Dive into a research article

2025-03-27

Mariachiara Grieco

C.G.B.

UNIVERSITÀ DEGLI STUDI DI MILANO

Deep dive into a paper

We will analyze the study
FOXA1 regulates alternative splicing in prostate cancer
published in *Cell Reports* in 2022.


Cell Reports

Volume 40, Issue 13, 27 September 2022, 111404

Article

FOXA1 regulates alternative splicing in prostate cancer

Marco Del Giudice [1 2 10], John G. Foster [3 10], Serena Peirone [1 4], Alberto Rissone [1 2], Livia Caizzi [1 2], Federica Gaudino [1 2], Caterina Parlato [1 2], Francesca Anselmi [1 5], Rebecca Arkell [3], Simonetta Guarrera [1 2], Salvatore Oliviero [1 5], Giuseppe Basso [1 2], Prabhakar Rajan [3 6 7 8 9 11] ✉, Matteo Cereda [1 4 11 12] ✉

In this analysis, we will:

- **Examine the study's objectives** – What hypotheses were tested?
- **Break down the methodology** – What experimental techniques were used?
- **Discuss key findings** – How does FOXA1 impact alternative splicing, and what are the implications for prostate cancer treatment?
- **Evaluate the significance** – How do these results contribute to our understanding of cancer biology and potential therapies?

Explore the title

Who

What

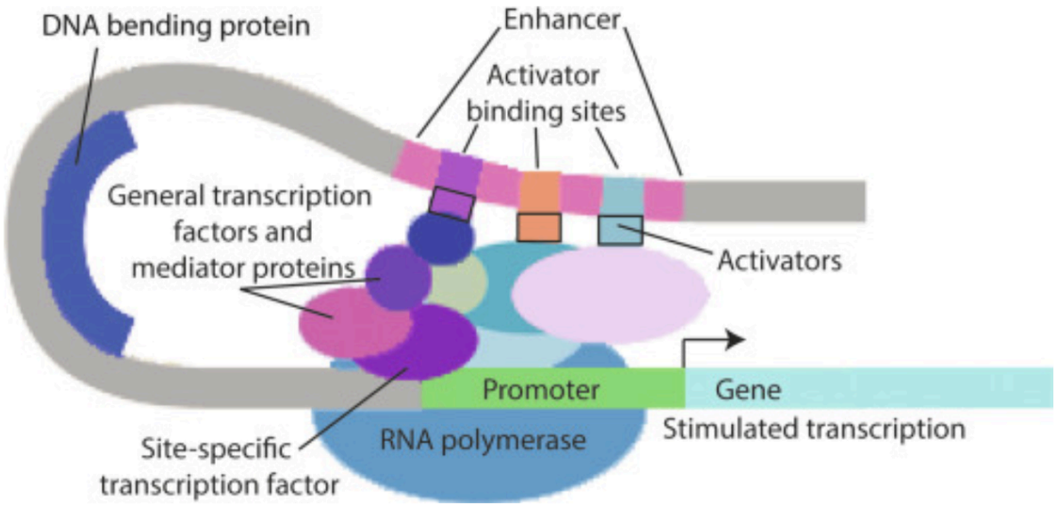
Where

Explore the title

Who What Where

FOXA1 regulates alternative splicing in prostate cancer

FOXA1

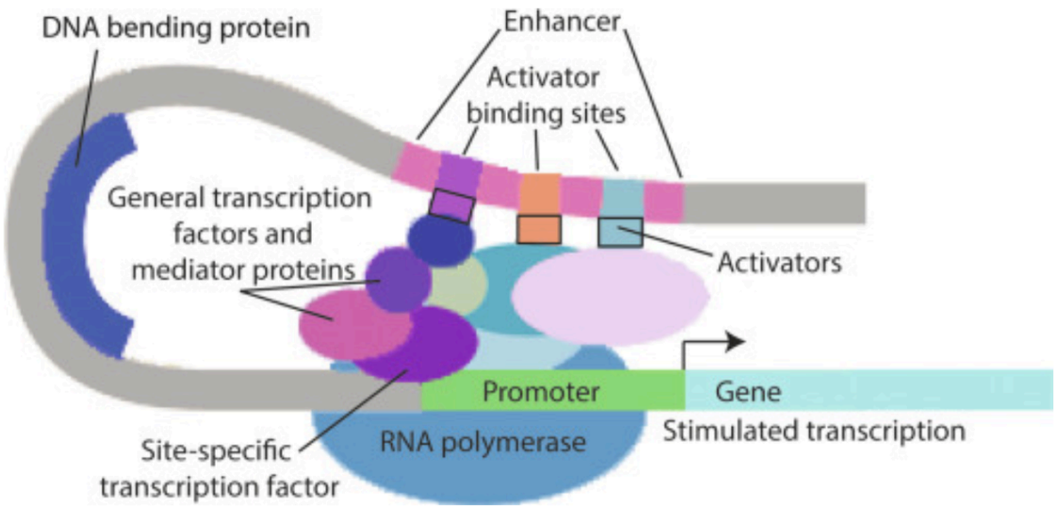


FOXA1 is a **pioneer transcription factor**, meaning it plays a crucial role in opening chromatin to **facilitate gene transcription** and **regulate gene expression.**

It regulates gene expression by binding to DNA regulatory sequences, with a strong **preference for enhancer regions** (distal regulatory elements).

FOXA1 is essential for the **development of multiple endoderm-derived organ** systems, including the liver, pancreas, lung, and prostate.

FOXA1

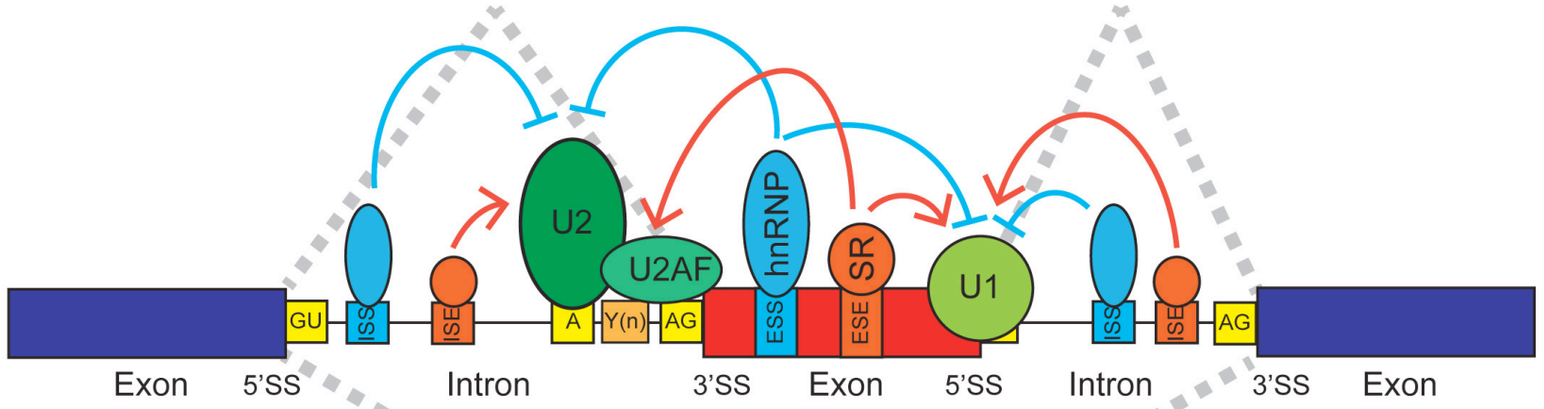


In **prostate cancer**, FOXA1 coordinates its activity with the **androgen receptor** (AR) to regulate gene expression.

However, it also has an **AR-independent role** in controlling epithelial-to-mesenchymal transition, a process linked to cancer progression and metastasis.

Mutations in prostate cancer frequently affect both the coding sequence and cis-regulatory elements (CREs) of FOXA1, leading to significant functional alterations that may contribute to tumor development and progression.

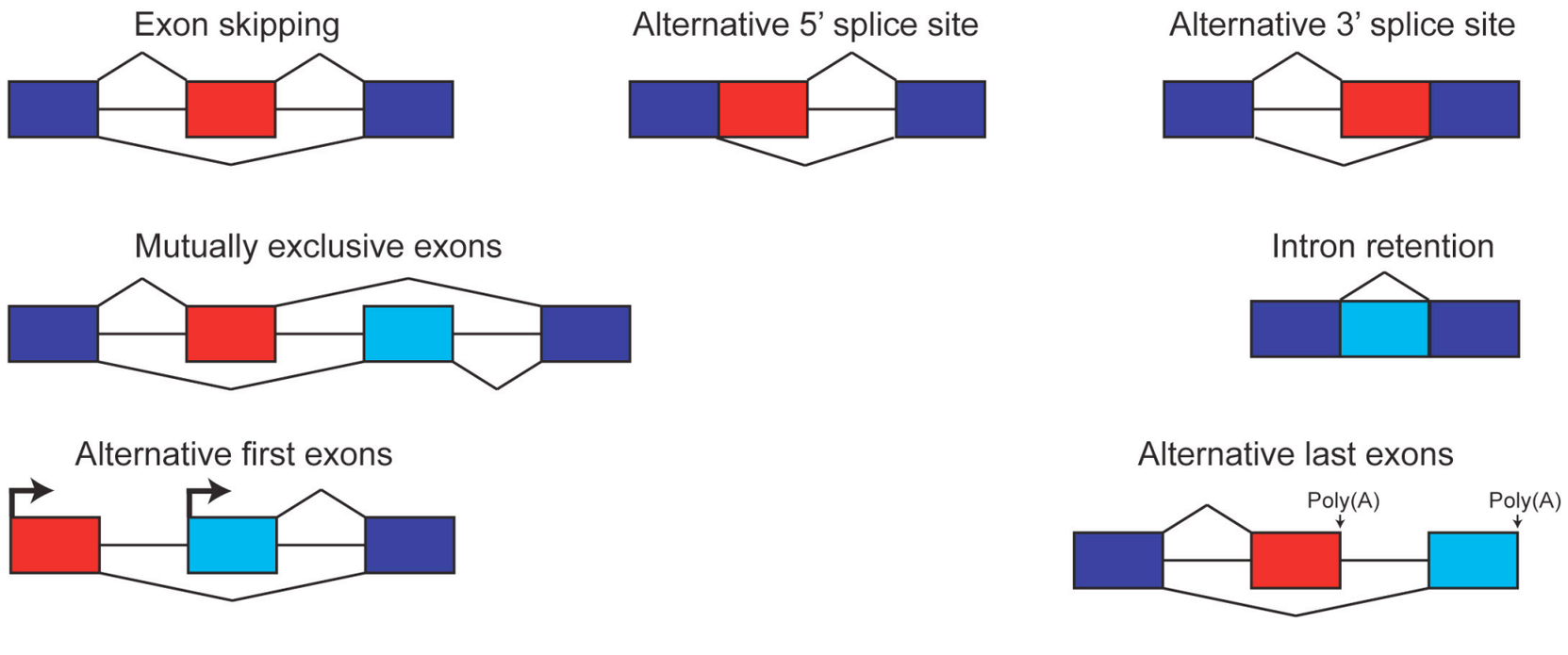
Alternative splicing



Splicing is a fundamental process in which introns are removed from a pre-mRNA molecule, and exons are joined together to form a **mature mRNA transcript.**

More than 95% of human genes produce multiple isoforms (mature mRNA variants) through a mechanism known as **alternative splicing.**

Alternative splicing



Alternative splicing significantly contributes to **proteomic diversity**, allowing a limited number of genes to encode a vast array of proteins.

This process is primarily regulated by **RNA-binding proteins** (RBPs), also known as splicing-related proteins (SRPs), which bind to specific RNA motifs to influence exon inclusion or exclusion.

Dysregulation of alternative splicing is frequently observed in **cancer**, leading to the production of aberrant protein isoforms that can contribute to tumor progression.

Prostate cancer

Prostate cancer is the second most common type of cancer worldwide and the leading cause of cancer-related death in men.

Despite significant advancements in diagnosis and treatment, end-stage metastatic castration-resistant prostate cancer (mCRPC) remains highly **challenging to treat.**

Prostate cancer exhibits **high heterogeneity**, contributing to treatment resistance and disease progression.

Recurrent activating alterations frequently occur in key oncogenic transcription factors, including **AR, ERG, FOXA1, and MYC**, playing a crucial role in tumor development and progression.

Author spotlight

Marco Del Giudice,[1,2,10] John G. Foster,[3,10] Serena Peirone,[1,4] Alberto Rissone,[1,2] Livia Caizzi,[1,2] Federica Gaudino,[1,2] Caterina Parlato,[1,2] Francesca Anselmi,[1,5] Rebecca Arkell,[3] Simonetta Guarrera,[1,2] Salvatore Oliviero,[1,5] Giuseppe Basso,[1,2] Prabhakar Rajan,[3,6,7,8,9,11,*] and Matteo Cereda[1,4,11,12,*]

[1]Italian Institute for Genomic Medicine, c/o IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy
[2]Candiolo Cancer Institute, FPO—IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy
[3]Centre for Cancer Cell and Molecular Biology, Barts Cancer Institute, Cancer Research UK Barts Centre, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK
[4]Department of Biosciences, Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy
[5]Department of Life Science and System Biology, Università degli Studi di Torino, via Accademia Albertina 13, 10123 Turin, Italy
[6]Division of Surgery and Interventional Science, University College London, Charles Bell House, 3 Road Floor, 43–45 Foley Street, London W1W 7TS, UK
[7]The Alan Turing Institute, British Library, 96 Euston Road, London NW1 2DB, UK
[8]Department of Urology, Barts Health NHS Trust, the Royal London Hospital, Whitechapel Road, London E1 1BB, UK
[9]Department of Uro-oncology, University College London NHS Foundation Trust, 47 Wimpole Street, London W1G 8SE, UK
[10]These authors contributed equally
[11]Senior author
[12]Lead contact
*Correspondence: p.rajan@qmul.ac.uk (P.R.), matteo.cereda1@unimi.it (M.C.)
https://doi.org/10.1016/j.celrep.2022.111404

The author list generally begins with the **first author(s)**, who contributed the most to the study, and continues with subsequent authors based on their level of contribution.

The **last author** is often the principal investigator or the leading researcher, who is usually responsible for the overall direction of the study.
In some fields, the last author holds significant seniority.

Author spotlight

Marco Del Giudice,[1,2,10] John G. Foster,[3,10] Serena Peirone,[1,4] Alberto Rissone,[1,2] Livia Caizzi,[1,2] Federica Gaudino,[1,2] Caterina Parlato,[1,2] Francesca Anselmi,[1,5] Rebecca Arkell,[3] Simonetta Guarrera,[1,2] Salvatore Oliviero,[1,5] Giuseppe Basso,[1,2] Prabhakar Rajan,[3,6,7,8,9,11,*] and Matteo Cereda[1,4,11,12,*]

[1]Italian Institute for Genomic Medicine, c/o IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy
[2]Candiolo Cancer Institute, FPO—IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy
[3]Centre for Cancer Cell and Molecular Biology, Barts Cancer Institute, Cancer Research UK Barts Centre, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK
[4]Department of Biosciences, Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy
[5]Department of Life Science and System Biology, Università degli Studi di Torino, via Accademia Albertina 13, 10123 Turin, Italy
[6]Division of Surgery and Interventional Science, University College London, Charles Bell House, 3 Road Floor, 43–45 Foley Street, London W1W 7TS, UK
[7]The Alan Turing Institute, British Library, 96 Euston Road, London NW1 2DB, UK
[8]Department of Urology, Barts Health NHS Trust, the Royal London Hospital, Whitechapel Road, London E1 1BB, UK
[9]Department of Uro-oncology, University College London NHS Foundation Trust, 47 Wimpole Street, London W1G 8SE, UK
[10]These authors contributed equally
[11]Senior author
[12]Lead contact
*Correspondence: p.rajan@qmul.ac.uk (P.R.), matteo.cereda1@unimi.it (M.C.)
https://doi.org/10.1016/j.celrep.2022.111404

Authors' affiliations with their institutions or universities are listed to provide **transparency** and **context** regarding where the research was conducted.
This is particularly important in collaborative studies involving multiple institutions or international research teams.

Author spotlight

Marco Del Giudice,[1,2,10] John G. Foster,[3,10] Serena Peirone,[1,4] Alberto Rissone,[1,2] Livia Caizzi,[1,2] Federica Gaudino,[1,2] Caterina Parlato,[1,2] Francesca Anselmi,[1,5] Rebecca Arkell,[3] Simonetta Guarrera,[1,2] Salvatore Oliviero,[1,5] Giuseppe Basso,[1,2] Prabhakar Rajan,[3,6,7,8,9,11,*] and Matteo Cereda[1,4,11,12,*]

[1]Italian Institute for Genomic Medicine, c/o IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy

[2]Candiolo Cancer Institute, FPO—IRCCS, Str. Prov. le 142, km 3.95, 10060 Candiolo (TO), Italy

[3]Centre for Cancer Cell and Molecular Biology, Barts Cancer Institute, Cancer Research UK Barts Centre, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK

[4]Department of Biosciences, Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy

[5]Department of Life Science and System Biology, Università degli Studi di Torino, via Accademia Albertina 13, 10123 Turin, Italy

[6]Division of Surgery and Interventional Science, University College London, Charles Bell House, 3 Road Floor, 43–45 Foley Street, London W1W 7TS, UK

[7]The Alan Turing Institute, British Library, 96 Euston Road, London NW1 2DB, UK

[8]Department of Urology, Barts Health NHS Trust, the Royal London Hospital, Whitechapel Road, London E1 1BB, UK

[9]Department of Uro-oncology, University College London NHS Foundation Trust, 47 Wimpole Street, London W1G 8SE, UK

[10]These authors contributed equally

[11]Senior author

[12]Lead contact

*Correspondence: p.rajan@qmul.ac.uk (P.R.), matteo.cereda1@unimi.it (M.C.)

https://doi.org/10.1016/j.celrep.2022.111404

Corresponding authors are typically marked with an asterisk (*) or another notation.
This authors are the **primary contact for the paper** and are responsible for managing the submission, communication with the journal, and addressing reviewer comments.
They handle reprints and are often **who to reach out** to if readers have any **questions or comments** regarding the study.
Their **email address** is usually provided in the author section, allowing readers to directly contact them for clarification or further information about the research.

Hot off the press or a blast from the past?

The **publication date** is a crucial factor when evaluating a research paper.

Scientific knowledge evolves rapidly, and newer studies often provide updated data, refined methodologies, and the latest discoveries in the field.

When assessing a paper, **ask yourself:**

- Is the research recent, or could it be outdated?
- Does it reflect the latest advancements in the field?
- Have there been newer studies that build upon or challenge these findings?

While older papers can still be valuable—especially if they are foundational or highly cited—it is essential to consider whether more recent studies provide additional insights or revised conclusions.

Abstract

In this case, we do not just have the traditional abstract (referred to as the “Summary"), but also additional key sections to enhance comprehension:

- **Brief description:** a concise overview of the research.
- **Graphical abstract:** a visual summary of the study’s main findings.
- **Highlights**: key takeaways from the paper, summarizing the most important points.

In brief

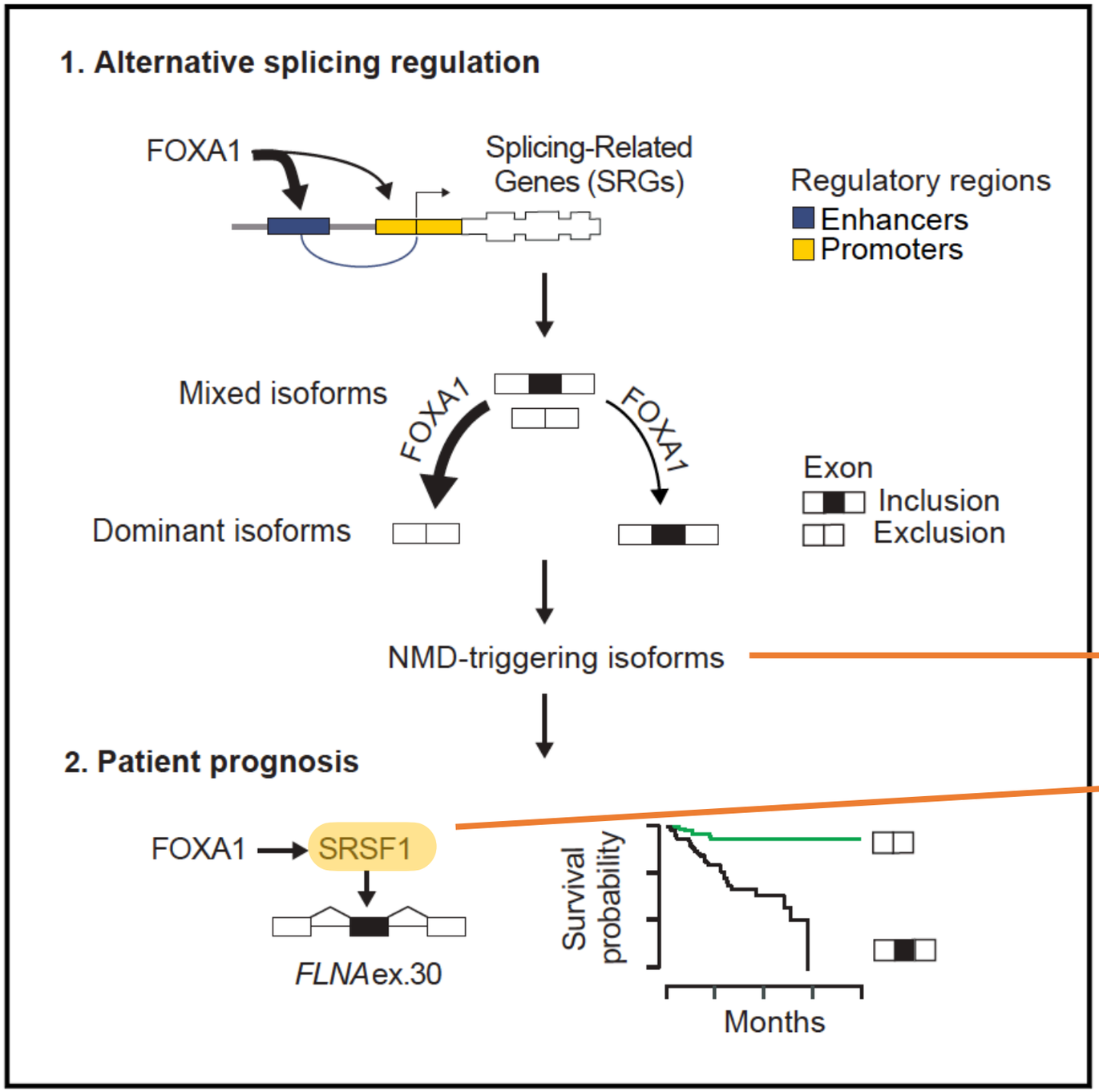
Del Giudice et al. identify the pioneer transcription factor FOXA1 as a master regulator of alternative splicing in prostate cancer. By controlling splicing factors, FOXA1 buffers the noise of isoform production toward a mRNA dominant product. This regulation impacts on splicing of nonsense-mediated decay-determinant exons influencing patient survival.

Glossary:
NMD is a translation-coupled mechanism that eliminates mRNAs containing premature translation-termination codons (PTCs).

PTCs arise from single nucleotide variations or alternative splicing events modifying RNA frame that convert a triplet nucleotide codon into one of three stop codons, i.e. TAG, TGA or TAA.

Abstract

Graphical abstract



Nonsense-mediated decay

Splicing-related protein

Abstract

Highlights

- FOXA1 is a master transcriptional regulator of splicing factors in prostate cancer
- FOXA1 drives splice isoform production toward an optimal dominant mRNA product
- FOXA1 controls exons triggering NMD, influencing prostate cancer patient prognosis
- FOXA1-controlled SRSF1 enhances inclusion of FLNA exon 30, promoting disease recurrence

Abstract

SUMMARY

Dysregulation of alternative splicing in prostate cancer is linked to transcriptional programs activated by AR, ERG, FOXA1, and MYC. Here, we show that FOXA1 functions as the primary orchestrator of alternative splicing dysregulation across 500 primary and metastatic prostate cancer transcriptomes. We demonstrate that FOXA1 binds to the regulatory regions of splicing-related genes, including *HNRNPK* and *SRSF1*. By controlling *trans*-acting factor expression, FOXA1 exploits an "exon definition" mechanism calibrating alternative splicing toward dominant isoform production. This regulation especially impacts splicing factors themselves and leads to a reduction of nonsense-mediated decay (NMD)-targeted isoforms. Inclusion of the NMD-determinant *FLNA* exon 30 by FOXA1-controlled oncogene SRSF1 promotes cell growth *in vitro* and predicts disease recurrence. Overall, we report a role for FOXA1 in rewiring the alternative splicing landscape in prostate cancer through a cascade of events from chromatin access, to splicing factor regulation, and, finally, to alternative splicing of exons influencing patient survival.

Discussion

1st paragraph: Overview of the study

In this study, by analysis of transcriptomics, protein-mRNA interactions, epigenomics, and chromosome conformation, we reveal that the pioneer TF FOXA1 orchestrates AS regulation in PC impacting on patient survival.

Discussion

2nd paragraph: Why is FOXA1 the predominant hallmark of SRGs dysregulation?

Collectively, our results indicate that *FOXA1* expression is a predominant hallmark of the transcriptional dysregulation of SRGs. As a pioneer factor, FOXA1 opens up nucleosomal domains for DNA binding by distinct TFs (Fei et al., 2019; Lupien et al., 2008). This pliant mechanism (Ramanand et al., 2020) may explain why FOXA1 hallmarks the global SRG dysregulation to a greater extent than the non-pioneer TFs, of which AR and MYC are documented to impact splicing regulation in PC (Phillips et al., 2020; Shah et al., 2020). Therefore, FOXA1 may open multiple channels to transmit transcriptional signals to SRG loci as exemplified by a common pioneer function for AR- and MYC-driven PC transcriptional programs (Barfeld et al., 2017).

Discussion

3rd paragraph: Mechanisms of FOXA1 regulation in alternative splicing events

By assessing AS changes in primary PC and cell lines, we demonstrate that FOXA1 calibrates the landscape of exon utilization toward an equilibrium that solidifies the production of dominant isoforms. This phenomenon is largely achieved by silencing lowly included exons in a consistent manner across tumors, but crucially also by enhancing highly included ones. Therefore, FOXA1 ultimately limits protein diversity toward isoforms that are functional for cells. We show that exons responding to FOXA1 are alternatively spliced by an "exon definition" mechanism, being shorter with longer flanking introns, strongly conserved across species, and, for a small fraction, marked by chromatin modifications (Agirre et al., 2021; Keren et al., 2010). A smaller exon size and higher intronic sequence conservation have been associated with a greater exon silencing, under evolutionary constraints, to control relative isoform frequencies (Baek and Green, 2005). By integrating analyses of *cis*-acting elements and *trans*-acting factors, we demonstrate that FOXA1 calibrates AS by enlisting splicing factors under its transcriptional control, including binding of PTBP1, U2AF2, and HNRNPC at 3′ ss (König et al., 2010; Sutandy et al., 2018; Xue et al., 2009), and HNRNPK at upstream intron-exon boundary and within downstream introns, respectively (Van Nostrand et al., 2020a, 2020b). It is fascinating that FOXA1 increases the inclusion of exons that are already highly included while reducing lowly included exons. This latter group indicates that FOXA1 is a genuine regulator of AS and not just an enhancer of splicing efficiency per se.

Main message

Discussion

4th paragraph: FOXA1-orchestrated auto-regulation of SRGs

It is likely significant that FOXA1-mediated AS preferentially impacts on SRGs themselves, suggesting that FOXA1 may be involved in a known regulatory feedback loop exploited by splicing factors to modulate their own protein expression levels (Lareau et al., 2007). Interestingly, our results indicate that high *FOXA1* expression in PC mostly inhibits the inclusion of NMD-determinant PTC-introducing "poison" exons. We hypothesize, therefore, that FOXA1-mediated AS restricts proteome diversity by influencing isoform degradation, particularly in SRGs. Recently, MYC has been implicated as a regulator of AS-coupled NMD in PC (Nasif et al., 2018; Pervouchine et al., 2019; Phillips et al., 2020). It is tempting to speculate that FOXA1, as a pliant regulator, may pioneer MYC to control transcription of specific SRGs and fine-tune AS in PC. Further functional studies are necessary to determine whether FOXA1 cooperates with specific TFs, chromatin modifiers, and RNA polymerase II, to rewire the AS landscape of PC.

Discussion

5th paragraph: Clinical impact

Clearly the systems-wide impact on AS mediated by FOXA1 is likely to have a profound effect on cancer severity. From a clinical perspective, we found that FOXA1 enhanced the inclusion of two NMD-determinant exons that are strong biomarkers of disease recurrence. Of these, we established a role for the FOXA1-enhanced PTC-preventing exon 30 in the cancer gene *FLNA* as a promoter of PC cell growth. We demonstrate that the inclusion of *FLNA* exon 30 is controlled primarily by SRSF1, which was the first proto-oncogenic splicing factor enacting some of the oncogenic functions of MYC (Das et al., 2012).

Discussion

6th paragraph: Summary of findings

1 In summary, we reveal a novel role for the pioneer TF FOXA1 in orchestrating AS regulation in PC at different stages of gene expression. By transcriptionally regulating *trans*-acting factors, 2 FOXA1 exploits an exon definition model to control relative isoform expression thereby fine-tuning proteome diversity. This splicing equilibrium favors the production of dominant isoforms, 3 especially including those that escape NMD. 4 FOXA1-mediated splicing regulation affects clinically relevant coding regions of the genome underlying PC patient survival. 5

Introduction

1st paragraph: Alternative splicing and its role in cancer

Pre-mRNA alternative splicing (AS) is a fundamental genetic process underpinning eukaryotic proteome diversity. AS is the selective inclusion of exons or introns into mature transcripts. Catalyzed by the macromolecular spliceosome complex comprising core spliceosomal factors, AS is finely regulated by auxiliary RNA-binding proteins (RBPs), which bind to sequence-specific nucleotide motifs to promote or repress a given splicing event (Cereda et al., 2014; Van Nostrand et al., 2020a). Genomic studies have also shown that somatic cells exploit RBP-mRNA interactions to promote tumor onset and progression (Pereira et al., 2017; Wang et al., 2018).

Introduction

2nd paragraph: The potential of targeting AS for novel cancer therapies

AS can be affected by somatic alterations leading to dysregulated expression of splicing-related genes (SRGs) (Sebestyén et al., 2016; Seiler et al., 2018). These alterations have uncovered novel cancer therapeutic targets (Lee and Abdel-Wahab, 2016). Small-molecule compounds targeting RBP-mRNA perturbations have entered clinical trials (Bonnal et al., 2020). For instance, pladienolide B derivatives inhibiting the SF3b splicing commitment complex have efficacy for blood and solid cancers (Zhang et al., 2020; Zhou et al., 2020). Similarly, antisense decoy oligonucleotides targeting RBPs have proven effective in preventing the activation of RBP-driven oncogenic programs (Denichenko et al., 2019). Finally, dysregulated AS has the potential to generate neo-epitopes to a greater extent than point mutations, thus potentially expanding the indications for immunotherapies (Frankiw et al., 2019; Kahles et al., 2018).

Introduction

3rd paragraph: Prostate cancer

The commonest cause of male-specific cancer death is prostate cancer (PC) (Rebello et al., 2021). Despite advances in the diagnosis and treatment of early disease, there are few therapeutic options for end-stage metastatic castration-resistant PC (mCRPC) (Rebello et al., 2021). The disease is difficult to tackle in part due to considerable phenotypic heterogeneity, underpinned by genomic alterations within different oncogenes or tumor suppressors. These impact on transcriptional and translational programs that are fundamental for the cell in complex ways (Rebello et al., 2021).

Introduction

4th paragraph: Aberrant AS in prostate cancer

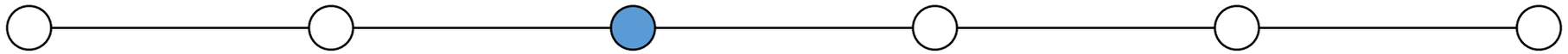
Interestingly, aberrant splicing can contribute to the heterogeneous phenotypes of PC (Paschalis et al., 2018; Rajan et al., 2009). The dysregulation of this mechanism increases with disease aggressiveness toward metastatic disease, with most SRGs being transcriptionally dysregulated throughout PC progression (Zhang et al., 2020). Consequently, the AS landscape fingerprints the spectrum of PC disease states, with many aberrant events associated with oncogenic signals driven by transcription factors (TFs), such as MYC and AR (Phillips et al., 2020; Shah et al., 2020). Consistently, novel therapeutic targeting of highly expressed SRGs (specifically members of the SF3 splicing commitment complex) has been shown to have antiproliferative effects in PC models (Kawamura et al., 2019; Zhang et al., 2020).

Introduction

5th paragraph: Role of TFs - AR, ERG, FOXA1, MYC - in prostate cancer

In the heterogeneous genetic landscape of PC, the only recurrent activating alterations occur within key oncogenic TFs: *AR*, *ERG*, *FOXA1*, and *MYC* (Rebello et al., 2021). Ligand-dependent activation of AR controls a tumorigenic cistrome of androgen-sensitive genes (Pomerantz et al., 2015). FOXA1 is a pioneer TF that reprograms the AR cistrome to drive PC initiation and progression to metastasis (Parolia et al., 2019). In the aggressive neuroendocrine PC (NEPC) subtype, where AR transcription is absent, FOXA1 is essential for proliferation (Baca et al., 2021). Similarly, overexpression of ERG redirects AR and FOXA1 binding to drive invasive PC, illustrating the cooperation between these TFs (Chen et al., 2013; Kron et al., 2017). Finally, aggressive PC is characterized by amplification of *MYC*, which is the most frequent genomic alteration in NEPCs (Rebello et al., 2021). MYC antagonizes AR transcriptional programs pioneered by FOXA1, underscoring the interdependence of PC on this handful of TFs (Hawksworth et al., 2010; Qiu et al., 2021).

Introduction



6th paragraph: Role of TFs in AS dysregulation in prostate cancer

Of these four TFs, all but FOXA1, have each been implicated in controlling splicing outcomes in PC by modulating SRG expression or influencing inclusion levels of functionally relevant exons (Phillips et al., 2020; Saulnier et al., 2021; Shah et al., 2020).

What has been done

These studies highlight the involvement of distinct TFs in the dysregulation of AS during PC progression. Nevertheless, in the context of PC transcriptional reprogramming cooperatively driven by these TFs, the magnitude of influence exerted by each individual TF to aberrant AS remains to be elucidated.

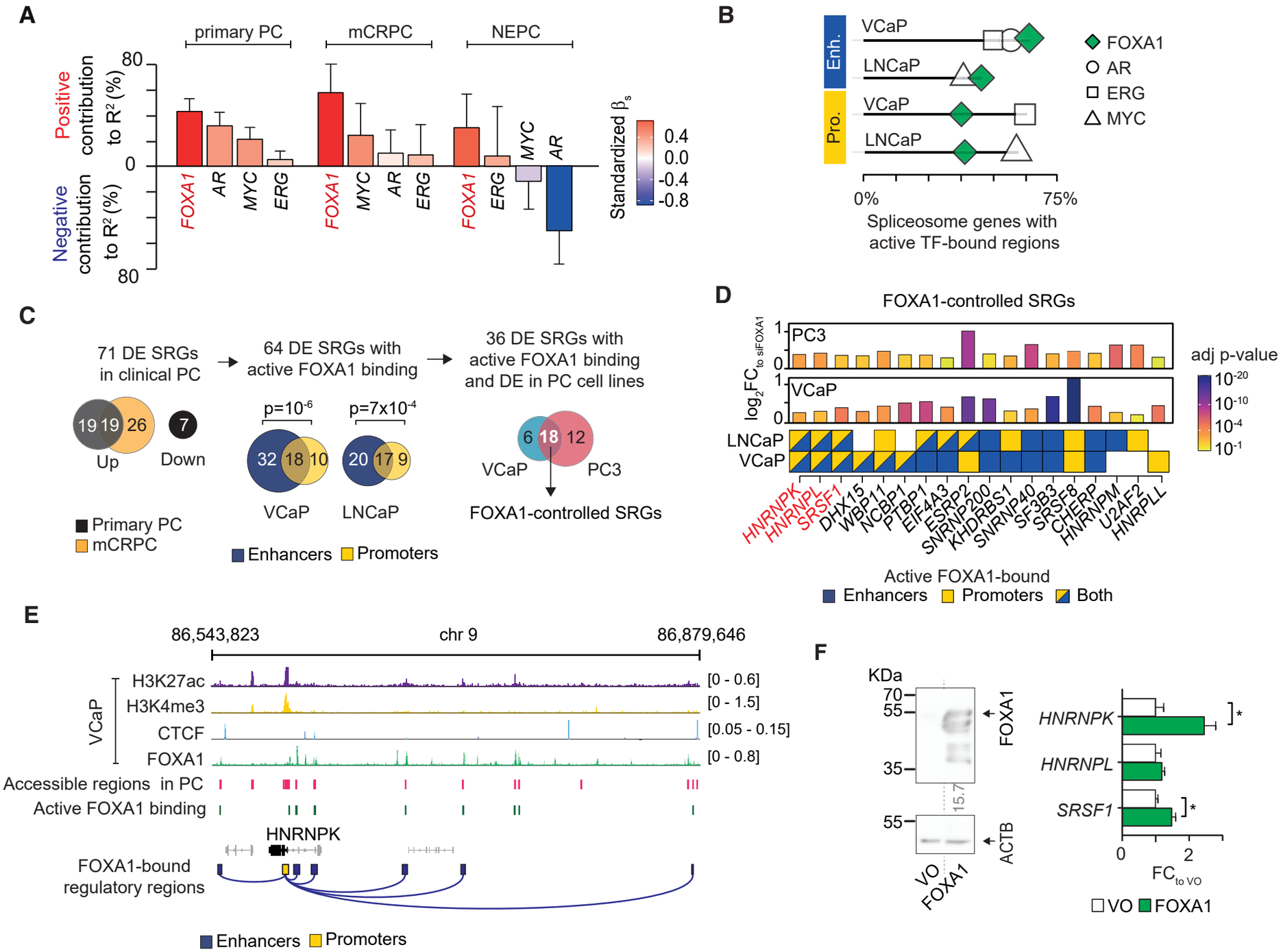
What is missing

Here, we systematically assess the impact of the four TFs on AS in primary PC and mCRPC patients.

Contribution of the paper

Results

Figure 1. FOXA1 transcriptionally controls splicing-related genes in PC



Results

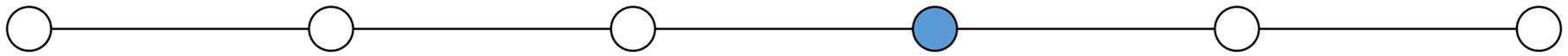
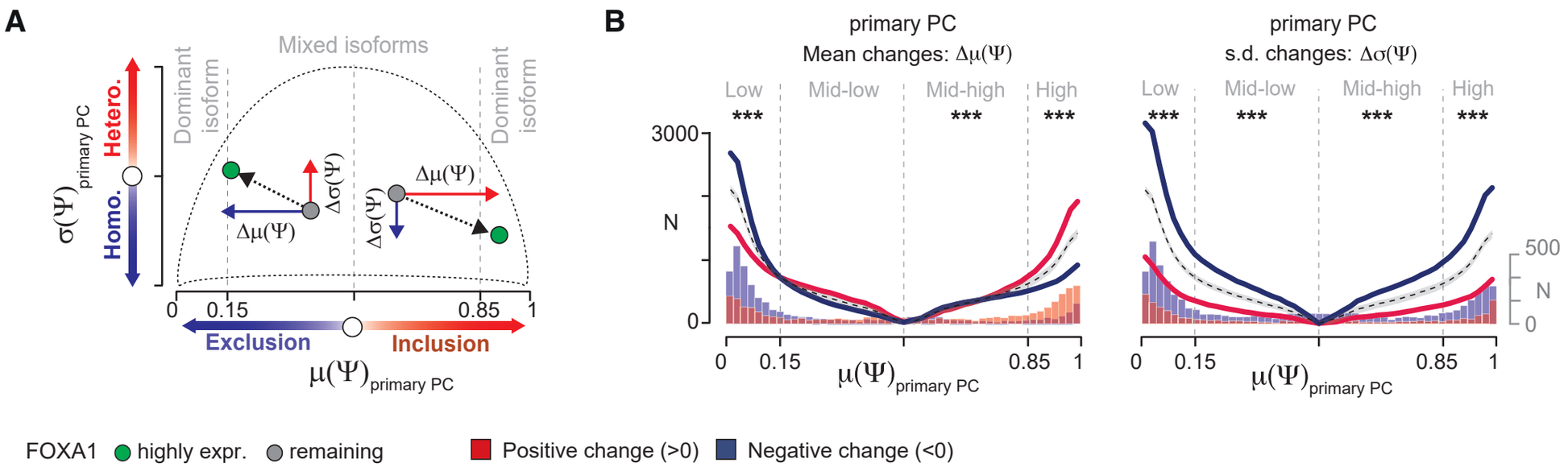
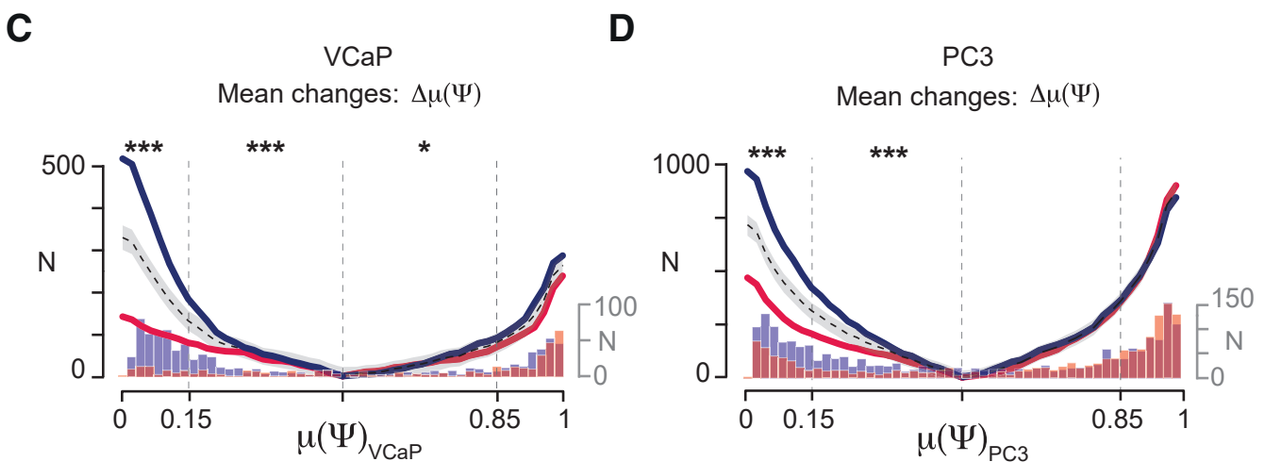
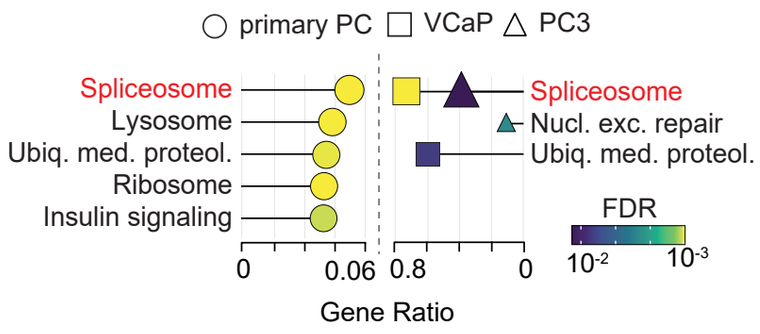


Figure 2. FOXA1 calibrates the alternative splicing equilibrium of PC by enhancing the production of dominant isoforms

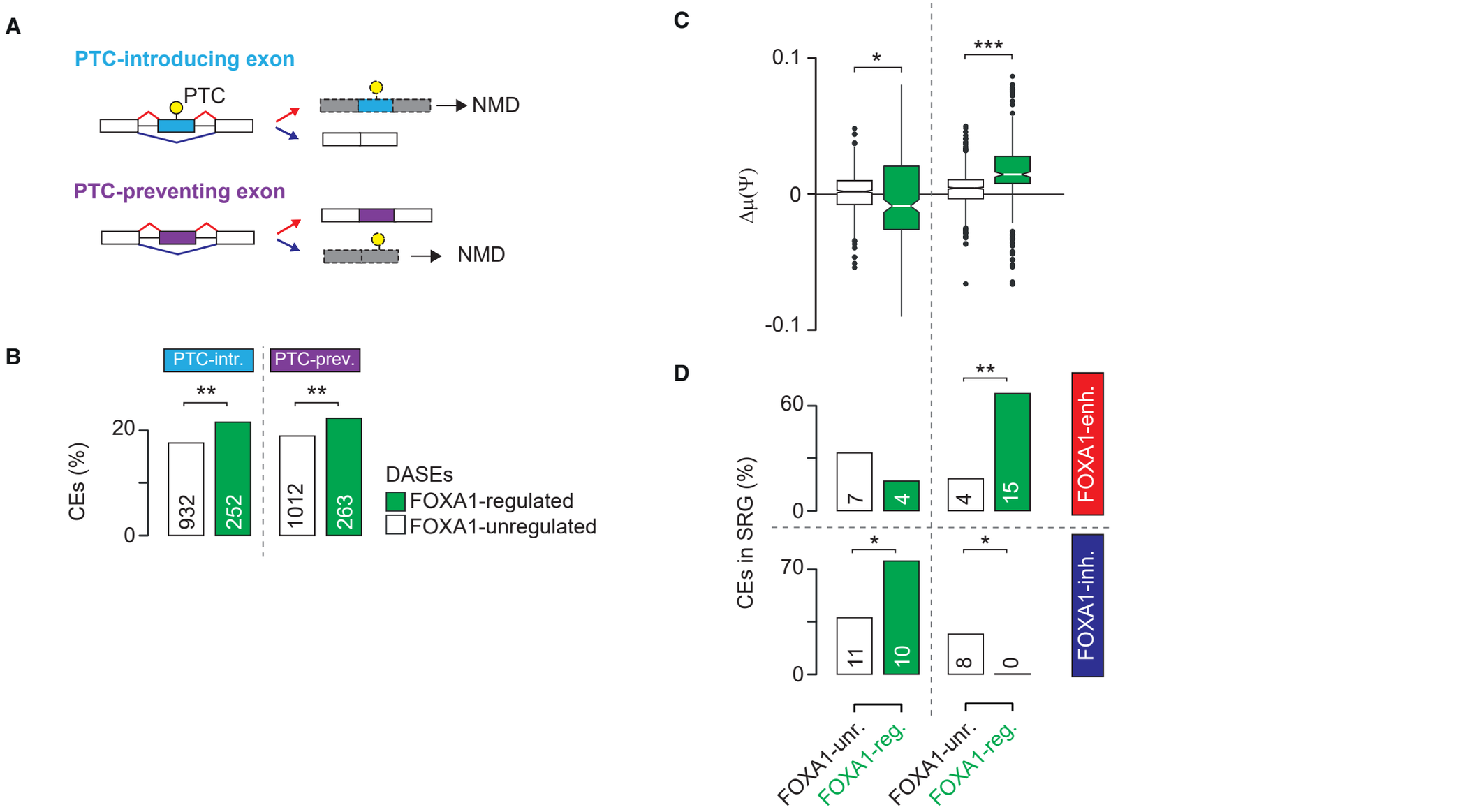






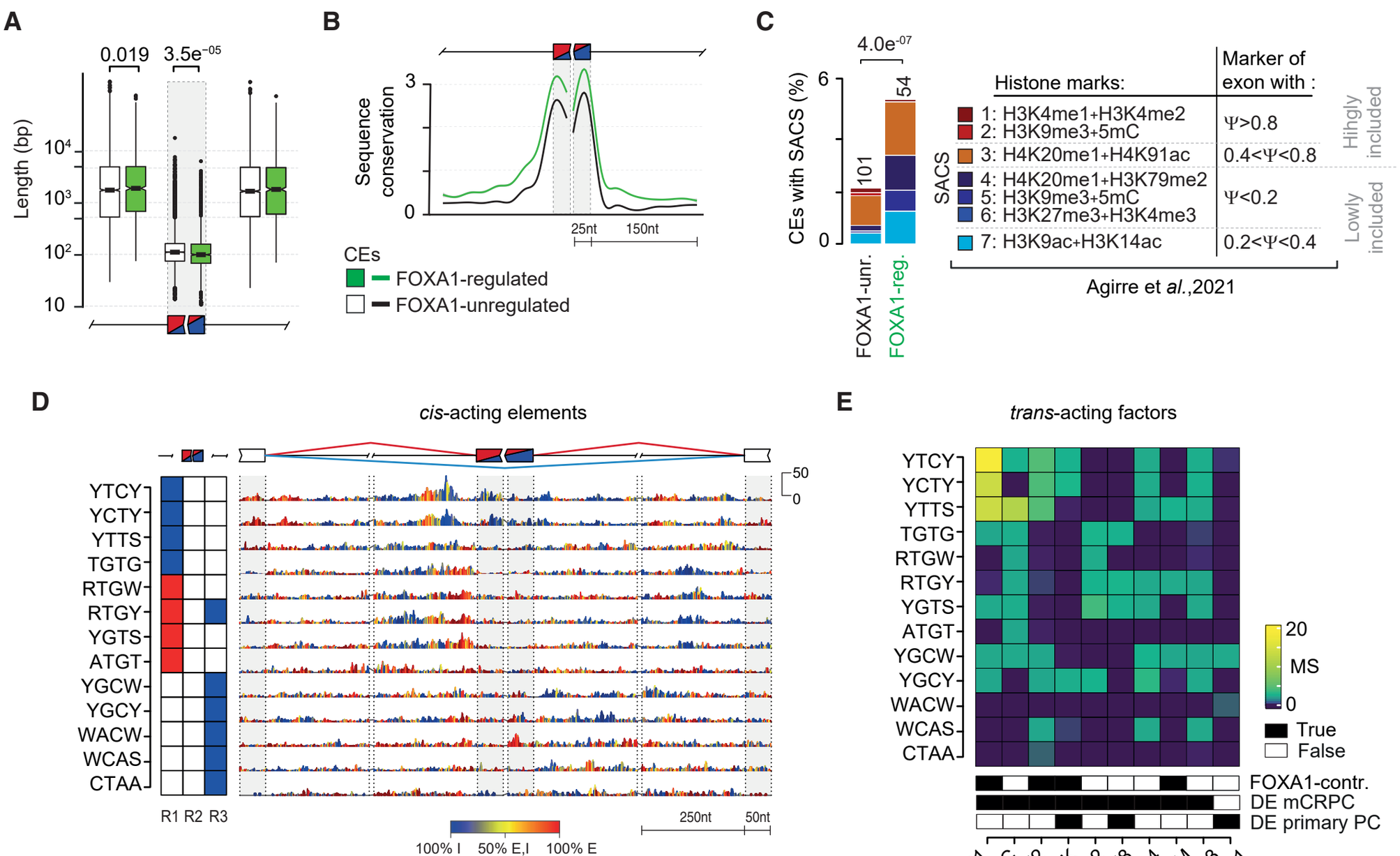
Results

Figure 3. FOXA1 controls nonsense-mediated decay determinant exons



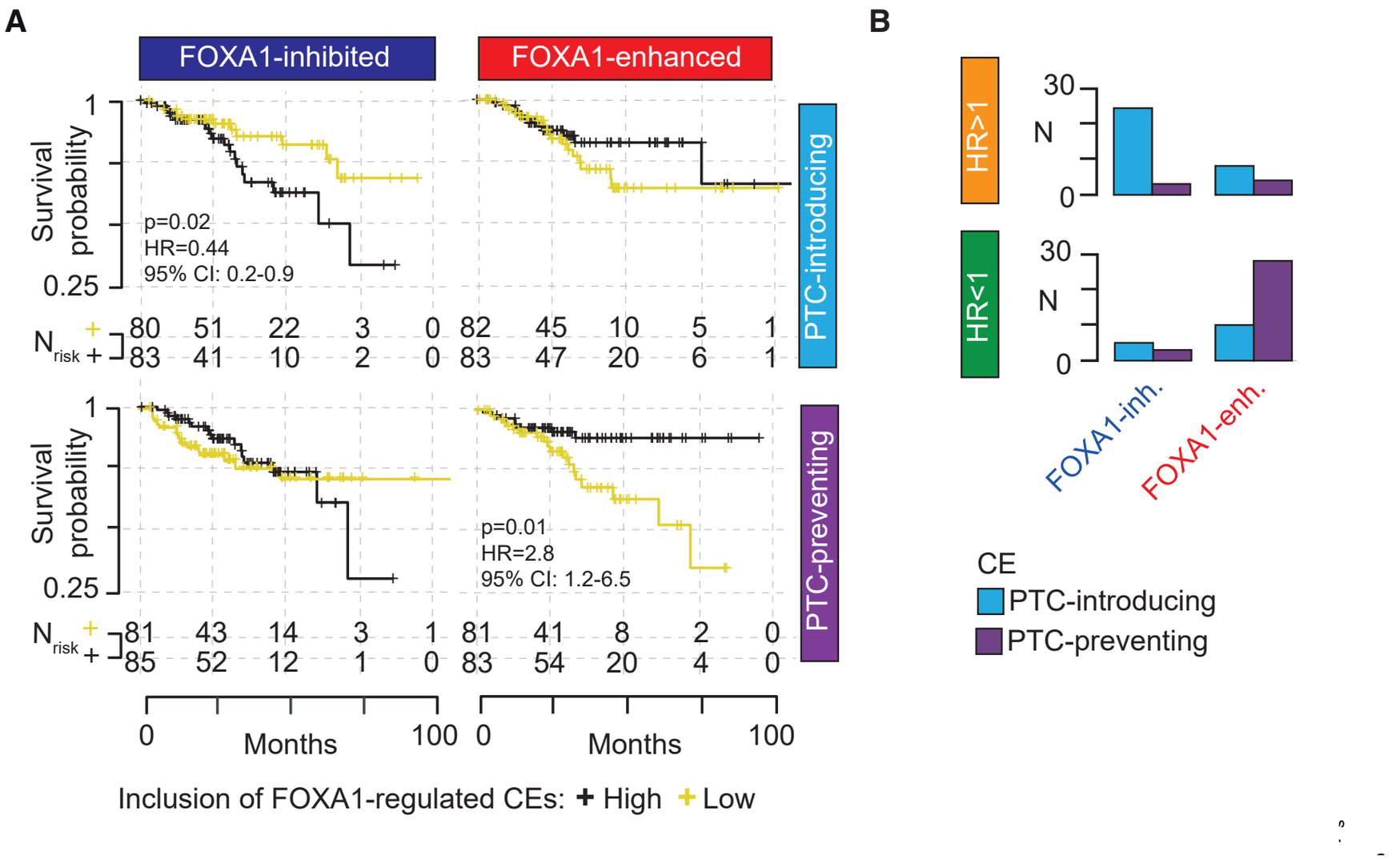
Results

Figure 4. FOXA1 mediates exon silencing by controlling *trans*-acting factors within an exon definition mechanism



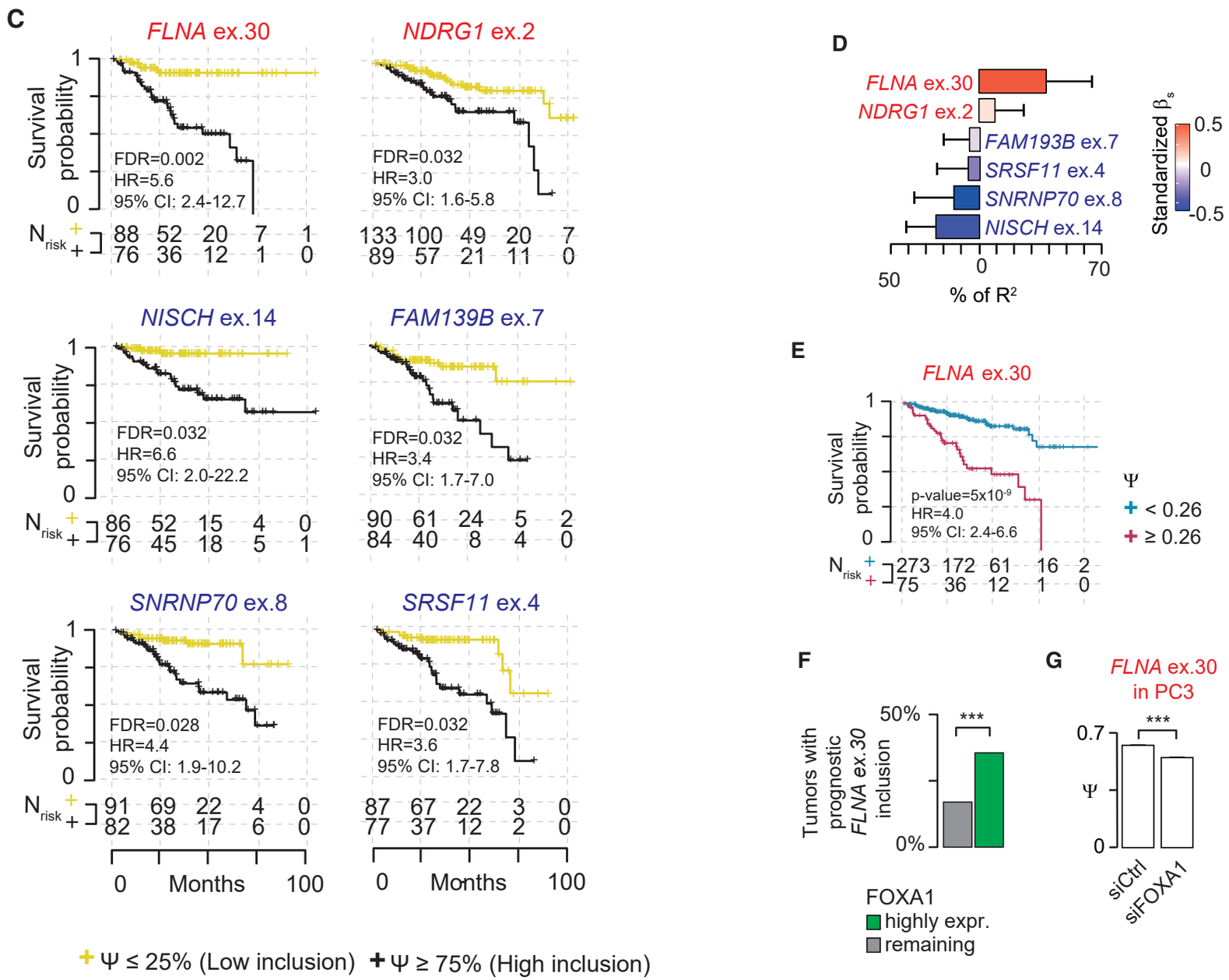
Results

Figure 5. FOXA1-regulated NMD-determinant exons predict PC patient prognosis



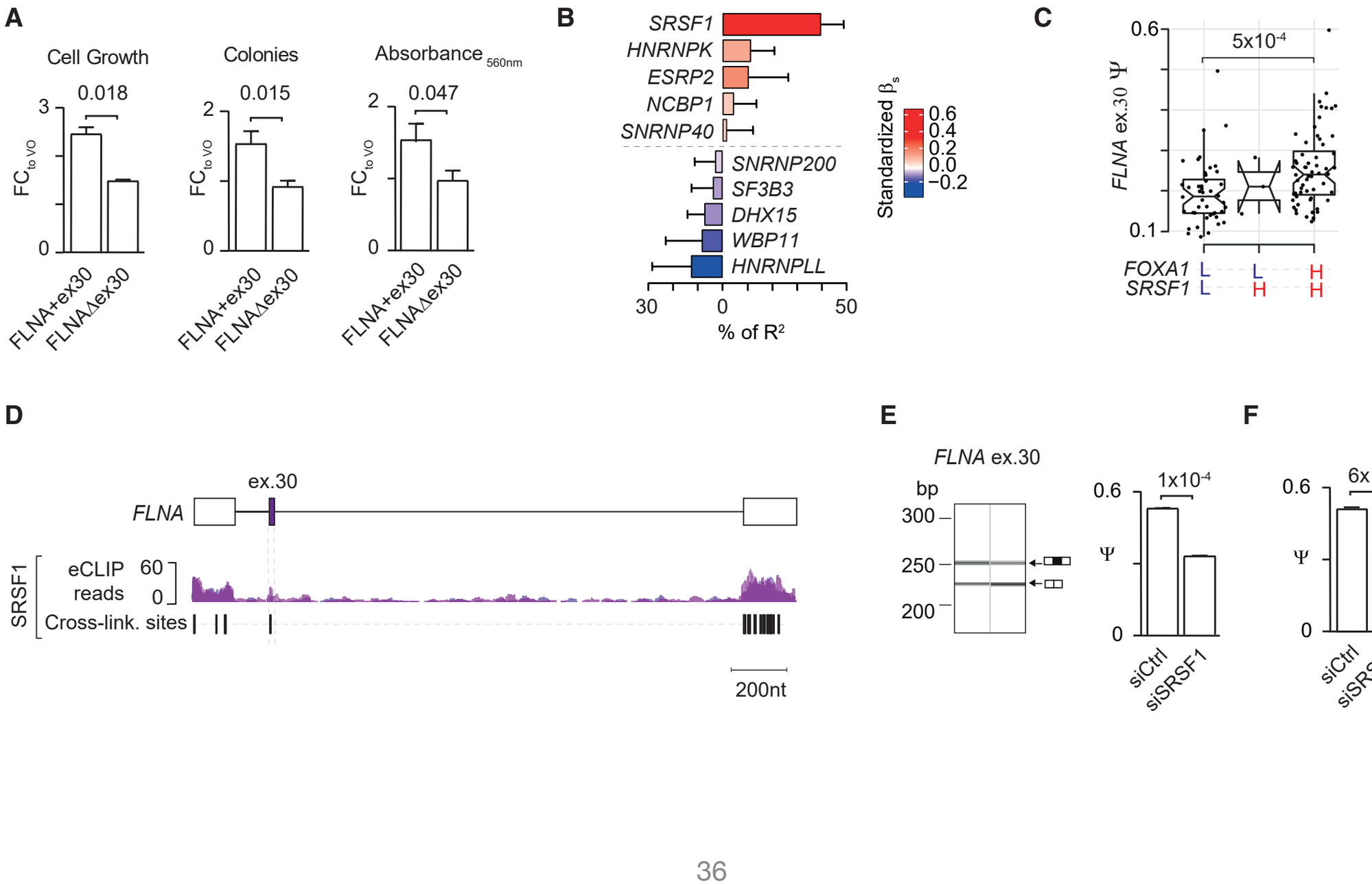
Results

Figure 5. FOXA1-regulated NMD-determinant exons predict PC patient prognosis



Results

Figure 6. *FLNA* exon 30 inclusion promotes PC cell growth and is controlled by SRSF1



METHOD DETAILS

RNA-seq patient datasets

RNA sequencing (RNA-seq) data were obtained from The Cancer Genome Atlas (TCGA) Data Matrix portal (Level 3, https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm) and cBioPortal (Beltran et al., 2016; Cerami et al., 2012; Chen et al., 2013) websites for 409 primary PCs, 118 mCRPCs and 15 NEPCs. The number of transcripts per million reads was measured starting from the scaled estimate expression values provided for 20,531 genes (Cereda et al., 2016). For the metastatic castration-resistant PC dataset, reads per kilobase of transcript per million mapped reads values were converted into transcripts per million. For each transcription factor, the distribution of expression levels across samples was measured. A transcription factor was considered as highly expressed if its transcripts per million value was $\geq 75^{th}$ percentile of its expression distribution across samples (Cereda et al., 2016) (Table S1).

Selection of splicing-related genes

A list of 128 genes in the Kyoto Encyclopedia of Genes and Genomes (KEGG) 'spliceosome' pathway was collected from MSigDb version 5 (Subramanian et al., 2005). An additional list of 66 RNA-binding proteins was obtained from the RNAcompete catalogue (Ray et al., 2013) and added to the 128 spliceosome genes. A final set of 148 genes with gene ontology terms related to splicing was retained for further analyses as splicing-related genes.

Multivariable covariance analysis

Relative contributions of expression, or inclusion, levels of multiple factors (*e.g.* genes, exons), namely regressors, to the correlation with a response variable (*e.g.* cumulative expression of splicing factors, FOXA1 expression) were measured using the following approach. Normalized expression, or inclusion levels, of regressors were normalized using a near-zero variance filter, Yeo-Johnson transformation, centering around their mean, and scaling by their standard deviation using the *preProcess* function in the R 'caret' package with parameters *method = c("center", "scale", "YeoJohnson", "nzv")*. A generalized linear regression model (GLM) was fitted to the response variable based on the normalized values of regressors using the *glm* function in the R 'stats' package. Relative importance of each regressor to the correlation measured by the model was calculated using the function *calc.relimp* in the R 'relaimpo' package (Grömping, 2006). This function divides the coefficient of determination R^2 into the contribution of each regressor using the averaging over orderings method (Lindeman, 1980). Confidence intervals were measured using a bootstrap procedure implemented in the function *boot.relimp*. For 1,000 iterations the full observation vectors were resampled and the regressor contributions were calculated.

Limitations

1st paragraph

1 Our characterization of AS regulation in PC is limited to the contribution of four key oncogenic TFs with recurrent activating alterations across PC patients. In light of a long tail of oncogenic drivers underpinning a heterogeneous disease, we cannot exclude the influence of other transcriptional regulators. The

2 analysis of FOXA1-mediated AS regulation was limited to primary PCs as splicing data for mCRPCs were not available. Although we recapitulated our results on metastatic PC cells, the generalizability of our findings to other clinical PC disease states remains to be elucidated.

Limitations

2nd paragraph

Our work is based on novel computational analyses that provide unique insights into AS regulation by FOXA1, including the involvement of candidate SRGs and, to a minor extent, chromatin regulators. However, the mechanistic details as to how FOXA1 modulates SRG expression, cooperates with epi-transcriptional regulators, and affects AS decisions remain questions to address in future studies. Although we highlighted candidate prognostic AS events that could be exploited as biomarkers and therapeutic targets, further studies are required to determine their value in the context of FOXA1. Furthermore, a lack of preclinical phenotyping in our study limits the immediate clinical translation of our findings.

3

4

5

Limitations

3rd paragraph

6 A potential confounder in the analysis of PC transcriptomes from bulk sequencing experiments is the contamination in low purity samples arising from benign prostatic epithelial, stromal, or immune cells. However, we performed computational validations showing that FOXA1 orchestrates AS regulation regardless of purity constraints (Figure S6; STAR Methods).