor Protein D52)	Role in proliferation and exo- and endocytic pathways	Well characterised role in numerous cancer types[46,66–69]	Known AR target, overexpressed and amplified in prostate cancer[70] Oncogene in prostate cancer[71] Neuroendocrine transdifferentiation of prostate cancer[72] Isoform produced by alternative promoter known as PrLZ and already linked to prostate cancer[47–49,73,74]
FDFT1 (Farnesyl-Diphosphate Farnesyltransferase 1)	squalene synthase	Role in lung cancer metastasis[75]	Linked to prostate cancer risk and aggressiveness[76]
TLE3 (Transducin Like Enhancer Of Split 3)	Negative regulator of Wnt/β-catenin signaling	Predictive marker for response to therapy in ovarian and breast cancer[77,78] Represses colon cancer proliferation[79]	Upregulated in prostate tumours[80] and linked to wnt signalling in castrate resistant disease[81]
CNNM2 (Cyclin & CBS Domain Divalent Metal Cation Transport Mediator 2)	Magnesium transporter	Proposed oncogenic role via increasing magnesium uptake[82]	Unknown
NUP93	Nucleoporin protein – role in apoptosis	Driver mutation linked to breast cancer[83]	Unknown
MAT2A Methionine adenosyltransferase II	Biosynthesis of S-adenosylmethionine, the principal biological methyl donor and precursor of polyamines and glutathione.	Upregulated in liver and colon cancer, potential drug target[84,85] Tumour suppressor in kidney carcinogenesis[86] Role in other cancer types[87]	Upregulated in prostate cancer and linked to cell migration via miR-34a and miR-34b[87,88]
PIK3R1	PI3K regulatory subunit	Underexpressed in breast cancer[89] High mutation frequency in endometrial cancer[90]	Controlled by androgens and repressed in prostate cancer cells[21]
SNAPC2 (Small Nuclear RNA Activating Complex Polypeptide 2)	Subunit of the snRNA-activating protein complex. Necessary for RNA polymerase II and III dependent small-nuclear RNA gene transcription	Epigenetic silencing is prognostic in glioblastoma[91]	Unknown
ZNF678 (Zinc Finger Protein 678)	Potential role in transcriptional regulation	Unknown	Unknown

Gene name	Function	Clinical importance and roles in other cancer types	Clinical importance and roles in prostate cancer
NDUFV3 (NADH:Ubiquinone Oxidoreductase Subunit V3)	Subunit of part of the mitochondrial respiratory chain	Unknown	Androgen regulated alternative splice isoform previously identified by our exon array study[10]
OSBPL1A (Oxysterol Binding Protein Like 1A)	Intracellular lipid receptor	Alternative promoter use in colorectal cancer[92]	Unknown
RDH13 (Retinol Dehydrogenase 13)	Role in retinoic acid production and protection against oxidative stress	Unknown	Unknown
ZNF121 (Zinc Finger Protein 121)	Potential role in transcriptional regulation	Interacts with MYC. Upregulated in breast cancer[93]	Unknown
SLC36A4.1 (Solute Carrier Family 36 Member 4)	amino acid transporter	Unknown	Unknown
RCAN1 (Regulator of Calcineurin 1)	Inhibits calcineurin-dependent signaling pathways	Inhibits NF-κB and suppresses lymphoma growth in mice[94]. Role in cancer cell migration[95]	Unknown
DCXR (Dicarbonyl & l-xylulose reductase)	Role in the uronate cycle of glucose metabolism	Low expression indicates poor prognosis for hepatocellular carcinoma[96]. Role in cell adhesion[97,98]	Upregulated and potential biomarker in prostate cancer[99]
NDUFAF4 (NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 4)	Role in the mitochondrial respiratory chain	Unknown	Unknown
MAPRE2 (Microtubule Associated Protein RP/EB Family Member 2)	Microtubule-associated protein that is necessary for spindle symmetry during mitosis	Role in the invasion of pancreatic cancer cells[100]	Unknown
PEX10 (Peroxisomal Biogenesis Factor 10)	Involved in import of peroxisomal matrix proteins	Unknown	Unknown
AP2S1 (Adaptor Related Protein Complex 2 Sigma 1 Subunit)	Function in protein transport across membranes	Unknown	Unknown
LINC01133 (long non-coding RNA)	Long non-coding RNA	Poor prognosis in colorectal cancer[101] Upregulated and linked to poor prognosis in lung cancer[102]	Unknown
ZNF226 (Zinc Finger Protein 226)	Potential role in transcriptional regulation	Unknown	Unknown
CDIP1 (Cell death inducing p53 target 1)	p53 apoptotic effector Regulates TNF-alpha-mediated apoptosis	sensitivity to TNFα-induced apoptosis in cancer cells[103]	Unknown

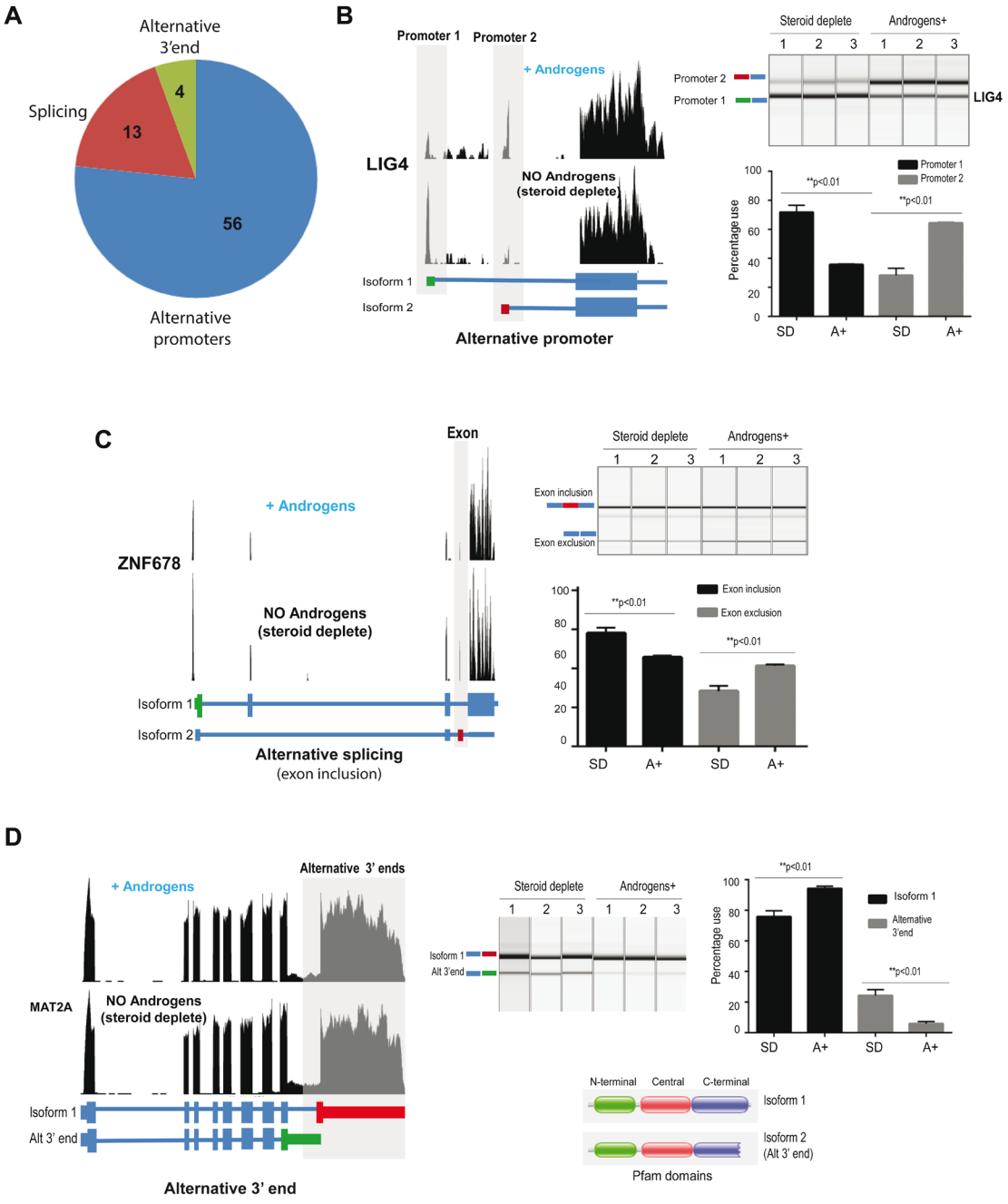

Figure 1. Global identification of androgen-dependent mRNA isoform production in prostate cancer cells predicts a major role for alternative promoter utilisation. (**A**) Analysis of RNAseq data from LNCaP cells grown with (A+) or without androgens (R1881) (steroid deplete, SD) for 24 hours identified 73 androgen regulated alternative mRNA isoforms. The 73 alternative events were generated via androgen-regulated utilisation of 56 alternative promoters, 4 alternative 3' ends and 13 alternative splicing events. (**B**) Androgens drive a promoter switch in the *LIG4* gene, which produces an mRNA isoform with an alternative 5'UTR. Visualisation of our LNCaP cell RNA-seq reads for the *LIG4* gene on the UCSC genome browser identified a switch from promoter 1 to alternative promoter 2 in cells grown in the presence of androgens. Promoter 2 is predicted to produce a different 5'UTR without influencing the protein sequence (left panel). Quantitative PCR using primers specific to each promoter indicate that in response to androgens there is repression of promoter 1 and induction of promoter 2 (right panel). (**C**) Androgens drive alternative splicing of the *ZNF678* gene. Visualisation of our LNCaP cell RNA-seq reads for the *ZNF678* gene on the UCSC genome browser identified a switch to inclusion of a cassette exon in the presence of androgens. Inclusion of the alternative cassette exon in the *ZNF678* gene is predicted to induce a switch to an alternative non-coding mRNA isoform (left panel). Quantitative PCR using primers in flanking exons confirmed increased inclusion of the alternative exon in LNCaP cells exposed to androgens (right panel). (**D**) Androgens promote selection of an alternative 3' end for the *MAT2A* gene. Visualisation of our LNCaP cell RNA-seq reads for the *MAT2A* gene on the UCSC genome browser indicates a switch to reduced usage of an alternative 3' end in the presence of androgens (left panel). Quantitative PCR using primers specific to each isoform confirmed down-regulation of an alternative 3' end ($p<0.01$). Alternative 3' ends for the *MAT2A* gene are predicted to produce proteins with different amino acid sequences and to influence a known Pfam domain (right panel).

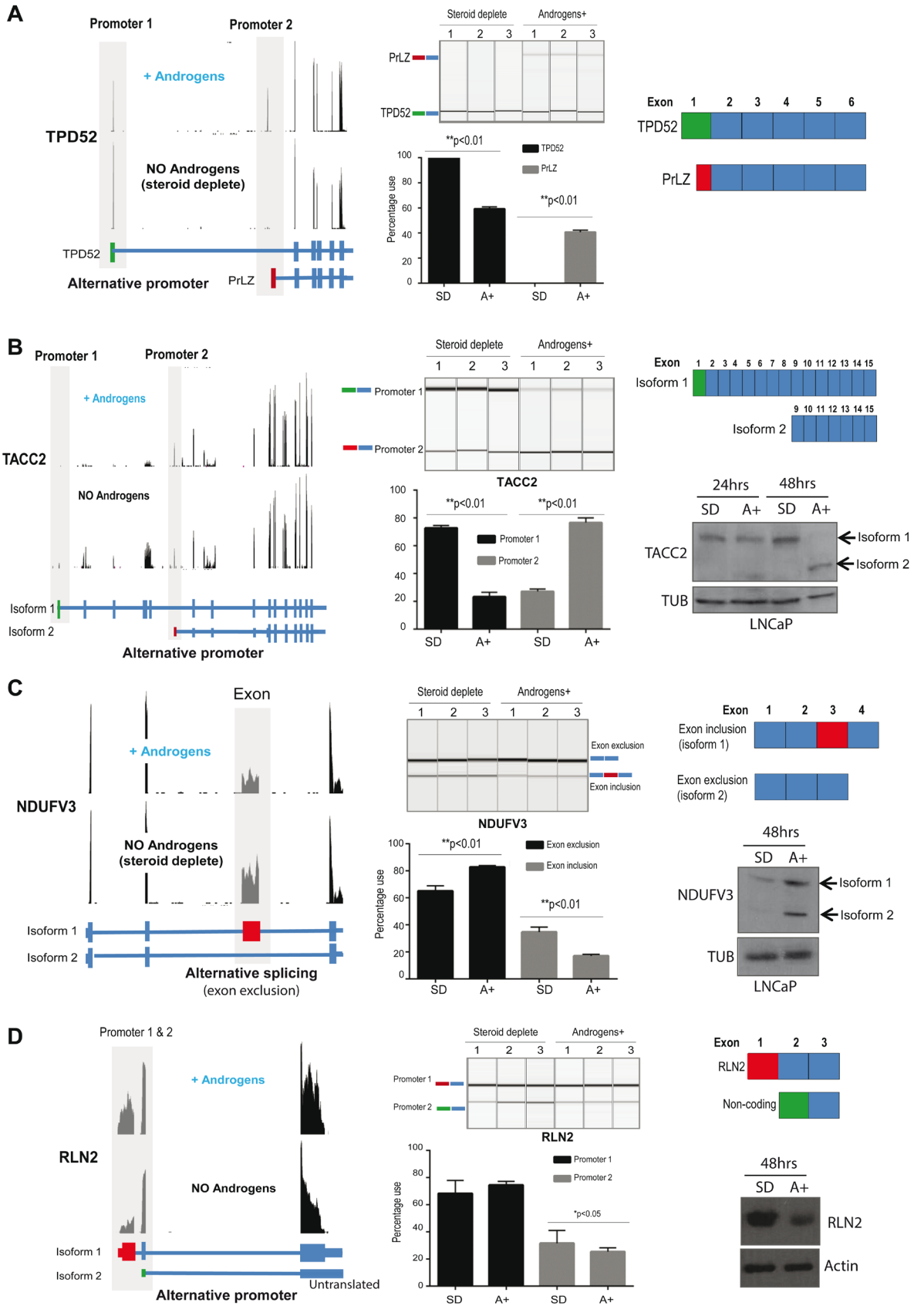
A
Promoter 1
Promoter 2
+ Androgens
TPD52
NO Androgens
(steroid deplete)
TPD52
Alternative promoter
PrLZ
Steroid deplete
Androgens+
1 2 3 1 2 3
PrLZ
TPD52
**p<0.01
TPD52
PrLZ
Percentage use
100
80
60
40
20
0
**p<0.01
SD
A+
SD
A+
Exon 1 2 3 4 5 6
TPD52
PrLZ
B
Promoter 1
Promoter 2
+ Androgens
TACC2
NO Androgens
Isoform 1
Isoform 2
Alternative promoter
Steroid deplete
Androgens+
1 2 3 1 2 3
Promoter 1
Promoter 2
TACC2
**p<0.01
**p<0.01
Promoter 1
Promoter 2
100
80
60
40
20
0
SD
A+
SD
A+
Exon 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Isoform 1
9 10 11 12 13 14 15
Isoform 2
24hrs
48hrs
SD A+ SD A+
TACC2
Isoform 1
Isoform 2
TUB
LNCaP
C
Exon
+ Androgens
NDUFV3
NO Androgens
(steroid deplete)
Isoform 1
Isoform 2
Alternative splicing
(exon exclusion)
Steroid deplete
Androgens+
1 2 3 1 2 3
Exon exclusion
Exon inclusion
NDUFV3
**p<0.01
Exon exclusion
Exon inclusion
**p<0.01
Percentage use
100
80
60
40
20
0
SD
A+
SD
A+
Exon 1 2 3 4
Exon inclusion
(isoform 1)
Exon exclusion
(isoform 2)
48hrs
SD A+
NDUFV3
Isoform 1
Isoform 2
TUB
LNCaP
D
Promoter 1 & 2
+ Androgens
RLN2
NO Androgens
Isoform 1
Isoform 2
Untranslated
Alternative promoter
Steroid deplete
Androgens+
1 2 3 1 2 3
Promoter 1
Promoter 2
RLN2
Promoter 1
Promoter 2
*p<0.05
Percentage use
100
80
60
40
20
0
SD
A+
SD
A+
Exon 1 2 3
RLN2
Non-coding
48hrs
SD A+
RLN2
Actin

Figure 2. Androgen regulated mRNA isoform switches control alternative protein isoforms and non-coding RNAs. (**A**) Androgens induce an alternative promoter in the oncogene *TPD52* that produces an isoform called PrLZ. Visualisation of our LNCaP cell RNA-seq reads for the *TPD52* gene on the UCSC genome browser identified a switch from promoter 1 to alternative promoter 2 in cells grown in the presence of androgens. Promoter 2 is known to produce an alternative protein isoform of TPD52 known as PrLZ (left panel). Quantitative PCR using primers specific to each promoter indicate an induction of the PrLZ isoform in response to androgens (middle panel). PrLZ has an alternative N-terminal amino acid sequence which results in an alternative protein isoform and disrupts a known Pfam domain (right panel). (**B**) Androgens induce an alternative promoter in the TACC2 gene that produces a novel alternative protein isoform. Visualisation of our LNCaP cell RNA-seq reads for the *TACC2* gene on the UCSC genome browser identified a switch from promoter 1 to alternative promoter 2 in cells grown in the presence of androgens. Promoter 2 is predicted to produce an alternative shorter protein isoform of TACC2 (isoform 2) (left panel). Quantitative PCR using primers specific to each promoter indicate a switch from isoform 1 to isoform 2 in response to androgens (middle panel). Detection of TACC2 protein in LNCaP by western blotting (cells were grown with or without androgens for 24 or 48 hours). Tubulin was used as a loading control. Exposure to androgens for 48 hours induces expression of the alternative TACC2 protein isoform (right panel). (**C**) Androgens drive alternative splicing of the *NDUFV3* gene. Visualisation of our LNCaP cell RNA-seq reads for the *NDUFV3* gene on the UCSC genome browser identified a switch to exclusion of a cassette exon in the presence of androgens (left panel). Quantitative PCR using primers in flanking exons confirmed less inclusion of the alternative exon in LNCaP cells exposed to androgens (middle panel). Exclusion of the alternative cassette exon is predicted to produce an alternative protein isoform. Detection of NDUFV3 protein in LNCaP cells using western blotting (right panel). (**D**) Androgens suppress an alternative promoter in the *RLN2* gene, which produces a shorter non-coding mRNA isoform. Visualisation of our LNCaP cell RNA-seq reads for the *RLN2* gene on the UCSC genome browser identified a switch from promoter 1 to alternative promoter 2 in cells grown in the presence of androgens. Promoter 2 is predicted to produce an untranslated non-coding mRNA isoform (left panel). Quantitative PCR using primers specific to each promoter indicated a significant switch in promoter usage in response to androgens (middle panel). Detection of RLN2 protein in LNCaP by western blotting (cells were grown with or without androgens for 48 hours). Actin was used as a loading control. As seen previously[55], androgens suppress RLN2 protein levels.

test whether prostate cancer cells turn off gene expression by switching between utilisation of promoters that generate coding and noncoding mRNAs, we analysed RLN2 protein levels. Consistent with our hypothesis and a previous study[55], RLN2 protein production was negatively regulated by androgens in parallel to the switch to the non-coding mRNA isoform (Figure 2D).

14 of the identified androgen-dependent mRNA isoforms lead to/result in coding mRNAs with altered 5' untranslated regions (5' UTR) with no impact on the coding sequence. These include a promoter switch in the *LIG4* gene (Figure 1B).

Differential expression of androgen-dependent mRNA isoforms in prostate adenocarcinoma versus normal tissue

To investigate potential links between androgen-dependent mRNA isoforms and tumourigenesis, we analysed the expression of 41 androgen-regulated mRNA isoform pairs in clinical prostate adenocarcinoma and normal prostate tissues. This analysis utilised transcriptomic data from 497 tumour samples and 52 normal samples in the PRAD TCGA cohort[104]. The remaining isoform pairs identified within our dataset have not been previously annotated by UCSC, therefore it was not possible to include them in our comparison. A description of the cohort used is summarised in Table 4.

33 of the 42 mRNA isoform pairs exhibited significant differences in the expression of at least one of the isoforms, or in the isoform expression ratio between tumour and normal tissues (Table 5). 13 of those tumour-specific alterations mimicked the effect of androgen stimulation in LNCaP cells: the changes were in form of alternative promoters for *TACC2*, *TPD52*, *NUP93*, *PIK3R1*, *RDH13*, *ZFAND6*, *CDIP1*, *YIF1B*, *LIMK2*, and *FDFT1*; an alternative 3´ end in *CNNM2*; and alternative exons in

Table 4. Description of the TCGA PRAD cohort.

Features	Total Cases
Cohort	497 patients
Tumour	497
Normal	52 (w/tumour matched sample available)
Gleason grade	
6	50
7	287
8	67
9	140
10	4
Tumour stage	
T2a	14
T2b	10
T2c	192
T3a	173
T3b	140
T4	12
Gleason grade (alternative gleason grade grouping)	
1 (primary + secondary score ≤ 6)	50
2 (3 + 4)	171
3 (4 + 3)	123
4 (4 + 4)	93
5 (primary + secondary score ≥ 9)	111

All tumours were hormone naive (not subject to ADT) at the time of sample collection

Table 5. Summarised results of the differential expression analysis of androgen-regulated isoforms between tumour and normal tissue samples in the TCGA PRAD cohort.

			Isoform 1			Isoform 2			PSI			
Gene	**Event type**	**Change with androgens (LNCap)**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**Delta PSI**	**Av. PSI**	**FDR**	**Consistency of change in tumours**
LIG4	Alternative promoter	Induction of promoter 2	**-0.81**	**1.77**	**4.31E-02**	**-1.53**	**1.28**	**4.48E-05**	**0.06**	**0.597300667**	**9.85E-02**	Opposite
TACC2	Alternative promoter	Repression of promoter 1	**-0.80**	**2.42**	**5.51E-03**	0.18	6.22	6.06E-01	**-0.16**	**0.284239843**	**2.95E-05**	**Consistent**
TPD52	Alternative promoter	Induction of promoter 2	-0.34	0.17	5.45E-01	**1.87**	**39.20**	**1.23E-09**	0.00	0.011365308	8.11E-06	**Consistent**
NUP93	Alternative promoter	Induction of promoter 1	**0.25**	**25.52**	**6.45E-04**	0.31	7.20	6.08E-01	0.01	0.828738669	7.52E-01	**Consistent**
RLN1	Alternative promoter	Repression of promoter 2	-0.45	133.50	4.97E-01	--	--	--	--	--	--	Not assessed
AP2S1	Alternative promoter	Induction of promoter 2	0.48	191.44	2.24E-05	--	--	--	--	--	--	Not assessed
RLN2	Alternative promoter	Induction of promoter 1	0.48	5.07	2.41E-01	--	--	--	--	--	--	Not assessed
PIK3R1	Alternative promoter	Repression of promoter 2	**-1.79**	**7.15**	**3.26E-12**	**-1.79**	**1.26**	**8.20E-06**	-0.02	0.820282185	7.52E-01	**Consistent**
MAPRE2	Alternative promoter	Switch to promoter 2	1.17	1.52	1.22E-01	-0.34	0.07	1.96E-01	**0.09**	**0.730349729**	**4.67E-02**	Opposite
NDUFAF4	Alternative promoter	Repression of promoter 2	0.55	0.06	5.86E-02	--	--	--	--	--	--	Not assessed
DCXR	Alternative promoter	Repression of promoter 2	0.68	623.07	2.05E-05	--	--	--	--	--	--	Not assessed
PEX10	Alternative promoter	Switch to promoter 2	0.92	75.55	7.84E-06	--	--	--	--	--	--	Not assessed
SNAPC2	Alternative promoter	Switch to promoter 2	0.38	5.42	1.23E-01	**0.22**	**37.58**	**3.20E-02**	-0.01	0.130583106	8.29E-01	Inconclusive
ATP6V0D1	Alternative promoter	Repression of promoter 2	-0.12	109.86	1.42E-01	--	--	--	--	--	--	Not assessed
ARRDC1	Alternative promoter	Induction of promoter 2	0.46	12.78	2.34E-05	--	--	--	--	--	--	Not assessed
DENND1A	Alternative promoter	Repression of promoter 2	0.04	7.09	9.11E-01	--	--	--	--	--	--	Not assessed
KLHL36	Alternative promoter	Induction of promoter 2	-0.38	10.58	4.61E-06	--	--	--	--	--	--	Not assessed
RAB3IL1	Alternative promoter	Repression of promoter 2	0.34	0.28	5.07E-01	0.05	4.68	6.91E-01	0.01	0.062673984	4.28E-01	Inconclusive
ACER3	Alternative promoter	Repression of promoter 2	0.13	6.32	8.52E-01	--	--	--	--	--	--	Not assessed
OSBPL1A	Alternative promoter	Induction of promoter 2	0.14	4.11	5.75E-01	**-1.06**	**3.56**	**3.44E-09**	**0.17**	**0.522207286**	**1.03E-08**	Opposite
TRIM16	Alternative promoter	Induction of promoter 2	-0.65	6.87	1.03E-14	--	--	--	--	--	--	Not assessed
VSIG10L	Alternative promoter	Induction of promoter 1	-1.01	1.91	5.49E-04	--	--	--	--	--	--	Not assessed

F1000Research 2018, 7:1189 Last updated: 17 MAY 2019

			Isoform 1			Isoform 2			PSI			
Gene	**Event type**	**Change with androgens (LNCap)**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**Delta PSI**	**Av. PSI**	**FDR**	**Consistency of change in tumours**
SEPT5	Alternative promoter	Repression of promoter 2	**0.80**	**11.47**	**1.79E-09**	**1.09**	**3.86**	**1.82E-06**	-0.03	0.749615358	1.90E-01	Opposite
HMGCR	Alternative promoter	Repression of promoter 1	-0.86	0.59	1.07E-01	**-0.55**	**17.41**	**1.09E-02**	0.00	0.029105295	9.62E-01	Inconclusive
RDH13	Alternative promoter	Induction of promoter 1	**1.67**	**2.10**	**1.31E-08**	**0.72**	**0.05**	**5.88E-03**	0.00	0.962155441	9.33E-02	**Consistent**
GPRIN2	Alternative promoter	Repression of promoter 2	--	--	--	-0.48	3.31	3.98E-02	--	--	--	Not assessed
CLK3	Alternative promoter	Repression of promoter 1	0.10	31.34	1.07E-01	--	0.04	--	0.00	0.998537929	6.18E-01	Inconclusive
RNH1	Alternative promoter	Induction of promoter 1	-0.16	4.38	7.95E-01	-0.19	6.56	5.74E-01	0.00	0.375368151	7.52E-01	Inconclusive
ZFAND6	Alternative promoter	Repression of promoter 2	-0.10	37.63	6.33E-01	**-1.51**	**2.29**	**5.59E-03**	**0.03**	**0.935657481**	**3.73E-02**	**Consistent**
CDIP1	Alternative promoter	Repression of promoter 2	0.77	0.35	1.16E-01	**-1.83**	**3.70**	**2.77E-11**	**0.06**	**0.142411928**	**1.46E-03**	**Consistent**
YIF1B	Alternative promoter	Switch to promoter 2	0.50	2.52	3.18E-01	**2.83**	**3.08**	**1.60E-04**	**-0.32**	**0.497841217**	**1.64E-02**	**Consistent**
LIMK2	Alternative promoter	Switch to promoter 2	**-0.90**	**6.80**	**1.50E-03**	**0.58**	**10.99**	**1.10E-05**	**-0.19**	**0.382613244**	**2.85E-06**	**Consistent**
TSC22D3	Alternative promoter	Repression of promoter 1	--	35.48	--	**-1.08**	**173.59**	**8.13E-15**	0.01	0.203019277	2.97E-01	Inconclusive
ALDH1A3	Alternative promoter	Repression of promoter 1	0.71	279.09	7.51E-03	--	--	--	--	--	--	Not assessed
TRABD	Alternative promoter	Switch to promoter 2	**1.57**	**21.80**	**3.42E-02**	0.87	0.54	1.18E-01	0.00	0.958501941	5.17E-01	Inconclusive
LIMCH1	Alternative promoter	Repression of promoter 2	--	0.01	--	--	--	--	--	--	--	Not assessed
GMFB	Alternative promoter	Induction of promoter 2	-0.11	11.91	7.54E-01	--	--	--	--	--	--	Not assessed
MLST8	Alternative promoter	Switch to promoter 1	**0.87**	**0.19**	**9.88E-04**	**1.51**	**4.90**	**9.60E-03**	0.02	0.121241399	5.81E-01	Inconclusive
TLE3	Alternative promoter	Induction of promoter 2	0.10	0.10	8.70E-01	-0.20	5.14	4.28E-01	0.00	0.02562604	6.14E-01	Inconclusive
UBA1	Alternative promoter	Repression of promoter 1	0.21	23.51	1.39E-01	0.01	131.71	9.46E-01	0.01	0.190009964	2.99E-01	Inconclusive
TNRC6B	Alternative promoter	Repression of promoter 2	**0.18**	**2.27**	**3.34E-02**	-0.43	0.03	4.15E-01	0.00	0.988593061	3.56E-02	Inconclusive
FDFT1	Alternative promoter	Repression of promoter 2	**-0.57**	**94.14**	**1.13E-07**	**-1.07**	**1.05**	**5.62E-12**	0.00	0.986642757	2.13E-02	**Consistent**
GREB1	Alternative promoter	Induction of promoter 2	**1.45**	**1.01**	**6.45E-04**	0.28	1.48	3.21E-01	**0.14**	**0.378280864**	**3.40E-02**	Inconclusive
NCAPD3	Alternative promoter	Induction of promoter 2	0.16	75.75	6.55E-01	--	--	--	--	--	--	Not assessed
SLC36A4	Alternative promoter	Induction of promoter 2	-0.91	2.15	1.60E-03	--	--	--	--	--	--	Not assessed
KLC2	Alternative promoter	Repression of promoter 1	0.47	0.27	4.16E-01	-0.76	3.64	8.12E-02	0.00	0.1048405	4.53E-01	Inconclusive
RAP1GAP	Alternative promoter	Repression of promoter 1	1.94	3.42	3.45E-08	--	--	--	--	--	--	Not assessed
TMEM79	Alternative promoter	Repression of promoter 1	0.21	3.77	7.91E-01	**-1.40**	**1.67**	**2.05E-05**	0.19	0.399443544	5.07E-02	Inconclusive
NR4A1	Alternative promoter	Induction of promoter 2	-0.40	1.86	2.34E-01	**-0.74**	**5.81**	**7.87E-03**	0.06	0.292753045	2.53E-01	Opposite
ZNF32	Alternative promoter	Repression of promoter 2	0.03	67.26	7.14E-01	0.03	4.12	7.14E-01	0.00	0.942446541	1.00E+00	Inconclusive

F1000Research 2018, 7:1189 Last updated: 17 MAY 2019

			Isoform 1			Isoform 2			PSI			
Gene	**Event type**	**Change with androgens (LNCap)**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**log2FC**	**Av.Expr. (TPM)**	**FDR**	**Delta PSI**	**Av. PSI**	**FDR**	**Consistency of change in tumours**
C1QTNF3	Alternative promoter	Induction of promoter 1	-0.30	3.41	4.67E-01	--	--	--	--	--	--	Not assessed
UBE2D3	Alternative promoter	Switch to promoter 2	**-0.50**	**8.00**	**5.09E-04**	-0.13	0.32	8.18E-01	-0.01	0.953413055	5.49E-01	Inconclusive
KRT8	Alternative promoter	Repression of promoter 1	-0.08	2.08	8.55E-01	**0.48**	**697.27**	**1.26E-05**	0.00	0.003455479	9.85E-02	Inconclusive
ELOVL1	Alternative promoter	Induction of promoter 2	-0.10	100.07	1.38E-01	--	--	--	--	--	--	Not assessed
RCAN1	Alternative promoter	Induction of promoter 2	-0.31	1.39	4.66E-01	**-1.40**	**6.90**	**4.40E-07**	**0.09**	**0.2372612**	**1.64E-02**	Opposite
SORBS3	Alternative promoter	Induction of promoter 2	0.21	6.33	6.20E-01	--	--	--	--	--	--	Not assessed
MAT2A	Alternative 3' end	Repression of isoform 2	-0.36	102.47	6.63E-02	0.27	13.41	2.87E-01	**-0.03**	**0.888519015**	**5.32E-03**	Inconclusive
CNNM2	Alternative 3' end	Induction of isoform 1	**0.67**	**0.44**	**2.73E-05**	**-0.79**	**1.22**	**5.96E-03**	**0.13**	**0.331082656**	**3.31E-05**	**Consistent**
TMEM125	Alternative 3' end	Induction of isoform 1	--	--	--	0.45	40.70	9.40E-04	--	--	--	Not assessed
CBWD2	Alternative 3' end	Induction of isoform 2	0.00	16.56	9.88E-01	--	--	--	--	--	--	Not assessed
NDUFV3	Alternative exon	Switch to isoform 2 (exon excluded)	-0.09	12.98	2.36E-01	**0.54**	**56.19**	**4.17E-07**	**-0.07**	**0.201011**	**2.54E-08**	**Consistent**
ZNF678	Alternative exon	Switch to isoform 2 (exon excluded)	0.32	0.97	2.23E-01	--	--	--	--	--	--	Not assessed
ZNF121	Alternative exon	Switch to isoform 2 (exon excluded)	**0.90**	**0.08**	**5.97E-03**	0.02	3.09	9.28E-01	0.00	0.037899858	9.85E-02	Inconclusive
SPATC1L	Alternative exon	Induction of isoform 2 (exon included)	0.35	36.98	4.71E-02	--	--	--	--	--	--	Not assessed
MOCOS	Alternative exon	Switch to isoform 2 (exon excluded)	-0.82	2.24	1.14E-09	--	--	--	--	--	--	Not assessed
RBM45	Alternative exon	Switch to isoform 2 (exon included)	0.25	7.85	9.96E-07	--	--	--	--	--	--	Not assessed
MIPEP	Alternative exon	Repression of isoform 2 (exon excluded)	0.87	49.00	9.53E-04	--	--	--	--	--	--	Not assessed
BBS4	Alternative exon	Induction of isoform 2 (exon included)	0.02	21.63	9.71E-01	--	--	--	--	--	--	Not assessed
FAM195A	Alternative exon	Switch to isoform 1 (exon exluded)	**0.87**	**43.81**	**4.03E-08**	**0.99**	**5.57**	**1.01E-08**	-0.01	0.884563881	2.50E-01	Inconclusive
LINC01133	Alternative exon	Induction of isoform 1 (exon excluded)	--	--	--	-1.58	2.77	1.39E-08	0.00	--	--	Not assessed
SS18	Alternative exon	Switch to isoform 2 (exon excluded)	-1.47	3.70	1.97E-02	-0.14	33.31	1.18E-02	**-0.07**	**0.087763421**	**2.88E-02**	**Consistent**
RHOC	Alternative exon	Switch to isoform 2 (exon excluded)	**0.62**	**1.48**	**3.71E-06**	0.13	153.20	1.96E-01	0.00	0.009830219	1.46E-03	Inconclusive
ZNF226	Retained intron	Switch to isoform 1 (intron included)	-0.13	2.48	5.37E-01	-0.08	13.49	7.40E-01	-0.01	0.184522223	8.77E-01	Inconclusive

F1000Research 2018, 7:1189 Last updated: 17 MAY 2019

NDUFV3 and *SS18* (Figure 3, Table 5 & Supplementary Figure 2). Two of the alternative promoters (*ZFAND6* and *CDIP1*) are predicted to introduce a change in the 5'UTR, whereas all the others are predicted to alter the resulting protein isoform. A number of mRNA isoforms that were androgen responsive in LNCaP cells showed tumour specific alterations opposite to the effect of androgen stimulation. These were *LIG4*, *MAPRE2*, *OSBPL1A*, *SEPT5*, *NR4A1*, and *RCAN1* (all predicted to alter the resulting protein isoform except *LIG4*). For the remaining 14 mRNA isoform pairs, the data was inconclusive according to the consistency conditions listed in the methods section (Table 5).

Changes in androgen-dependent mRNA isoform expression during tumour progression

We next investigated whether the identified androgen-dependent mRNA isoforms are differentially expressed during prostate cancer progression by correlating the expression levels of each isoform with Gleason scores and prostate tumour grades within the PRAD TCGA cohort (Figure 4 & Figure 5, Table 6 & Table 7 and Supplementary Figure 3 & Supplementary Figure 4). For 6 of the alternative mRNA isoforms responsive to androgens (made from alternative promoters in *LIG4, OSBPL1A, CLK3, TSC22D3 & ZNF32* and utilising an alternative exon in *ZNF121*), the expression changed significantly with Gleason score and showed specific alterations consistent with the effect of androgen stimulation. Conversely, 9 alternative isoforms (which were androgen responsive in LNCaP cells) showed tumour specific alterations opposite to the effect of androgen stimulation (including an alternative promoters in *NUP93* and the alternative 3´end of *MAT2A)*. 3 androgen regulated mRNA isoforms (*OSBPL1A*, *CLK3* and *TSC22D3*) change significantly with both Gleason grade and tumour stage.

Dataset 1. Real-time PCR raw Ct values

http://dx.doi.org/10.5256/f1000research.15604.d212873

Dataset 2. Raw unedited western blot images

http://dx.doi.org/10.5256/f1000research.15604.d212874

Discussion

The main function of the androgen receptor (AR) is as a DNA binding transcription factor that regulates gene expression. Here we show the AR can couple hormone induced gene transcription to alternative mRNA isoform expression in prostate cancer. In response to androgens, the AR can induce the use of alternative promoters, induce the expression of alternatively spliced mRNA isoforms, regulate the expression of non-coding mRNA transcripts, and promote the transcription of mRNA isoforms encoding different protein isoforms. Importantly, we also find that some of these alternative mRNA isoforms are differentially regulated in prostate cancer versus normal tissue and also significantly change expression during tumour progression. Our data suggest that most androgen regulated alternative mRNA isoforms are generated through alternative promoter selection rather than through internal alternative exon splicing mechanisms. This suggests expression of alternative isoforms of specific genes can be a consequence of RNA polymerase being recruited to different promoters in response to androgen stimulation. Alternative promoter usage has been observed for many genes and is believed to play a significant role in the control of gene expression[4,105,106]. Alternative promoter use can also generate mRNA isoforms with distinct functional activities from the same gene, sometimes having opposing functions[11].

Androgen exposure further drives a smaller number of alternative splicing events suggesting that the AR could contribute to altered patterns of splicing in prostate cancer cells. Tumour progression is believed to be associated with a coordinated change in splicing patterns which is regulated by several factors including signalling molecules[7]. We also identified 4 AR regulated alternative mRNA 3' end isoform switches. This is the first time that regulation of 3' mRNA end processing has been shown to be controlled by androgens. The selection of alternative 3' ends can produce mRNA isoforms differing in the length of their 3' UTRs (which can lead to the inclusion or exclusion of regulatory elements and influence gene expression), or in their C-terminal coding region (which can contribute to proteome diversity)[107–114]. Defective 3' mRNA processing of numerous genes has been linked to an oncogenic phenotype[115–119], and the 3' mRNA end profiles of samples from multiple cancer types significantly differ from those of healthy tissue samples[115,119–121].

Based on the findings presented in this study, we propose that activated AR has the ability to coordinate both transcriptional activity and mRNA isoform decisions through the recruitment of co-regulators to specific promoters. The genomic action of the AR is influenced by a large number of collaborating transcription factors[122–124]. Specifically, Sam68 and p68 have been shown to modulate AR dependent alternative splicing of specific genes and are significantly overexpressed in prostate cancer[31,32]. In future work it will be important to define the role of specific AR co-regulators in AR mediated isoform selection.

Some of the androgen dependent mRNA isoforms identified here are predicted to yield protein isoforms that may be clinically important, or to switch off protein production via generation of noncoding mRNA isoforms. Although the functional significance of the alternative mRNA isoforms identified in this study is yet largely unexplored, as is their role in the cellular response to androgens, the presented results emphasize the importance of analysing gene regulation and function at the mRNA isoform level.

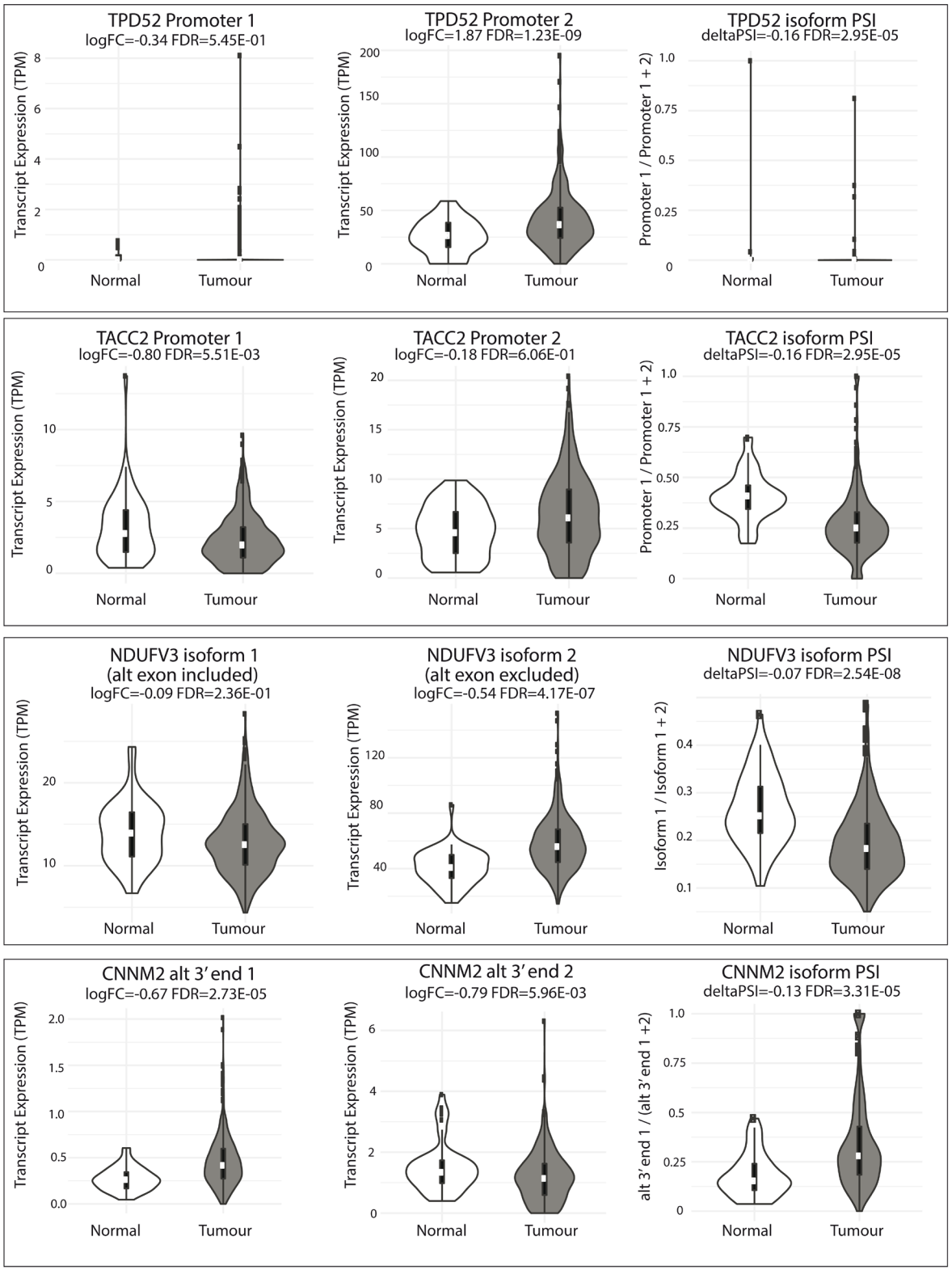

Figure 3. Differential expression of androgen dependent mRNA isoforms in prostate cancer versus normal tissue within the PRAD TCGA cohort for *TPD52*, *TACC2*, *NDUFV3* and *CNNM2*. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio in PSI (right panel) in normal and tumour samples. The mean log2 fold-change (logFC) in expression between tumour and normal samples and the associated FDR-adjusted p-value for the moderated t-statistic of differential expression are shown for both isoforms (left and central panels). The mean difference in PSI (deltaPSI) between tumour and normal samples and the associated FDR-adjusted p-value for the Mann-Whitney U test of differential splicing are shown (right panel).

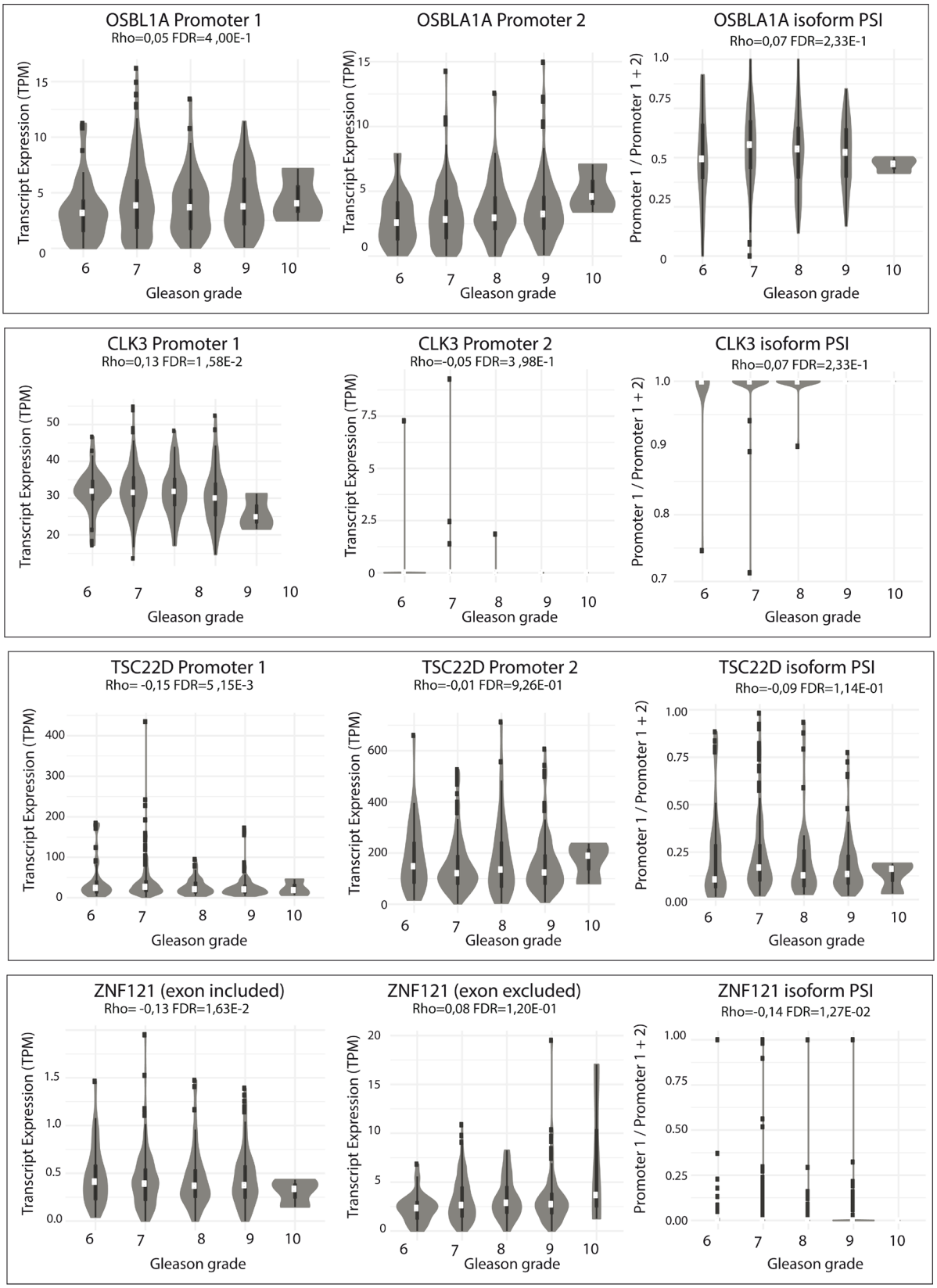

Figure 4. Differential alternative mRNA isoform expression in the TGCA PRAD cohort across different Gleason grades for *OSBPL1A*, *CLK3*, *TSC22D* and *ZNF121*. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio (right panel) by Gleason grade. Their respective Spearman's correlation coefficient (Rho) with grade and associated FDR-adjusted p-value are shown.

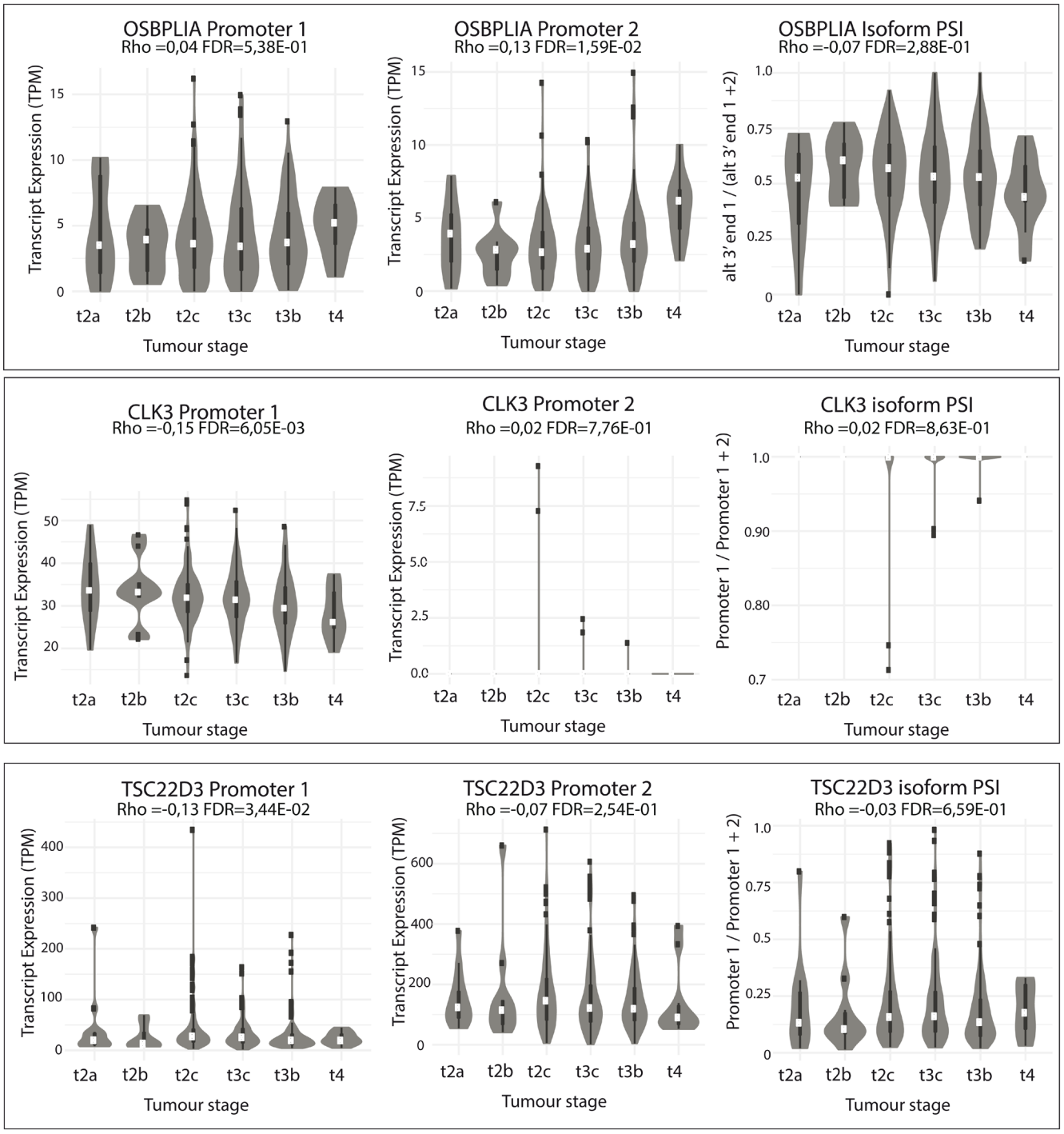

Figure 5. Differential alternative mRNA isoform expression in the TGCA PRAD cohort across different tumour stages for *OSBPL1A*, *CLK3* and *TSC22D3*. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio (right panel) by tumour stage. Their respective Spearman's correlation coefficient (Rho) with stage and associated FDR-adjusted p-value are shown.

Table 6. Summarised results of the correlation analysis of androgen-regulated isoforms expression with Gleason score in the TCGA PRAD cohort.

			Isoform 1		Isoform 2		PSI		
Gene	**Event type**	**Change with androgens (LNCap)**	**Rho**	**FDR**	**Rho**	**FDR**	**Rho**	**FDR**	**Consistency of change with Gleason**
LIG4	Alternative promoter	Induction of promoter 2	-0.07	1.92E-01	0.09	1.07E-01	**-0.18**	**4.21E-04**	Consistent -
TACC2	Alternative promoter	Repression of promoter 1	-0.08	1.55E-01	0.01	9.26E-01	-0.08	1.88E-01	Inconclusive
TPD52	Alternative promoter	Induction of promoter 2	0.00	9.51E-01	0.02	7.73E-01	0.00	9.46E-01	Inconclusive
NUP93	Alternative promoter	Induction of promoter 1	**-0.18**	**7.92E-04**	-0.07	1.81E-01	0.04	4.75E-01	Opposite
RLN1	Alternative promoter	Repression of promoter 2	-0.16	1.98E-03	--	--	--	--	Not assessed
AP2S1	Alternative promoter	Induction of promoter 2	-0.01	8.72E-01	--	--	--	--	Not assessed
RLN2	Alternative promoter	Induction of promoter 1	-0.10	6.03E-02	--	--	--	--	Not assessed
PIK3R1	Alternative promoter	Repression of promoter 2	-0.07	2.51E-01	0.09	1.20E-01	-0.17	1.29E-03	Inconclusive
MAPRE2	Alternative promoter	Switch to promoter 2	-0.07	1.92E-01	-0.06	2.73E-01	0.06	3.23E-01	Inconclusive
NDUFAF4	Alternative promoter	Repression of promoter 2	0.00	9.79E-01	--	--	--	--	Not assessed
DCXR	Alternative promoter	Repression of promoter 2	-0.29	4.07E-09	--	--	--	--	Not assessed
PEX10	Alternative promoter	Switch to promoter 2	0.08	1.50E-01	--	--	--	--	Not assessed
SNAPC2	Alternative promoter	Switch to promoter 2	**0.15**	**5.48E-03**	**-0.18**	**3.55E-04**	**0.21**	**5.13E-05**	Opposite
ATP6V0D1	Alternative promoter	Repression of promoter 2	-0.11	3.43E-02	--	--	--	--	Not assessed
ARRDC1	Alternative promoter	Induction of promoter 2	0.12	2.00E-02	--	--	--	--	Not assessed
DENND1A	Alternative promoter	Repression of promoter 2	-0.02	8.10E-01	--	--	--	--	Not assessed
KLHL36	Alternative promoter	Induction of promoter 2	-0.13	1.67E-02	--	--	--	--	Not assessed
RAB3IL1	Alternative promoter	Repression of promoter 2	0.06	3.17E-01	**0.32**	**9.13E-12**	-0.02	7.15E-01	Opposite
ACER3	Alternative promoter	Repression of promoter 2	0.16	3.79E-03	--	--	--	--	Not assessed
OSBPL1A	Alternative promoter	Induction of promoter 2	0.05	4.00E-01	**0.13**	**1.58E-02**	-0.07	2.33E-01	**Consistent**
TRIM16	Alternative promoter	Induction of promoter 2	0.10	6.06E-02	--	--	--	--	Not assessed
VSIG10L	Alternative promoter	Induction of promoter 1	-0.16	1.98E-03	--	--	--	--	Not assessed
SEPT5	Alternative promoter	Repression of promoter 2	0.17	1.12E-03	**0.12**	**1.93E-02**	-0.04	4.91E-01	Opposite
HMGCR	Alternative promoter	Repression of promoter 1	0.03	6.56E-01	-0.05	4.54E-01	0.07	2.33E-01	Inconclusive
RDH13	Alternative promoter	Induction of promoter 1	0.03	7.01E-01	0.08	1.20E-01	-0.10	1.00E-01	Inconclusive
GPRIN2	Alternative promoter	Repression of promoter 2	--	--	-0.01	8.93E-01	--	--	Not assessed
CLK3	Alternative promoter	Repression of promoter 1	**-0.13**	**1.58E-02**	-0.05	3.98E-01	0.07	2.33E-01	**Consistent**
RNH1	Alternative promoter	Induction of promoter 1	0.05	4.41E-01	0.07	1.83E-01	-0.01	9.23E-01	Inconclusive
ZFAND6	Alternative promoter	Repression of promoter 2	0.07	1.87E-01	0.05	3.82E-01	-0.03	6.36E-01	Inconclusive
CDIP1	Alternative promoter	Repression of promoter 2	0.02	8.10E-01	0.03	6.81E-01	-0.01	9.23E-01	Inconclusive
YIF1B	Alternative promoter	Switch to promoter 2	0.02	8.10E-01	-0.04	5.42E-01	0.05	4.39E-01	Inconclusive
LIMK2	Alternative promoter	Switch to promoter 2	-0.02	8.10E-01	-0.03	6.30E-01	0.00	9.49E-01	Inconclusive
TSC22D3	Alternative promoter	Repression of promoter 1	**-0.15**	**5.15E-03**	-0.01	9.26E-01	-0.09	1.14E-01	**Consistent**
ALDH1A3	Alternative promoter	Repression of promoter 1	-0.12	2.00E-02	--	--	--	--	Not assessed
TRABD	Alternative promoter	Switch to promoter 2	0.14	8.04E-03	-0.04	5.43E-01	0.05	4.39E-01	Inconclusive
LIMCH1	Alternative promoter	Repression of promoter 2	0.05	4.34E-01	--	--	--	--	Not assessed
GMFB	Alternative promoter	Induction of promoter 2	0.08	1.55E-01	--	--	--	--	Not assessed
MLST8	Alternative promoter	Switch to promoter 1	0.19	5.32E-04	0.19	2.05E-04	0.07	2.14E-01	Inconclusive
TLE3	Alternative promoter	Induction of promoter 2	0.05	4.28E-01	-0.10	7.19E-02	0.07	2.33E-01	Inconclusive

			Isoform 1		Isoform 2		PSI		
Gene	**Event type**	**Change with androgens (LNCap)**	**Rho**	**FDR**	**Rho**	**FDR**	**Rho**	**FDR**	**Consistency of change with Gleason**
UBA1	Alternative promoter	Repression of promoter 1	0.09	8.99E-02	0.03	5.95E-01	0.01	8.68E-01	Inconclusive
TNRC6B	Alternative promoter	Repression of promoter 2	-0.05	4.00E-01	-0.09	1.19E-01	0.09	1.11E-01	Inconclusive
FDFT1	Alternative promoter	Repression of promoter 2	-0.02	7.41E-01	0.07	2.07E-01	-0.07	2.14E-01	Inconclusive
GREB1	Alternative promoter	Induction of promoter 2	-0.05	4.41E-01	**-0.14**	**5.45E-03**	0.04	4.60E-01	Opposite
NCAPD3	Alternative promoter	Induction of promoter 2	-0.23	3.61E-06	--	--	--	--	Not assessed
SLC36A4	Alternative promoter	Induction of promoter 2	0.12	1.88E-02	--	--	--	--	Not assessed
KLC2	Alternative promoter	Repression of promoter 1	-0.02	8.10E-01	0.13	1.58E-02	-0.04	4.60E-01	Inconclusive
RAP1GAP	Alternative promoter	Repression of promoter 1	0.01	8.79E-01	--	--	--	--	Not assessed
TMEM79	Alternative promoter	Repression of promoter 1	-0.04	4.70E-01	0.15	3.46E-03	-0.09	1.11E-01	Inconclusive
NR4A1	Alternative promoter	Induction of promoter 2	0.10	5.44E-02	0.00	9.79E-01	0.10	7.40E-02	Inconclusive
ZNF32	Alternative promoter	Repression of promoter 2	**-0.22**	**1.32E-05**	**-0.22**	**1.11E-05**	-0.09	1.31E-01	Consistent -
C1QTNF3	Alternative promoter	Induction of promoter 1	0.08	1.58E-01	--	--	--	--	Not assessed
UBE2D3	Alternative promoter	Switch to promoter 2	0.18	7.24E-04	0.08	1.27E-01	-0.02	7.15E-01	Inconclusive
KRT8	Alternative promoter	Repression of promoter 1	-0.05	3.81E-01	-0.16	2.07E-03	0.01	8.68E-01	Inconclusive
ELOVL1	Alternative promoter	Induction of promoter 2	0.18	7.24E-04	--	--	--	--	Not assessed
RCAN1	Alternative promoter	Induction of promoter 2	0.10	5.13E-02	-0.01	8.70E-01	0.12	3.69E-02	Inconclusive
SORBS3	Alternative promoter	Induction of promoter 2	0.12	2.21E-02	--	--	--	--	Not assessed
MAT2A	Alternative 3' end	Repression of isoform 2	0.04	5.39E-01	**0.27**	**3.68E-08**	**-0.33**	**8.82E-13**	Opposite
CNNM2	Alternative 3' end	Induction of isoform 1	-0.06	3.30E-01	0.03	5.87E-01	-0.08	2.04E-01	Inconclusive
TMEM125	Alternative 3' end	Induction of isoform 1	--	--	-0.19	2.05E-04	--	--	Not assessed
CBWD2	Alternative 3' end	Induction of isoform 2	0.13	1.37E-02	--	--	--	--	Not assessed
NDUFV3	Alternative exon	Switch to isoform 2 (exon excluded)	**0.14**	**8.04E-03**	-0.07	2.48E-01	**0.13**	**2.23E-02**	Opposite
ZNF678	Alternative exon	Switch to isoform 2 (exon excluded)	-0.07	1.87E-01	--	--	--	--	Not assessed
ZNF121	Alternative exon	Switch to isoform 2 (exon excluded)	**-0.13**	**1.63E-02**	0.08	1.20E-01	**-0.14**	**1.27E-02**	**Consistent**
SPATC1L	Alternative exon	Induction of isoform 2 (exon included)	-0.13	1.58E-02	--	--	--	--	Not assessed
MOCOS	Alternative exon	Switch to isoform 2 (exon excluded)	-0.01	8.72E-01	--	--	--	--	Not assessed
RBM45	Alternative exon	Switch to isoform 2 (exon included)	0.12	2.45E-02	--	--	--	--	Not assessed
MIPEP	Alternative exon	Repression of isoform 2 (exon excluded)	-0.14	9.92E-03	--	--	--	--	Not assessed
BBS4	Alternative exon	Induction of isoform 2 (exon included)	-0.08	1.87E-01	--	--	--	--	Not assessed
FAM195A	Alternative exon	Switch to isoform 1 (exon exluded)	0.04	5.43E-01	0.14	5.35E-03	**-0.18**	**4.65E-04**	Opposite
LINC01133	Alternative exon	Induction of isoform 1 (exon excluded)	--	--	-0.02	7.51E-01	--	--	Not assessed
SS18	Alternative exon	Switch to isoform 2 (exon excluded)	0.04	4.86E-01	-0.06	2.51E-01	0.07	2.33E-01	Inconclusive
RHOC	Alternative exon	Switch to isoform 2 (exon excluded)	**0.29**	**4.07E-09**	0.15	4.24E-03	**0.21**	**3.63E-05**	Opposite
ZNF226	Retained intron	Switch to isoform 1 (intron included)	0.01	8.67E-01	-0.10	7.49E-02	0.11	6.74E-02	Inconclusive

Table 7. Summarised results of the correlation analysis of androgen-regulated isoforms expression with tumour stage in the TCGA PRAD cohort (related to Figure 4 and Supplementary Figure 5).

			Isoform 1		Isoform 2		PSI		
Gene	**Event type**	**Change with androgens (LNCap)**	**Rho**	**FDR**	**Rho**	**FDR**	**Rho**	**FDR**	**Consistency of change with stage**
LIG4	Alternative promoter	Induction of promoter 2	-0.04	6.05E-01	0.02	6.82E-01	-0.09	1.82E-01	Inconclusive
TACC2	Alternative promoter	Repression of promoter 1	-0.08	1.74E-01	-0.05	4.47E-01	-0.04	5.65E-01	Inconclusive
TPD52	Alternative promoter	Induction of promoter 2	-0.02	7.85E-01	-0.02	6.82E-01	-0.02	7.95E-01	Inconclusive
NUP93	Alternative promoter	Induction of promoter 1	**-0.12**	**3.95E-02**	0.03	6.65E-01	-0.05	4.43E-01	Opposite
RLN1	Alternative promoter	Repression of promoter 2	-0.22	1.82E-05	--	--	--	--	Not assessed
AP2S1	Alternative promoter	Induction of promoter 2	-0.04	5.51E-01	--	--	--	--	Not assessed
RLN2	Alternative promoter	Induction of promoter 1	-0.16	5.68E-03	--	--	--	--	Not assessed
PIK3R1	Alternative promoter	Repression of promoter 2	-0.02	7.92E-01	0.11	5.92E-02	**-0.14**	**3.27E-02**	Opposite -
MAPRE2	Alternative promoter	Switch to promoter 2	-0.02	7.56E-01	-0.02	6.82E-01	0.03	1.00E+00	Inconclusive
NDUFAF4	Alternative promoter	Repression of promoter 2	0.08	1.89E-01	--	--	--	--	Not assessed
DCXR	Alternative promoter	Repression of promoter 2	-0.30	6.32E-10	--	--	--	--	Not assessed
PEX10	Alternative promoter	Switch to promoter 2	0.10	9.95E-02	--	--	--	--	Not assessed
SNAPC2	Alternative promoter	Switch to promoter 2	**0.13**	**2.87E-02**	**-0.23**	**5.57E-06**	**0.20**	**2.40E-04**	Opposite
ATP6V0D1	Alternative promoter	Repression of promoter 2	-0.11	5.43E-02	--	--	--	--	Not assessed
ARRDC1	Alternative promoter	Induction of promoter 2	0.08	2.06E-01	--	--	--	--	Not assessed
DENND1A	Alternative promoter	Repression of promoter 2	-0.01	8.49E-01	--	--	--	--	Not assessed
KLHL36	Alternative promoter	Induction of promoter 2	-0.10	1.04E-01	--	--	--	--	Not assessed
RAB3IL1	Alternative promoter	Repression of promoter 2	0.08	1.71E-01	**0.33**	**4.58E-12**	0.00	9.75E-01	Opposite
ACER3	Alternative promoter	Repression of promoter 2	0.16	4.77E-03	--	--	--	--	Not assessed
OSBPL1A	Alternative promoter	Induction of promoter 2	0.04	5.38E-01	**0.13**	**1.59E-02**	-0.07	2.88E-01	**Consistent**
TRIM16	Alternative promoter	Induction of promoter 2	0.06	3.95E-01	--	--	--	--	Not assessed
VSIG10L	Alternative promoter	Induction of promoter 1	-0.12	5.43E-02	--	--	--	--	Not assessed
SEPT5	Alternative promoter	Repression of promoter 2	0.11	7.96E-02	0.07	2.54E-01	-0.01	8.89E-01	Inconclusive
HMGCR	Alternative promoter	Repression of promoter 1	0.00	9.91E-01	-0.04	5.77E-01	0.04	6.25E-01	Inconclusive
RDH13	Alternative promoter	Induction of promoter 1	-0.03	7.33E-01	0.10	7.19E-02	-0.12	9.32E-02	Inconclusive
GPRIN2	Alternative promoter	Repression of promoter 2	--	--	0.03	6.48E-01	--	--	Not assessed
CLK3	Alternative promoter	Repression of promoter 1	**-0.15**	**6.05E-03**	0.02	7.76E-01	0.02	8.63E-01	**Consistent**
RNH1	Alternative promoter	Induction of promoter 1	-0.02	7.92E-01	0.10	6.12E-02	-0.08	2.28E-01	Inconclusive
ZFAND6	Alternative promoter	Repression of promoter 2	0.03	6.50E-01	0.04	5.78E-01	-0.04	6.05E-01	Inconclusive
CDIP1	Alternative promoter	Repression of promoter 2	0.10	1.04E-01	0.02	7.82E-01	0.06	3.78E-01	Inconclusive
YIF1B	Alternative promoter	Switch to promoter 2	-0.01	8.87E-01	-0.10	6.71E-02	0.06	3.97E-01	Inconclusive
LIMK2	Alternative promoter	Switch to promoter 2	0.00	9.67E-01	-0.05	4.72E-01	0.00	9.75E-01	Inconclusive
TSC22D3	Alternative promoter	Repression of promoter 1	**-0.13**	**3.44E-02**	-0.07	2.54E-01	-0.03	6.59E-01	**Consistent**
ALDH1A3	Alternative promoter	Repression of promoter 1	-0.18	7.69E-04	--	--	--	--	Not assessed
TRABD	Alternative promoter	Switch to promoter 2	0.06	3.95E-01	-0.03	6.48E-01	0.03	7.83E-01	Inconclusive
LIMCH1	Alternative promoter	Repression of promoter 2	0.02	7.85E-01	--	--	--	--	Not assessed
GMFB	Alternative promoter	Induction of promoter 2	0.07	2.57E-01	--	--	--	--	Not assessed
MLST8	Alternative promoter	Switch to promoter 1	0.10	8.19E-02	0.15	6.14E-03	0.02	7.83E-01	Inconclusive
TLE3	Alternative promoter	Induction of promoter 2	0.03	6.38E-01	**-0.11**	**3.84E-02**	0.04	5.65E-01	Opposite
UBA1	Alternative promoter	Repression of promoter 1	0.12	5.43E-02	0.00	9.72E-01	0.06	3.99E-01	Inconclusive

			Isoform 1		Isoform 2		PSI		
Gene	**Event type**	**Change with androgens (LNCap)**	**Rho**	**FDR**	**Rho**	**FDR**	**Rho**	**FDR**	**Consistency of change with stage**
TNRC6B	Alternative promoter	Repression of promoter 2	-0.04	6.31E-01	-0.03	6.48E-01	0.02	7.83E-01	Inconclusive
FDFT1	Alternative promoter	Repression of promoter 2	-0.05	4.82E-01	0.04	5.46E-01	-0.08	2.28E-01	Inconclusive
GREB1	Alternative promoter	Induction of promoter 2	-0.11	7.48E-02	**-0.18**	**7.01E-04**	0.01	8.96E-01	Opposite
NCAPD3	Alternative promoter	Induction of promoter 2	-0.23	1.82E-05	--	--	--	--	Not assessed
SLC36A4	Alternative promoter	Induction of promoter 2	0.07	2.59E-01	--	--	--	--	Not assessed
KLC2	Alternative promoter	Repression of promoter 1	-0.03	6.33E-01	0.13	1.81E-02	-0.08	2.78E-01	Inconclusive
RAP1GAP	Alternative promoter	Repression of promoter 1	0.02	7.85E-01	--	--	--	--	Not assessed
TMEM79	Alternative promoter	Repression of promoter 1	-0.08	1.71E-01	0.16	1.97E-03	-0.10	1.20E-01	Inconclusive
NR4A1	Alternative promoter	Induction of promoter 2	0.01	8.49E-01	-0.06	3.69E-01	0.08	2.62E-01	Inconclusive
ZNF32	Alternative promoter	Repression of promoter 2	-0.15	6.70E-03	0.02	7.34E-01	-0.08	2.33E-01	Inconclusive
C1QTNF3	Alternative promoter	Induction of promoter 1	0.03	6.74E-01	--	--	--	--	Not assessed
UBE2D3	Alternative promoter	Switch to promoter 2	0.20	2.96E-04	0.07	2.17E-01	-0.02	7.83E-01	Inconclusive
KRT8	Alternative promoter	Repression of promoter 1	-0.04	6.05E-01	-0.24	2.72E-06	0.04	6.05E-01	Inconclusive
ELOVL1	Alternative promoter	Induction of promoter 2	0.13	2.87E-02	--	--	--	--	Not assessed
RCAN1	Alternative promoter	Induction of promoter 2	0.09	1.26E-01	-0.01	8.69E-01	0.10	1.20E-01	Inconclusive
SORBS3	Alternative promoter	Induction of promoter 2	0.11	7.96E-02	--	--	--	--	Not assessed
MAT2A	Alternative 3' end	Repression of isoform 2	0.01	9.35E-01	**0.18**	**7.83E-04**	**-0.21**	**8.42E-05**	Opposite
CNNM2	Alternative 3' end	Induction of isoform 1	0.05	3.95E-01	0.05	4.47E-01	-0.04	6.05E-01	Inconclusive
TMEM125	Alternative 3' end	Induction of isoform 1	--	--	-0.16	2.80E-03	--	--	Not assessed
CBWD2	Alternative 3' end	Induction of isoform 2	0.08	1.74E-01	--	--	--	--	Not assessed
NDUFV3	Alternative exon	Switch to isoform 2 (exon excluded)	0.11	7.48E-02	-0.05	4.72E-01	0.11	1.00E-01	Inconclusive
ZNF678	Alternative exon	Switch to isoform 2 (exon excluded)	-0.02	7.43E-01	--	--	--	--	Not assessed
ZNF121	Alternative exon	Switch to isoform 2 (exon excluded)	-0.08	1.80E-01	0.03	6.48E-01	-0.09	1.82E-01	Inconclusive
SPATC1L	Alternative exon	Induction of isoform 2 (exon included)	-0.10	9.95E-02	--	--	--	--	Not assessed
MOCOS	Alternative exon	Switch to isoform 2 (exon excluded)	0.03	6.33E-01	--	--	--	--	Not assessed
RBM45	Alternative exon	Switch to isoform 2 (exon included)	0.08	1.71E-01	--	--	--	--	Not assessed
MIPEP	Alternative exon	Repression of isoform 2 (exon excluded)	-0.16	4.48E-03	--	--	--	--	Not assessed
BBS4	Alternative exon	Induction of isoform 2 (exon included)	-0.06	3.85E-01	--	--	--	--	Not assessed
FAM195A	Alternative exon	Switch to isoform 1 (exon exluded)	0.06	3.37E-01	0.10	6.85E-02	-0.10	1.20E-01	Inconclusive
LINC01133	Alternative exon	Induction of isoform 1 (exon excluded)	--	--	0.00	9.72E-01	--	--	Not assessed
SS18	Alternative exon	Switch to isoform 2 (exon excluded)	0.04	5.68E-01	-0.04	5.46E-01	0.06	3.97E-01	Inconclusive
RHOC	Alternative exon	Switch to isoform 2 (exon excluded)	0.15	6.05E-03	0.11	3.84E-02	0.11	1.00E-01	Inconclusive
ZNF226	Retained intron	Switch to isoform 1 (intron included)	-0.03	6.64E-01	-0.09	1.23E-01	0.07	3.35E-01	Inconclusive

Data availability

The RNASeq data from LNCaP cells has been published previously https://doi.org/10.1016/j.ebiom.2016.04.018[25]

The RNAseq custom tracks are available in Supplementary File 1. To view these files please load them onto the UCSC website using the 'My data' tab and 'custom tracks'. Then 'Paste URLs or data'. The data is aligned to Feb 2009 (GRCh37/hg19).

Prostate adenocarcinoma cohort RNA-Seq data was downloaded from the Broad Institute TCGA Genome Analysis Center: Firehose 16/01/28 run https://doi.org/10.7908/C11G0KM9[43]

Dataset 1: Real-time PCR raw Ct values 10.5256/f1000research.15604.d212873[41]

Dataset 2: Raw unedited western blot images 10.5256/f1000research.15604.d212874[125]

Competing interests

No competing interests were disclosed.

Grant information

This work was funded by Prostate Cancer UK [PG12-34, S13-020 and RIA16-ST2-011].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary Table 1: Details of primer sequences used.

Click here to access the data.

Supplementary File 1: RNA-Seq reads custom tracks for visualisation on UCSC genome browser.

Click here to access the data.

Supplementary Figure 1: PCR validation of 17 androgen regulated alternative events.

Click here to access the data.

Supplementary Figure 2: Differential alternative mRNA isoform expression in theTGCA PRAD cohort. Normal vs. tumour (unpaired samples) analysis. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio in PSI (right panel) in normal and tumour samples. The mean log2 fold-change (logFC) in expression between tumour and normal samples and the associated FDR-adjusted p-value for the moderated t-statistic of differential expression are shown for both isoforms (left and central panels). The mean difference in PSI (deltaPSI) between tumour and normal samples and the associated FDR-adjusted p-value for the Mann-Whitney U test of differential splicing are shown (right panel).

Click here to access the data.

Supplementary Figure 3: Differential alternative mRNA isoform expression in the TGCA PRAD cohort across different Gleason grades. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio (right panel) by Gleason grade. Their respective Spearman's correlation coefficient (Rho) with grade and associated FDR-adjusted p-value are shown.

Click here to access the data.

Supplementary Figure 4: Differential alternative mRNA isoform expression in the TGCA PRAD cohort across different tumour stages. Violin-boxplots of expression in transcripts per million mapped reads (TPM) of Isoforms 1 (left panel) and 2 (central panel), and of their expression ratio (right panel) by tumour stage. Their respective Spearman's correlation coefficient (Rho) with stage and associated FDR-adjusted p-value are shown.

Click here to access the data.

References

1. Johnson JM, Castle J, Garrett-Engele P, *et al.*: **Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays.** *Science.* 2003; **302**(5653): 2141–4.
PubMed Abstract | Publisher Full Text

2. Pan Q, Shai O, Lee LJ, *et al.*: **Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing.** *Nat Genet.* 2008; **40**(12): 1413–5.
PubMed Abstract | Publisher Full Text

3. Wang ET, Sandberg R, Luo S, *et al.*: **Alternative isoform regulation in human tissue transcriptomes.** *Nature.* 2008; **456**(7221): 470–6.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Davuluri RV, Suzuki Y, Sugano S, *et al.*: **The functional consequences of alternative promoter use in mammalian genomes.** *Trends Genet.* 2008; **24**(4): 167–77.
PubMed Abstract | Publisher Full Text

5. Trapnell C, Williams BA, Pertea G, *et al.*: **Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation.** *Nat Biotechnol.* 2010; **28**(5): 511–5.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation.** *Cell.* 2011; **144**(5): 646–74.
PubMed Abstract | Publisher Full Text

7. Oltean S, Bates DO: **Hallmarks of alternative splicing in cancer.** *Oncogene.* 2014; **33**(46): 5311–8.
PubMed Abstract | Publisher Full Text

8. Ladomery M: **Aberrant alternative splicing is another hallmark of cancer.** *Int J Cell Biol.* 2013; **2013**: 463786.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Rajan P, Elliott DJ, Robson CN, *et al.*: **Alternative splicing and biological heterogeneity in prostate cancer.** *Nat Rev Urol.* 2009; **6**(8): 454–60.
PubMed Abstract | Publisher Full Text

10. Rajan P, Dalgliesh C, Carling PJ, *et al.*: **Identification of novel androgen-regulated pathways and mRNA isoforms through genome-wide exon-specific profiling of the LNCaP transcriptome.** *PLoS One.* 2011; **6**(12): e29088.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Munkley J, Livermore K, Rajan P, *et al.*: **RNA splicing and splicing regulator changes in prostate cancer pathology.** *Hum Genet.* 2017; **136**(9): 1143–54.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Livermore KE, Munkley J, Elliott DJ: **Androgen receptor and prostate cancer.** *AIMS Mol Sci.* 2016; **3**(2): 280–99.
Publisher Full Text

13. Mills IG: **Maintaining and reprogramming genomic androgen receptor activity in prostate cancer.** *Nat Rev Cancer.* 2014; **14**(3): 187–98.
PubMed Abstract | Publisher Full Text

14. Snoek R, Cheng H, Margiotti K, *et al.*: ***In vivo* knockdown of the androgen receptor results in growth inhibition and regression of well-established, castration-resistant prostate tumors.** *Clin Cancer Res.* 2009; **15**(1): 39–47.
PubMed Abstract | Publisher Full Text

15. Hara T, Miyazaki H, Lee A, *et al.*: **Androgen receptor and invasion in prostate cancer.** *Cancer Res.* 2008; **68**(4): 1128–35.
PubMed Abstract | Publisher Full Text

16. Hååg P, Bektic J, Bartsch G, *et al.*: **Androgen receptor down regulation by small interference RNA induces cell growth inhibition in androgen sensitive as well as in androgen independent prostate cancer cells.** *J Steroid Biochem Mol Biol.* 2005; **96**(3–4): 251–8.
PubMed Abstract | Publisher Full Text

17. Munkley J, Oltean S, Vodák D, *et al.*: **The androgen receptor controls expression of the cancer-associated sTn antigen and cell adhesion through induction of ST6GalNAc1 in prostate cancer.** *Oncotarget.* 2015; **6**(33): 34358–74.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Munkley J, Rajan P, Lafferty NP, *et al.*: **A novel androgen-regulated isoform of the TSC2 tumour suppressor gene increases cell proliferation.** *Oncotarget.* 2014; **5**(1): 131–9.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Massie CE, Lynch A, Ramos-Montoya A, *et al.*: **The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis.** *EMBO J.* 2011; **30**(13): 2719–33.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Munkley J, Lafferty NP, Kalna G, *et al.*: **Androgen-regulation of the protein tyrosine phosphatase PTPRR activates ERK1/2 signalling in prostate cancer cells.** *BMC Cancer.* 2015; **15**: 9.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Munkley J, Livermore KE, McClurg UL, *et al.*: **The PI3K regulatory subunit gene *PIK3R1* is under direct control of androgens and repressed in prostate cancer cells.** *Oncoscience.* 2015; **2**(9): 755–64.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Karantanos T, Corn PG, Thompson TC: **Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches.** *Oncogene.* 2013; **32**(49): 5501–11.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Cai C, He HH, Chen S, *et al.*: **Androgen receptor gene expression in prostate cancer is directly suppressed by the androgen receptor through recruitment of lysine-specific demethylase 1.** *Cancer Cell.* 2011; **20**(4): 457–71.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Yuan X, Cai C, Chen S, *et al.*: **Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis.** *Oncogene.* 2014; **33**(22): 2815–25.
PubMed Abstract | Publisher Full Text | Free Full Text

25. Munkley J, Vodak D, Livermore KE, *et al.*: **Glycosylation is an Androgen-Regulated Process Essential for Prostate Cancer Cell Viability.** *EBioMedicine.* 2016; **8**: 103–16.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Auboeuf D, Hönig A, Berget SM, *et al.*: **Coordinate regulation of transcription and splicing by steroid receptor coregulators.** *Science.* 2002; **298**(5592): 416–9.
PubMed Abstract | Publisher Full Text

27. Satoh T, Katano-Toki A, Tomaru T, *et al.*: **Coordinated regulation of transcription and alternative splicing by the thyroid hormone receptor and its associating coregulators.** *Biochem Biophys Res Commun.* 2014; **451**(1): 24–9.
PubMed Abstract | Publisher Full Text

28. Dutertre M, Gratadou L, Dardenne E, *et al.*: **Estrogen regulation and physiopathologic significance of alternative promoters in breast cancer.** *Cancer Res.* 2010; **70**(9): 3760–70.
PubMed Abstract | Publisher Full Text

29. Dowhan DH, Hong EP, Auboeuf D, *et al.*: **Steroid hormone receptor coactivation and alternative RNA splicing by U2AF^{65}-related proteins CAPERalpha and CAPERbeta.** *Mol Cell.* 2005; **17**(3): 429–39.
PubMed Abstract | Publisher Full Text

30. Cochrane DR, Wang Z, Muramaki M, *et al.*: **Differential regulation of clusterin and its isoforms by androgens in prostate cells.** *J Biol Chem.* 2007; **282**(4): 2278–87.
PubMed Abstract | Publisher Full Text

31. Rajan P, Gaughan L, Dalgliesh C, *et al.*: **The RNA-binding and adaptor protein Sam68 modulates signal-dependent splicing and transcriptional activity of the androgen receptor.** *J Pathol.* 2008; **215**(1): 67–77.
PubMed Abstract | Publisher Full Text

32. Clark EL, Coulson A, Dalgliesh C, *et al.*: **The RNA helicase p68 is a novel androgen receptor coactivator involved in splicing and is overexpressed in prostate cancer.** *Cancer Res.* 2008; **68**(19): 7938–46.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Salama SA, Mohammad MA, Diaz-Arrastia CR, *et al.*: **Estradiol-17β upregulates pyruvate kinase M2 expression to coactivate estrogen receptor-α and to integrate metabolic reprogramming with the mitogenic response in endometrial cells.** *J Clin Endocrinol Metab.* 2014; **99**(10): 3790–9.
PubMed Abstract | Publisher Full Text

34. Lal S, Allan A, Markovic D, *et al.*: **Estrogen alters the splicing of type 1 corticotropin-releasing hormone receptor in breast cancer cells.** *Sci Signal.* 2013; **6**(282): ra53.
PubMed Abstract | Publisher Full Text

35. Bhat-Nakshatri P, Song EK, Collins NR, *et al.*: **Interplay between estrogen receptor and AKT in estradiol-induced alternative splicing.** *BMC Med Genomics.* 2013; **6**: 21.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Munkley J, McClurg UL, Livermore KE, *et al.*: **The cancer-associated cell migration protein TSPAN1 is under control of androgens and its upregulation increases prostate cancer cell migration.** *Sci Rep.* 2017; **7**(1): 5249.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Trapnell C, Roberts A, Goff L, *et al.*: **Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.** *Nat Protoc.* 2012; **7**(3): 562–78.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Kim D, Pertea G, Trapnell C, *et al.*: **TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions.** *Genome Biol.* 2013; **14**(4): R36.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2.** *Nat Methods.* 2012; **9**(4): 357–9.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Trapnell C, Hendrickson DG, Sauvageau M, *et al.*: **Differential analysis of gene regulation at transcript resolution with RNA-seq.** *Nature Biotechnol.* 2013; **31**(1): 46–53.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Munkley J, Maia TM, Ibarluzea N, *et al.*: **Dataset 1 in: Androgen-dependent alternative mRNA isoform expression in prostate cancer cells.** *F1000Research.* 2018.
http://www.doi.org/10.5256/f1000research.15604.d212873

42. Young MD, Wakefield MJ, Smyth GK, *et al.*: **Gene ontology analysis for RNA-seq: accounting for selection bias.** *Genome Biol.* 2010; **11**(2): R14.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Center BITGDA: **Analysis-ready standardized TCGA data from Broad GDAC Firehose 2016_01_28 run.** *Broad Institute of MIT and Harvard Dataset.* 2016.
Publisher Full Text

44. Ritchie ME, Phipson B, Wu D, *et al.*: ***limma* powers differential expression analyses for RNA-sequencing and microarray studies.** *Nucleic Acids Res.* 2015; **43**(7): e47.
PubMed Abstract | Publisher Full Text | Free Full Text

45. Tyner C, Barber GP, Casper J, *et al.*: **The UCSC Genome Browser database: 2017 update.** *Nucleic Acids Res.* 2017; **45**(D1): D626–D34.
PubMed Abstract | Publisher Full Text | Free Full Text

46. Roslan N, Bieche I, Bright RK, *et al.*: **TPD52 represents a survival factor in *ERBB2*-amplified breast cancer cells.** *Mol Carcinog.* 2014; **53**(10): 807–19.
PubMed Abstract | Publisher Full Text

47. Zhang H, Wang J, Pang B, *et al.*: **PC-1/PrLZ contributes to malignant progression in prostate cancer.** *Cancer Res.* 2007; **67**(18): 8906–13.
PubMed Abstract | Publisher Full Text

48. Zhang D, He D, Xue Y, *et al.*: **PrLZ protects prostate cancer cells from apoptosis induced by androgen deprivation via the activation of Stat3/Bcl-2 pathway.** *Cancer Res.* 2011; **71**(6): 2193–202.
PubMed Abstract | Publisher Full Text | Free Full Text

49. Yu L, Shang ZF, Wang J, *et al.*: **PC-1/PrLZ confers resistance to rapamycin in prostate cancer cells through increased 4E-BP1 stability.** *Oncotarget.* 2015; **6**(24): 20356–69.
PubMed Abstract | Publisher Full Text | Free Full Text

50. Feng S, Agoulnik IU, Bogatcheva NV, *et al.*: **Relaxin promotes prostate cancer progression.** *Clin Cancer Res.* 2007; **13**(6): 1695–702.
PubMed Abstract | Publisher Full Text

51. Feng S, Agoulnik IU, Li Z, *et al.*: **Relaxin/RXFP1 signaling in prostate cancer progression.** *Ann N Y Acad Sci.* 2009; **1160**: 379–80.
PubMed Abstract | Publisher Full Text | Free Full Text

52. Neschadim A, Summerlee AJ, Silvertown JD: **Targeting the relaxin hormonal pathway in prostate cancer.** *Int J Cancer.* 2015; **137**(10): 2287–95.
PubMed Abstract | Publisher Full Text

53. Silvertown JD, Ng J, Sato T, *et al.*: **H2 relaxin overexpression increases *in vivo* prostate xenograft tumor growth and angiogenesis.** *Int J Cancer.* 2006; **118**(1): 62–73.
PubMed Abstract | Publisher Full Text

54. Silvertown JD, Symes JC, Neschadim A, *et al.*: **Analog of H2 relaxin exhibits antagonistic properties and impairs prostate tumor growth.** *FASEB J.* 2007; **21**(3): 754–65.
PubMed Abstract | Publisher Full Text

55. Thompson VC, Morris TG, Cochrane DR, *et al.*: **Relaxin becomes upregulated during prostate cancer progression to androgen independence and is negatively regulated by androgens.** *Prostate.* 2006; **66**(16): 1698–709.
PubMed Abstract | Publisher Full Text

56. Onodera Y, Takagi K, Miki Y, *et al.*: **TACC2 (transforming acidic coiled-coil protein 2) in breast carcinoma as a potent prognostic predictor associated with cell proliferation.** *Cancer Med.* 2016; **5**(8): 1973–82.
PubMed Abstract | Publisher Full Text | Free Full Text

57. Takayama K, Horie-Inoue K, Suzuki T, *et al.*: **TACC2 is an androgen-responsive cell cycle regulator promoting androgen-mediated and castration-resistant growth of prostate cancer.** *Mol Endocrinol.* 2012; **26**(5): 748–61.
PubMed Abstract | Publisher Full Text | Free Full Text

58. Kim DH, Oh SY, Kim SY, *et al.*: **DNA ligase4 as a prognostic marker in nasopharyngeal cancer patients treated with radiotherapy.** *Asian Pac J Cancer Prev.* 2014; **15**(24): 10985–9.
PubMed Abstract | Publisher Full Text

59. Jun S, Jung YS, Suh HN, *et al.*: ***LIG4* mediates Wnt signalling-induced radioresistance.** *Nat Commun.* 2016; **7**: 10994.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Grupp K, Roettger L, Kluth M, *et al.*: **Expression of DNA ligase IV is linked to poor prognosis and characterizes a subset of prostate cancers harboring TMPRSS2:ERG fusion and PTEN deletion.** *Oncol Rep.* 2015; **34**(3): 1211–20.
PubMed Abstract | Publisher Full Text

61. Cao WH, Liu HM, Liu X, *et al.*: **Relaxin enhances *in-vitro* invasiveness of breast cancer cell lines by upregulation of S100A4/MMPs signaling.** *Eur Rev Med Pharmacol Sci.* 2013; **17**(5): 609–17.
PubMed Abstract

62. Binder C, Hagemann T, Husen B, *et al.*: **Relaxin enhances *in-vitro* invasiveness of breast cancer cell lines by up-regulation of matrix metalloproteases.** *Mol Hum Reprod.* 2002; **8**(9): 789–96.
PubMed Abstract | Publisher Full Text

63. Ma J, Niu M, Yang W, *et al.*: **Role of relaxin-2 in human primary osteosarcoma.** *Cancer Cell Int.* 2013; **13**(1): 59.
PubMed Abstract | Publisher Full Text | Free Full Text

64. Hombach-Klonisch S, Bialek J, Trojanowicz B, *et al.*: **Relaxin enhances the oncogenic potential of human thyroid carcinoma cells.** *Am J Pathol.* 2006; **169**(2): 617–32.
PubMed Abstract | Publisher Full Text | Free Full Text

65. Radestock Y, Willing C, Kehlen A, *et al.*: **Relaxin enhances S100A4 and promotes growth of human thyroid carcinoma cell xenografts.** *Mol Cancer Res.* 2010; **8**(4): 494–506.
PubMed Abstract | Publisher Full Text

66. Byrne JA, Frost S, Chen Y, *et al.*: **Tumor protein D52 (TPD52) and cancer-oncogene understudy or understudied oncogene?** *Tumour Biol.* 2014; **35**(8): 7369–82.
PubMed Abstract | Publisher Full Text

67. Byrne JA, Balleine RL, Schoenberg Fejzo M, *et al.*: **Tumor protein D52 (TPD52) is overexpressed and a gene amplification target in ovarian cancer.** *Int J Cancer.* 2005; **117**(6): 1049–54.
PubMed Abstract | Publisher Full Text

68. Zhao Z, Liu H, Hou J, *et al.*: **Tumor Protein D52 (TPD52) Inhibits Growth and Metastasis in Renal Cell Carcinoma Cells Through the PI3K/Akt Signaling Pathway.** *Oncol Res.* 2017; **25**(5): 773–9.
PubMed Abstract | Publisher Full Text

69. Li J, Li Y, Liu H, *et al.*: **The four-transmembrane protein MAL2 and tumor protein D52 (TPD52) are highly expressed in colorectal cancer and correlated with poor prognosis.** *PLoS One.* 2017; **12**(5): e0178515.
PubMed Abstract | Publisher Full Text | Free Full Text

70. Rubin MA, Varambally S, Beroukhim R, *et al.*: **Overexpression, amplification, and androgen regulation of TPD52 in prostate cancer.** *Cancer Res.* 2004; **64**(11): 3814–22.
PubMed Abstract | Publisher Full Text

71. Goto Y, Nishikawa R, Kojima S, *et al.*: **Tumour-suppressive *microRNA-224* inhibits cancer cell migration and invasion via targeting oncogenic *TPD52* in prostate cancer.** *FEBS Lett.* 2014; **588**(10): 1973–82.
PubMed Abstract | Publisher Full Text

72. Moritz T, Venz S, Junker H, *et al.*: **Isoform 1 of TPD52 (PC-1) promotes neuroendocrine transdifferentiation in prostate cancer cells.** *Tumour Biol.* 2016; **37**(8): 10435–46.
PubMed Abstract | Publisher Full Text

73. Shang ZF, Wei Q, Yu L, *et al.*: **Suppression of PC-1/PrLZ sensitizes prostate cancer cells to ionizing radiation by attenuating DNA damage repair and inducing autophagic cell death.** *Oncotarget.* 2016; **7**(38): 62340–51.
PubMed Abstract | Publisher Full Text | Free Full Text

74. Li L, Xie H, Liang L, *et al.*: **Increased PrLZ-mediated androgen receptor transactivation promotes prostate cancer growth at castration-resistant stage.** *Carcinogenesis.* 2013; **34**(2): 257–67.
PubMed Abstract | Publisher Full Text | Free Full Text

75. Yang YF, Jan YH, Liu YP, *et al.*: **Squalene synthase induces tumor necrosis factor receptor 1 enrichment in lipid rafts to promote lung cancer metastasis.** *Am J Respir Crit Care Med.* 2014; **190**(6): 675–87.
PubMed Abstract | Publisher Full Text

76. Fukuma Y, Matsui H, Koike H, *et al.*: **Role of squalene synthase in prostate cancer risk and the biological aggressiveness of human prostate cancer.** *Prostate Cancer Prostatic Dis.* 2012; **15**(4): 339–45.
PubMed Abstract | Publisher Full Text

77. Susini T, Berti B, Carriero C, *et al.*: **Topoisomerase II alpha and TLE3 as predictive markers of response to anthracycline and taxane-containing regimens for neoadjuvant chemotherapy in breast cancer.** *Onco Targets Ther.* 2014; **7**: 2111–20.
PubMed Abstract | Publisher Full Text | Free Full Text

78. Samimi G, Ring BZ, Ross DT, *et al.*: **TLE3 expression is associated with sensitivity to taxane treatment in ovarian carcinoma.** *Cancer Epidemiol Biomarkers Prev.* 2012; **21**(2): 273–9.
PubMed Abstract | Publisher Full Text

79. Yang RW, Zeng YY, Wei WT, *et al.*: **TLE3 represses colorectal cancer proliferation by inhibiting MAPK and AKT signaling pathways.** *J Exp Clin Cancer Res.* 2016; **35**(1): 152.
PubMed Abstract | Publisher Full Text | Free Full Text

80. Nakaya HI, Beckedorff FC, Baldini ML, *et al.*: **Splice variants of *TLE* family genes and up-regulation of a *TLE3* isoform in prostate tumors.** *Biochem Biophys Res Commun.* 2007; **364**(4): 918–23.
PubMed Abstract | Publisher Full Text

81. Guan H, Liu C, Fang F, *et al.*: **MicroRNA-744 promotes prostate cancer progression through aberrantly activating Wnt/β-catenin signaling.** *Oncotarget.* 2017; **8**(9): 14693–707.
PubMed Abstract | Publisher Full Text | Free Full Text

82. Giménez-Mascarell P, Oyenarte I, Hardy S, *et al.*: **Structural Basis of the Oncogenic Interaction of Phosphatase PRL-1 with the Magnesium Transporter CNNM2.** *J Biol Chem.* 2017; **292**(3): 786–801.
PubMed Abstract | Publisher Full Text | Free Full Text

83. Lee JH, Zhao XM, Yoon I, *et al.*: **Integrative analysis of mutational and transcriptional profiles reveals driver mutations of metastatic breast cancers.** *Cell Discov.* 2016; **2**: 16025.
PubMed Abstract | Publisher Full Text | Free Full Text

84. Zhang W, Sviripa V, Chen X, *et al.*: **Fluorinated *N,N*-dialkylaminostilbenes repress colon cancer by targeting methionine *S*-adenosyltransferase 2A.** *ACS Chem Biol.* 2013; **8**(4): 796–803.
PubMed Abstract | Publisher Full Text | Free Full Text

85. Frau M, Feo F, Pascale RM: **Pleiotropic effects of methionine adenosyltransferases deregulation as determinants of liver cancer progression and prognosis.** *J Hepatol.* 2013; **59**(4): 830–41.
PubMed Abstract | Publisher Full Text

86. Wang X, Guo X, Yu W, *et al.*: **Expression of methionine adenosyltransferase 2A in renal cell carcinomas and potential mechanism for kidney carcinogenesis.** *BMC Cancer.* 2014; **14**: 196.
PubMed Abstract | Publisher Full Text | Free Full Text

87. Maldonado LY, Arsene D, Mato JM, *et al.*: **Methionine adenosyltransferases in cancers: Mechanisms of dysregulation and implications for therapy.** *Exp Biol Med (Maywood).* 2018; **243**(2): 107–17.
PubMed Abstract | Publisher Full Text | Free Full Text

88. Tomasi ML, Cossu C, Spissu Y, *et al.*: **S-adenosylmethionine and methylthioadenosine inhibit cancer metastasis by targeting microRNA 34a/b-methionine adenosyltransferase 2A/2B axis.** *Oncotarget.* 2017; **8**(45): 78851–69.
PubMed Abstract | Publisher Full Text | Free Full Text

89. Cizkova M, Vacher S, Meseure D, *et al.*: ***PIK3R1* underexpression is an independent prognostic marker in breast cancer.** *BMC Cancer.* 2013; **13**: 545.
PubMed Abstract | Publisher Full Text | Free Full Text

90. Cheung LW, Hennessy BT, Li J, *et al.*: **High frequency of *PIK3R1* and *PIK3R2* mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability.** *Cancer Discov.* 2011; **1**(2): 170–85.
PubMed Abstract | Publisher Full Text | Free Full Text

91. Ma J, Hou X, Li M, *et al.*: **Genome-wide methylation profiling reveals new biomarkers for prognosis prediction of glioblastoma.** *J Cancer Res Ther.* 2015; **11 Suppl 2**: C212–5.
PubMed Abstract | Publisher Full Text

92. Thorsen K, Schepeler T, Øster B, *et al.*: **Tumor-specific usage of alternative**

transcription start sites in colorectal cancer identified by genome-wide exon array analysis. *BMC Genomics.* 2011; **12**: 505.
PubMed Abstract | Publisher Full Text | Free Full Text

93. Luo A, Zhang X, Fu L, *et al.*: **Zinc finger factor ZNF121 is a MYC-interacting protein functionally affecting MYC and cell proliferation in epithelial cells.** *J Genet Genomics.* 2016; **43**(12): 677–85.
PubMed Abstract | Publisher Full Text

94. Liu C, Zheng L, Wang H, *et al.*: **The RCAN1 inhibits NF-κB and suppresses lymphoma growth in mice.** *Cell Death Dis.* 2015; **6**(10): e1929.
PubMed Abstract | Publisher Full Text | Free Full Text

95. Espinosa AV, Shinohara M, Porchia LM, *et al.*: **Regulator of calcineurin 1 modulates cancer cell migration *in vitro*.** *Clin Exp Metastasis.* 2009; **26**(6): 517–26.
PubMed Abstract | Publisher Full Text | Free Full Text

96. Hang X, Wu Z, Chu K, *et al.*: **Low expression of DCXR protein indicates a poor prognosis for hepatocellular carcinoma patients.** *Tumour Biol.* 2016; **37**(11): 15079–85.
PubMed Abstract | Publisher Full Text

97. Ebert B, Kisiela M, Maser E: **Human DCXR - another 'moonlighting protein' involved in sugar metabolism, carbonyl detoxification, cell adhesion and male fertility?.** *Biol Rev Camb Philos Soc.* 2015; **90**(1): 254–78.
PubMed Abstract | Publisher Full Text

98. Cho-Vega JH, Vega F, Schwartz MR, *et al.*: **Expression of dicarbonyl/L-xylulose reductase (DCXR) in human skin and melanocytic lesions: morphological studies supporting cell adhesion function of DCXR.** *J Cutan Pathol.* 2007; **34**(7): 535–42.
PubMed Abstract | Publisher Full Text

99. Cho-Vega JH, Tsavachidis S, Do KA, *et al.*: **Dicarbonyl/L-xylulose reductase: a potential biomarker identified by laser-capture microdissection-micro serial analysis of gene expression of human prostate adenocarcinoma.** *Cancer Epidemiol Biomarkers Prev.* 2007; **16**(12): 2615–22.
PubMed Abstract | Publisher Full Text

100. Abiatari I, Gillen S, DeOliveira T, *et al.*: **The microtubule-associated protein MAPRE2 is involved in perineural invasion of pancreatic cancer cells.** *Int J Oncol.* 2009; **35**(5): 1111–6.
PubMed Abstract | Publisher Full Text

101. Zhang JH, Li AY, Wei N: **Downregulation of long non-coding RNA LINC01133 is predictive of poor prognosis in colorectal cancer patients.** *Eur Rev Med Pharmacol Sci.* 2017; **21**(9): 2103–7.
PubMed Abstract

102. Zhang J, Zhu N, Chen X: **A novel long noncoding RNA LINC01133 is upregulated in lung squamous cell cancer and predicts survival.** *Tumour Biol.* 2015; **36**(10): 7465–71.
PubMed Abstract | Publisher Full Text

103. Brown-Endres L, Schoenfeld D, Tian F, *et al.*: **Expression of the p53 target CDIP correlates with sensitivity to TNFα-induced apoptosis in cancer cells.** *Cancer Res.* 2012; **72**(9): 2373–82.
PubMed Abstract | Publisher Full Text | Free Full Text

104. Fraser M, Sabelnykova VY, Yamaguchi TN, *et al.*: **Genomic hallmarks of localized, non-indolent prostate cancer.** *Nature.* 2017; **541**(7637): 359–64.
PubMed Abstract | Publisher Full Text

105. Ayoubi TA, Van De Ven WJ: **Regulation of gene expression by alternative promoters.** *FASEB J.* 1996; **10**(4): 453–60.
PubMed Abstract | Publisher Full Text

106. Baek D, Davis C, Ewing B, *et al.*: **Characterization and predictive discovery of evolutionarily conserved mammalian alternative promoters.** *Genome Res.* 2007; **17**(2): 145–55.
PubMed Abstract | Publisher Full Text | Free Full Text

107. Millevoi S, Vagner S: **Molecular mechanisms of eukaryotic pre-mRNA 3' end processing regulation.** *Nucleic Acids Res.* 2010; **38**(9): 2757–74.
PubMed Abstract | Publisher Full Text | Free Full Text

108. Hollerer I, Grund K, Hentze MW, *et al.*: **mRNA 3'end processing: A tale of the tail reaches the clinic.** *EMBO Mol Med.* 2014; **6**(1): 16–26.
PubMed Abstract | Publisher Full Text | Free Full Text

109. Tian B, Manley JL: **Alternative cleavage and polyadenylation: the long and short of it.** *Trends Biochem Sci.* 2013; **38**(6): 312–20.
PubMed Abstract | Publisher Full Text | Free Full Text

110. Moore MJ: **From birth to death: the complex lives of eukaryotic mRNAs.** *Science.* 2005; **309**(5740): 1514–8.
PubMed Abstract | Publisher Full Text

111. Miyamoto S, Chiorini JA, Urcelay E, *et al.*: **Regulation of gene expression for translation initiation factor eIF-2 alpha: importance of the 3' untranslated region.** *Biochem J.* 1996; **315**(Pt 3): 791–8.
PubMed Abstract | Publisher Full Text | Free Full Text

112. Takagaki Y, Seipelt RL, Peterson ML, *et al.*: **The polyadenylation factor CstF-64 regulates alternative processing of IgM heavy chain pre-mRNA during B cell differentiation.** *Cell.* 1996; **87**(5): 941–52.
PubMed Abstract | Publisher Full Text

113. Edwalds-Gilbert G, Veraldi KL, Milcarek C: **Alternative poly(A) site selection in complex transcription units: means to an end?** *Nucleic Acids Res.* 1997; **25**(13): 2547–61.
PubMed Abstract | Publisher Full Text | Free Full Text

114. Lutz CS: **Alternative polyadenylation: a twist on mRNA 3' end formation.** *ACS Chem Biol.* 2008; **3**(10): 609–17.
PubMed Abstract | Publisher Full Text

115. Mayr C, Bartel DP: **Widespread shortening of 3'UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells.** *Cell.* 2009; **138**(4): 673–84.
PubMed Abstract | Publisher Full Text | Free Full Text

116. Enciso-Mora V, Hosking FJ, Di Stefano AL, *et al.*: **Low penetrance susceptibility to glioma is caused by the *TP53* variant rs78378222.** *Br J Cancer.* 2013; **108**(10): 2178–85.
PubMed Abstract | Publisher Full Text | Free Full Text

117. Stacey SN, Sulem P, Jonasdottir A, *et al.*: **A germline variant in the *TP53* polyadenylation signal confers cancer susceptibility.** *Nat Genet.* 2011; **43**(11): 1098–103.
PubMed Abstract | Publisher Full Text | Free Full Text

118. Wiestner A, Tehrani M, Chiorazzi M, *et al.*: **Point mutations and genomic deletions in *CCND1* create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival.** *Blood.* 2007; **109**(11): 4599–606.
PubMed Abstract | Publisher Full Text | Free Full Text

119. Lin Y, Li Z, Ozsolak F, *et al.*: **An in-depth map of polyadenylation sites in cancer.** *Nucleic Acids Res.* 2012; **40**(17): 8460–71.
PubMed Abstract | Publisher Full Text | Free Full Text

120. Morris AR, Bos A, Diosdado B, *et al.*: **Alternative cleavage and polyadenylation during colorectal cancer development.** *Clin Cancer Res.* 2012; **18**(19): 5256–66.
PubMed Abstract | Publisher Full Text

121. Fu Y, Sun Y, Li Y, *et al.*: **Differential genome-wide profiling of tandem 3' UTRs among human breast cancer and normal cells by high-throughput sequencing.** *Genome Res.* 2011; **21**(5): 741–7.
PubMed Abstract | Publisher Full Text | Free Full Text

122. Wang Q, Li W, Liu XS, *et al.*: **A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth.** *Mol Cell.* 2007; **27**(3): 380–92.
PubMed Abstract | Publisher Full Text | Free Full Text

123. Andreu-Vieyra C, Lai J, Berman BP, *et al.*: **Dynamic nucleosome-depleted regions at androgen receptor enhancers in the absence of ligand in prostate cancer cells.** *Mol Cell Biol.* 2011; **31**(23): 4648–62.
PubMed Abstract | Publisher Full Text | Free Full Text

124. Zhao JC, Fong KW, Jin HJ: **FOXA1 acts upstream of GATA2 and AR in hormonal regulation of gene expression.** *Oncogene.* 2016; **35**(33): 4335–44.
PubMed Abstract | Publisher Full Text | Free Full Text

125. Munkley J, Maia TM, Ibarluzea N, *et al.*: **Dataset 2 in: Androgen-dependent alternative mRNA isoform expression in prostate cancer cells.** *F1000Research.* 2018.
http://www.doi.org/10.5256/f1000research.15604.d212874

Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Version 1

Reviewer Report 04 October 2018

https://doi.org/10.5256/f1000research.17022.r37702

✔ **Jennifer Byrne**
Children's Cancer Research Unit, Kids Research Institute, The Children's Hospital at Westmead, Westmead, NSW, Australia

Prostate cancer is a common cancer in men that is driven in part through deregulated androgen signalling. The importance of androgen inhibitors in prostate cancer therapy and the clinical challenges posed by the development of androgen-resistant disease both justify the detailed description of the effects of androgen treatment on gene transcription and alternative splicing in prostate cancer cells. In this sense, the analyses reported by Munkley and colleagues represent valuable additions to the literature. However, further explanation of the results presented would increase the reader's ability to understand these results and their significance, and to identify which results should be prioritised for further research. I have therefore provided some specific suggestions to increase the accessibility of the data as they are currently presented.

Specific comments

1. The genes shown in Tables 1, 2, 3 and 5 are not shown in alphabetical order. It is unclear how these genes are ranked and why they are shown in the orders displayed. It would be helpful for any groupings of genes to be more clearly displayed in these tables where this is relevant.
2. It would be helpful to more clearly indicate which findings are novel and which are supported by the literature in Tables and/or Figures.
3. In Table 1, a number of genes are shown in bold, but this is not explained.
4. In Table 1, it would be helpful to annotate the isoform ID's shown (columns towards the right side of Table). What does "comparable" mean here?
5. It is challenging to show data for a large number of genes, most of which the authors will not be familiar with. However, in Figure 2, incorrect information is shown for the TPD52 gene (panel A). The PrLZ isoform is actually longer than the TPD52 isoform (through an extended N-terminal sequence specific to PrLZ), yet the sizes of these isoforms indicated in the diagram at the right have been switched (TPD52 is incorrectly shown to be the longer isoform). The authors should check whether this is an isolated error or whether other data for the TPD52 and PrLZ isoforms have been switched (for example in Figure 3).
6. It would be helpful for Table 4 to include percentages as well as sample numbers so that readers can compare the composition of the TCGA PRAD cohort with other published cohorts.

7. Analyses compared differential isoform expression in prostate cancer and normal tissue. The cohort included 497 prostate cancer patients, for which 52 had matched normal tissue (Table 5, Figure 3). I've assumed that these analyses compared transcript levels in the 497 prostate cancer cases with those in the 52 normal tissue cases. However, given that the 52 normal tissue cases had matched tumour samples available, were analyses conducted to compare the 52 matched cases? These analyses could be argued to be more robust through comparing matched samples, albeit in a smaller cohort.
8. Table 5 should indicate the numbers of tumour and normal tissue samples compared.
9. Some data in Tables 5, 6, and 6 are shown in bold, but this is not explained.
10. I could not open Dataset 2. Could this be made available as a pdf file?
11. All violin plots (Figures 3-5, also supplementary data) should specify the sample numbers compared, either below the X axis or in the figure legend if the same sample numbers apply to every plot shown.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 18 September 2018

https://doi.org/10.5256/f1000research.17022.r38286

Cyril F. Bourgeois
INSERM U1210, CNRS UMR 5239, Laboratory of Biology and Modelling of the Cell, Ecole Normale Supérieure de Lyon (ENS Lyon), University of Lyon, Lyon, France

This paper by Munkley and colleagues identifies in a comprehensive manner novel alternative mRNA isoforms regulated by androgens. Interestingly most isoforms result from a choice between alternative promoters, suggesting that regulation takes place mostly at the transcriptional level, but they identified also a few alternative cassette exons and 3' ends. They show experimental validation for 17 isoforms. Beside increasing the number of identified genes in the context of androgen-treated prostate cancer LNCaP cells, the authors analysed the expression of those new isoforms in a large cohort of prostate tumours. They found the expression of some of the mRNA isoforms is positively correlated in the androgen-treated cell and in cancer versus normal samples, and find further correlation with the tumour grade and stage for 3 alternative isoforms.

Overall this is an interesting work that clearly deserves to be published, as it reveals new potentially interesting target genes for prostate cancer. I have only a couple of comments/questions that may help to improve the strength of the manuscript.

Did the authors try to experimentally validate the regulation of alternative isoforms for the 3 most interesting genes, i.e. *OSBPL1A*, *CLK3* and *TSC22D3*, which is correlated to tumour stage ? As these new isoforms are predicted to alter the protein sequence, is it possible to discuss or predict what could be the impact of these modifications for these proteins, with regards to what is known about their function and/or in the context of prostate cancer?

Looking at the RNA-seq profiles for the validated examples, it seems to me that in some cases, especially for RLN1 and RLN2, regulation of promoter choice correspond also to changes in the 3 end of the transcript (the peak seems to be shifted to the 3' end). Such examples may have escaped the in silico prediction, but can you make any comment on this ?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Transcription and alternative splicing, transcriptomics

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 05 September 2018

https://doi.org/10.5256/f1000research.17022.r36819

Sebastian Oltean
Institute of Biomedical and Clinical Sciences, Medical School, University of Exeter, Exeter, UK

Prostate cancer (PCa) is still a significant health problem in UK and across the world. Though a small minority of patients progress to aggressive forms, the absolute number is quite significant due to the high incidence of PCa among men. Therefore, investigation of molecular mechanisms of PCa progression is very important and will hopefully unravel novel therapeutic targets.

Alternative splicing (AS) has been shown to occur in over 94% of genes in humans. It is therefore a crucial level of gene regulation and not surprisingly involved in virtually every physiological and pathological process. AS de-regulation has been implicated in many diseases, including cancer and in particular PCa, and interestingly, many times it has been shown to drive cancer pathology independently of transcription.

Since androgens are main players in PCa, the idea of analysing global changes in AS in response to androgens is very welcome to the field. The authors found 10 times more AS isoforms regulated by androgens than previously reported in data from cell culture, most of them occurring through alternative promoter mechanism. They have confirmed and validated part of these changes. They have also analysed the isoforms changes between adenocarcinoma and normal tissues as well as during progression through the Gleason stages of PCa.

This is a very well thought and executed study, with may informative results. I have a suggestion for the discussion part:

- one issue in global analysis of splice isoforms is which ones are causal (ie maintain and aggravate the phenotype) and which ones are just associated with the pathological progression; while a full answer to this would need experimental evidence on each individual splicing event, could the authors discuss 1-2 examples, if possible, where the changes at protein level (either sequence or expression level of a particular isoform) would hypothetically have a causal role

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: alternative splicing; prostate cancer; diabetes (renal complications)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.