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Supplementary Materials for

SRSF6 modulates histone-chaperone HIRA splicing to orchestrate AR and E2F activity in prostate cancer

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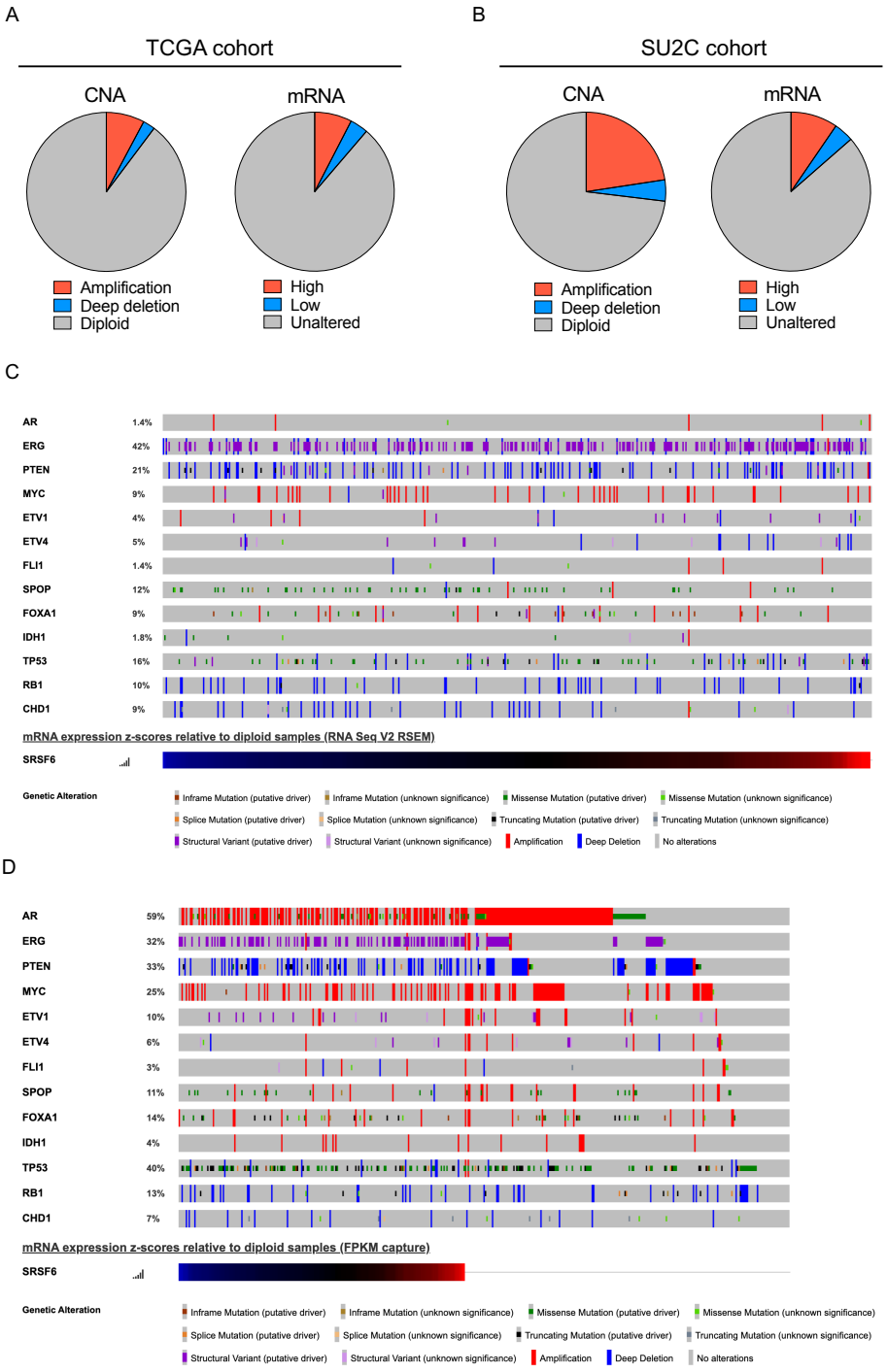
Legends for data S1 to S4

Other Supplementary Material for this manuscript includes the following:

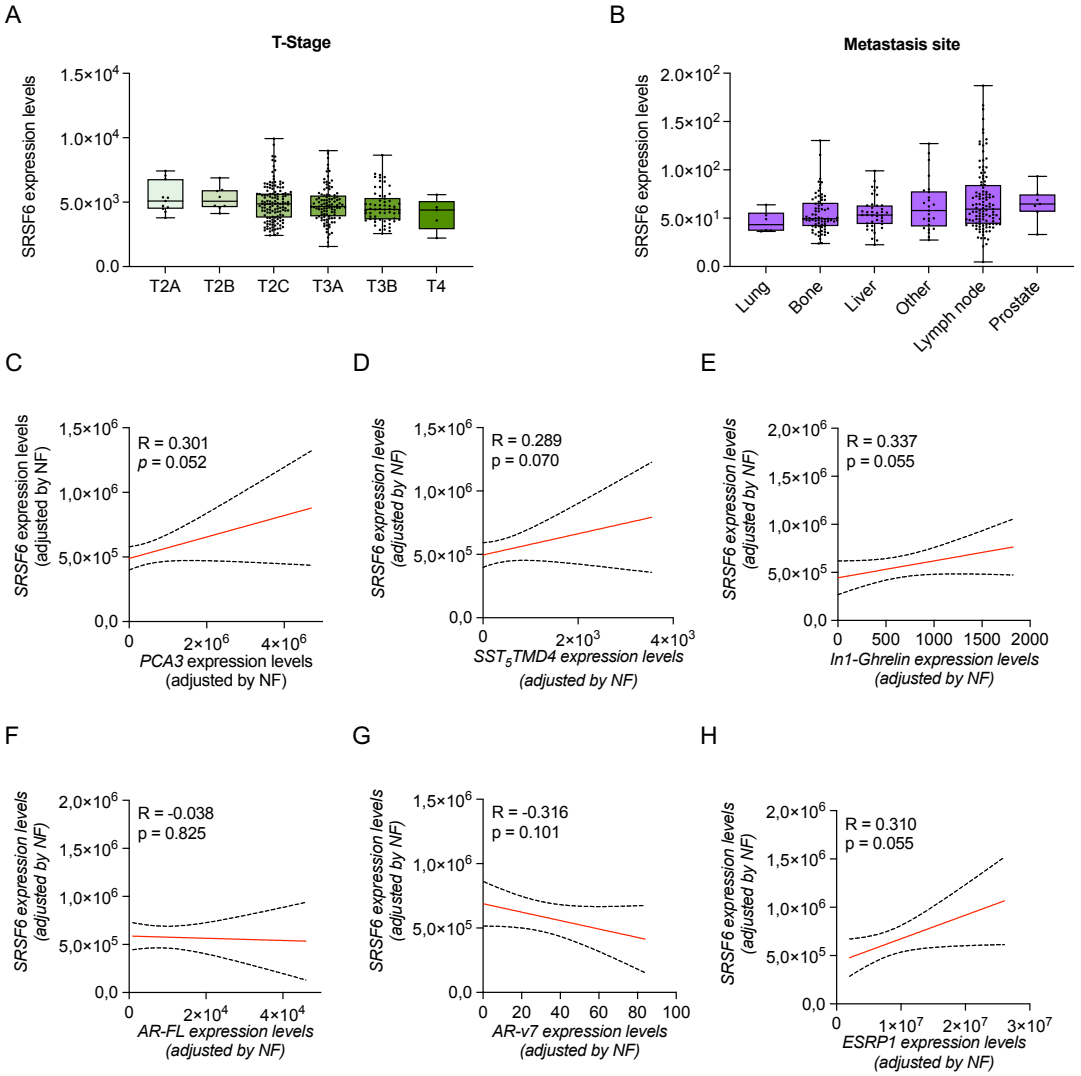
Data S1 to S4

Fig. S1.



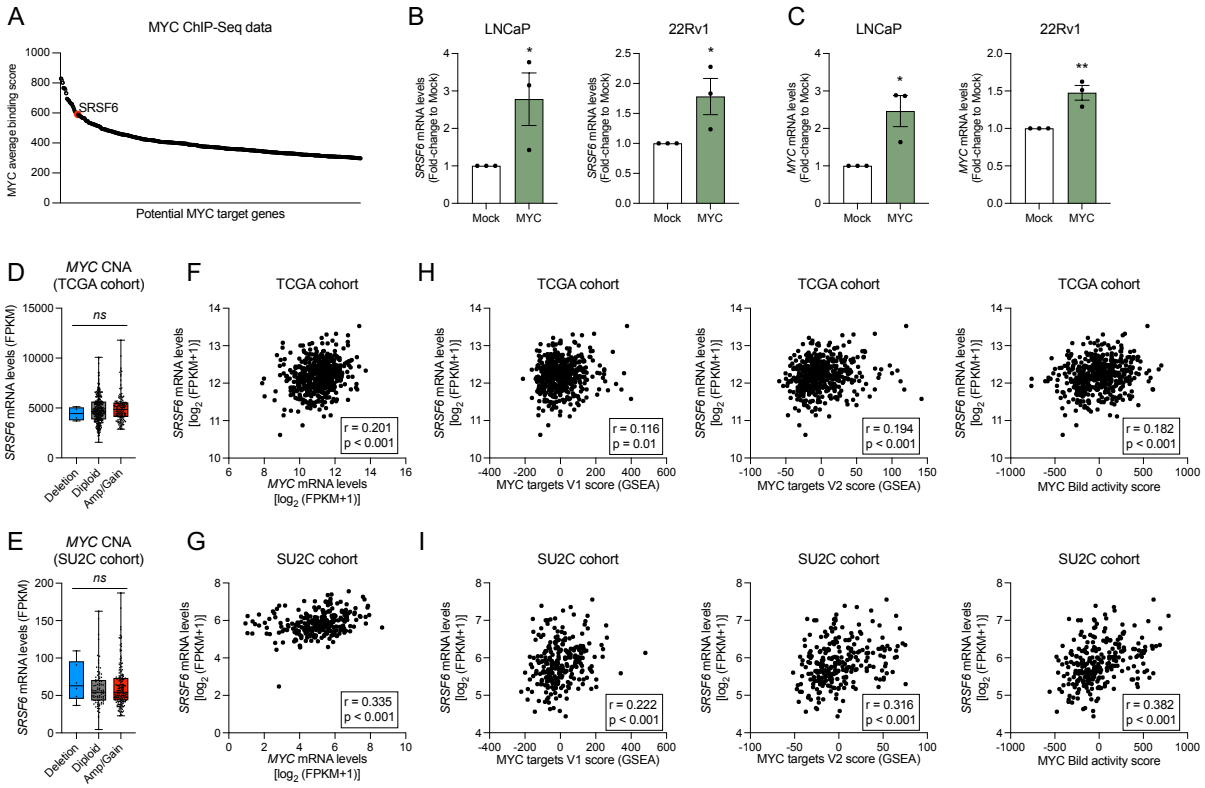
***SRSF6* genomic alterations in PCa.** (A-B) Percentage of patients with *SRSF6* copy number alteration (CNA; left panels) and mRNA alterations [samples with high and low expression of *SRSF6* were defined as those with two standard deviations away from the mean of expression of all profiled samples (Z-score ±2, respectively); right panels] from TCGA (A) and SU2C (B) cohort. (C-D) Association between *SRSF6* mRNA levels and common genetic alterations of PCa in TCGA cohort (C) and SU2C (D) cohort.

Fig. S2.



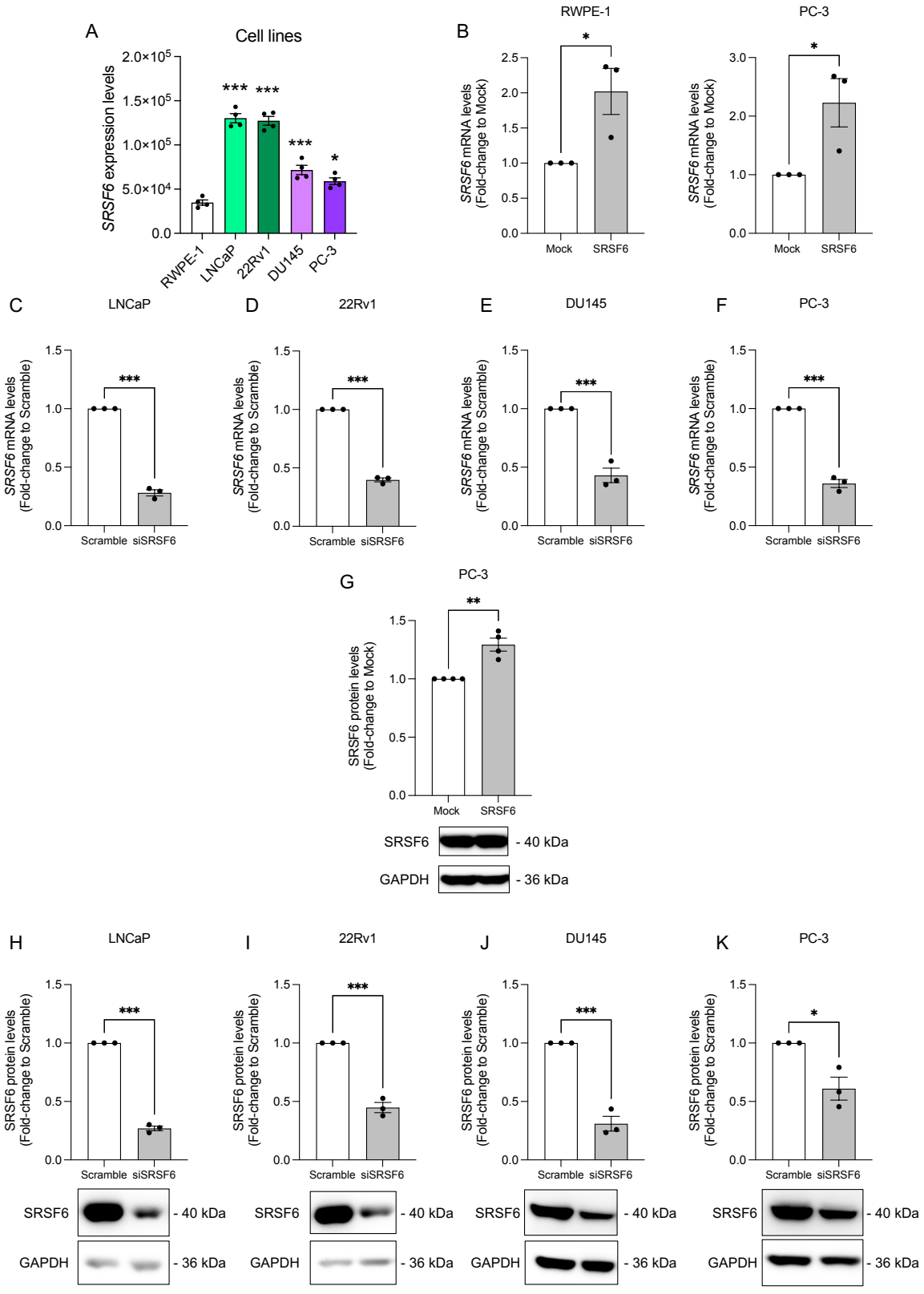
Associations and correlations of *SRSF6* expression levels with clinical and molecular parameters of PCa aggressiveness. (A) Association between *SRSF6* mRNA levels and T-Stage in TCGA cohort. Data are represented as min to max boxplot, with median. (B) Association between *SRSF6* mRNA levels and metastasis site in SU2C cohort. Data are represented as min to max boxplot, with median. (C-H) Correlation between *SRSF6* mRNA levels and *PCA3* (C), *SST5TMD4* (D), *In1-Ghrelin* (E), *AR-FL* (F), *AR-v7* (G), and *ESRP1* (H) expression levels in biopsies cohort. mRNA levels are adjusted by normalization factor (calculated from *ACTB* and *GAPDH* expression levels). Correlations data are represented by mean (connecting line) and error bands (pointed line).

Fig. S3.



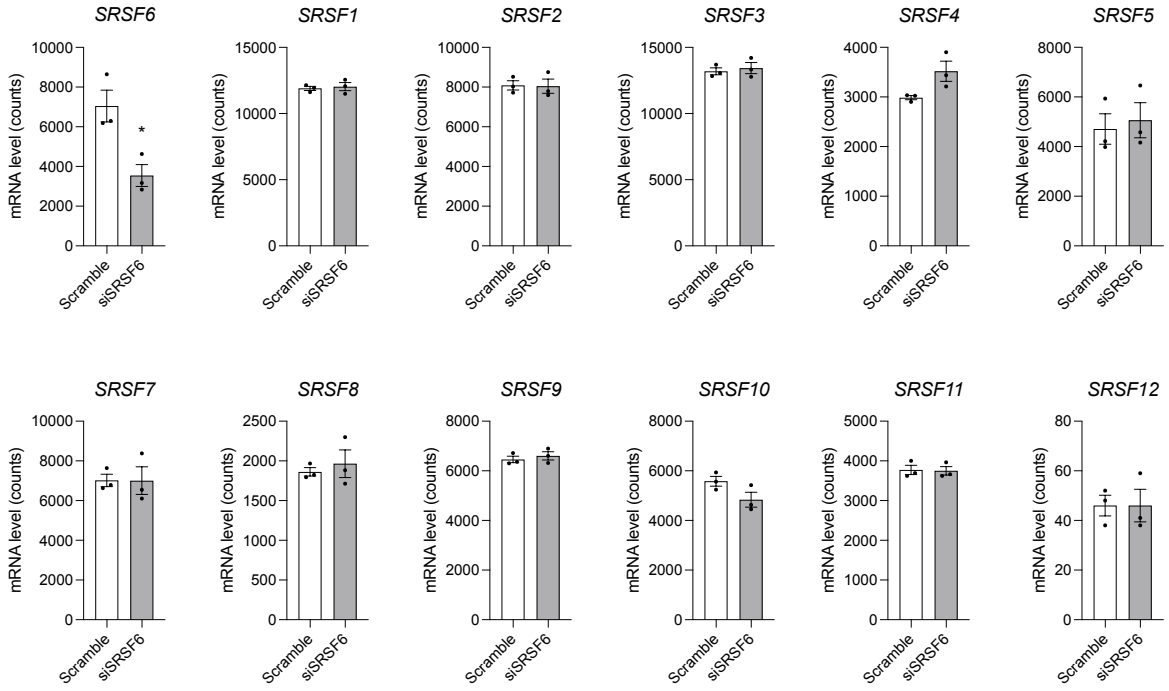
Association between MYC pathway and *SRSF6* expression levels. (A) Top-scoring potential MYC target genes among different cell lines. MYC ChIP-Seq data derived from ChIP-Atlas database by filtering binding regions ±1 kb from transcription starting site. (B-C) Expression of *SRSF6* (B) and *MYC* (C) in response to *MYC* overexpression in LNCaP and 22Rv1 cells. (D-E) Association between *SRSF6* mRNA levels and copy-number alteration (CNA) of the *MYC* gene in TCGA (D) and SU2C (E) cohorts. (F-G) Correlation between *MYC* and *SRSF6* mRNA levels in TCGA (F) and SU2C (G) cohorts. (H-I) Correlation between MYC activity scores and *SRSF6* mRNA levels in TCGA (H) and SU2C (I) cohorts. Asterisks (*p<0.05; **p<0.01) indicate statistically significant differences between groups.

Fig. S4.


A
Cell lines
SRSF6 expression levels
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0.0
***
***
***
*
RWPE-1
LNCaP
22Rv1
DU145
PC-3
B
RWPE-1
*
SRSF6 mRNA levels (Fold-change to Mock)
Mock
SRSF6
PC-3
*
SRSF6 mRNA levels (Fold-change to Mock)
Mock
SRSF6
C
LNCaP
***
SRSF6 mRNA levels (Fold-change to Scramble)
Scramble
siSRSF6
D
22Rv1
***
SRSF6 mRNA levels (Fold-change to Scramble)
Scramble
siSRSF6
E
DU145
***
SRSF6 mRNA levels (Fold-change to Scramble)
Scramble
siSRSF6
F
PC-3
***
SRSF6 mRNA levels (Fold-change to Scramble)
Scramble
siSRSF6
G
PC-3
**
SRSF6 protein levels (Fold-change to Mock)
Mock
SRSF6
SRSF6
- 40 kDa
GAPDH
- 36 kDa
H
LNCaP
***
SRSF6 protein levels (Fold-change to Scramble)
Scramble
siSRSF6
SRSF6
- 40 kDa
GAPDH
- 36 kDa
I
22Rv1
***
SRSF6 protein levels (Fold-change to Scramble)
Scramble
siSRSF6
SRSF6
- 40 kDa
GAPDH
- 36 kDa
J
DU145
***
SRSF6 protein levels (Fold-change to Scramble)
Scramble
siSRSF6
SRSF6
- 40 kDa
GAPDH
- 36 kDa
K
PC-3
*
SRSF6 protein levels (Fold-change to Scramble)
Scramble
siSRSF6
SRSF6
- 40 kDa
GAPDH
- 36 kDa

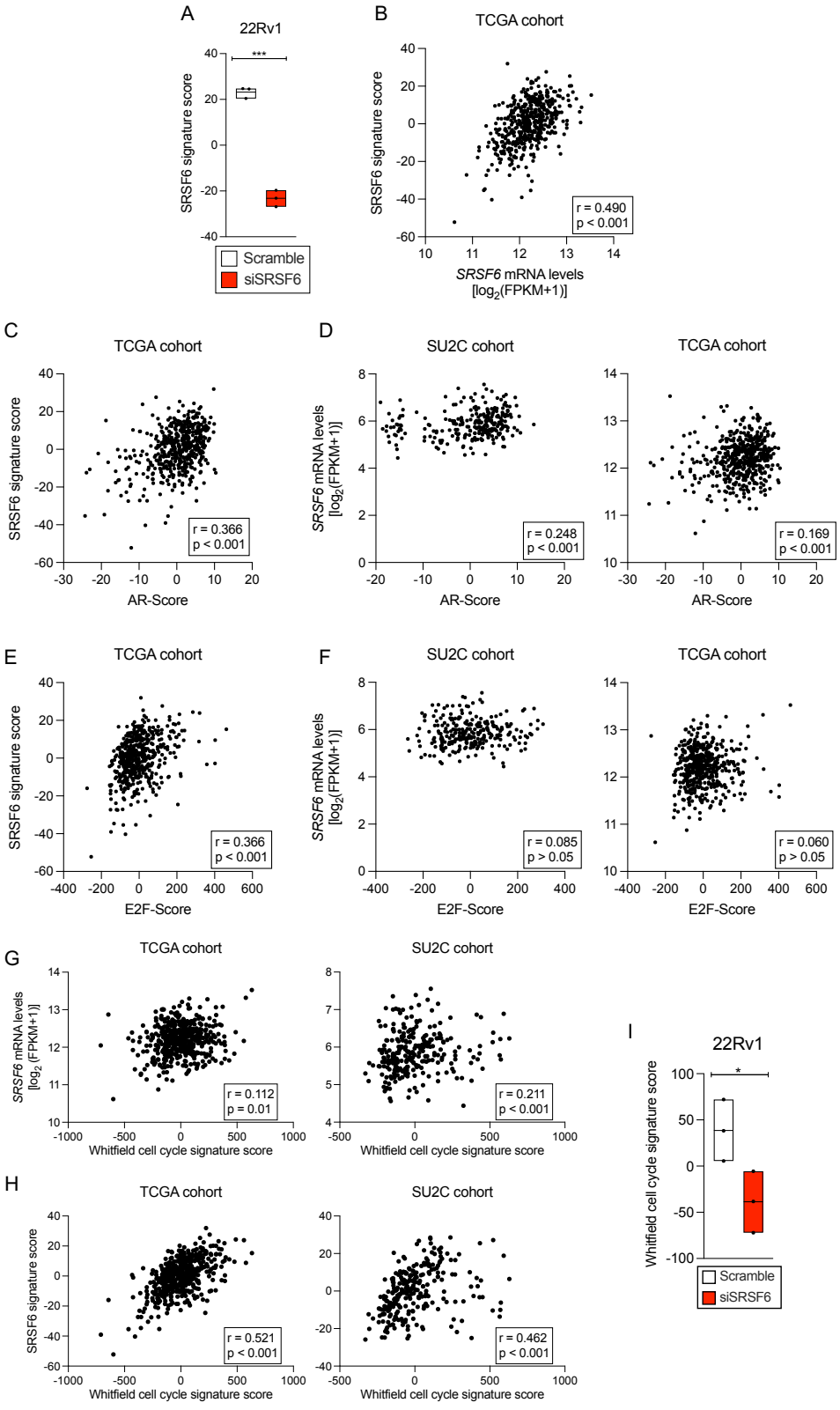
***SRSF6* mRNA levels and validation of SRSF6 modulation in human prostate-derived cell lines.** (A) Comparison of *SRSF6* expression levels between a non-tumor prostate cell line (RWPE-1) and PCa cell lines LNCaP, 22Rv1, DU145 and PC-3 (n=3). Data represent the mean ± SEM of *SRSF6* expression levels determined by qPCR and adjusted by normalization factor calculated from *ACTB* and *GAPDH* expression levels. (B) Validation of *SRSF6* mRNA overexpression in RWPE-1 (left panel) and PC-3 cells (right panel). (C-F) Validation of *SRSF6* mRNA silencing in LNCaP (C), 22Rv1 (D), DU145 (E), and PC-3 (F) cells. *SRSF6* mRNA levels were determined by qPCR and are adjusted by *ACTB* expression levels. (G) Validation of SRSF6 protein overexpression in PC-3 cells. (H-K) Validation of SRSF6 protein silencing in LNCaP (H), 22Rv1 (I), DU145 (J), and PC-3 (K) cells. SRSF6 protein levels were determined by Western blot and adjusted by GAPDH. Data are represented as fold-change of control (RWPE-1, mock, or scramble) cells (mean ± SEM). Representative images of Western blot are depicted on bottom panels. Asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.001$) indicate statistically significant differences between groups.

Fig. S5.



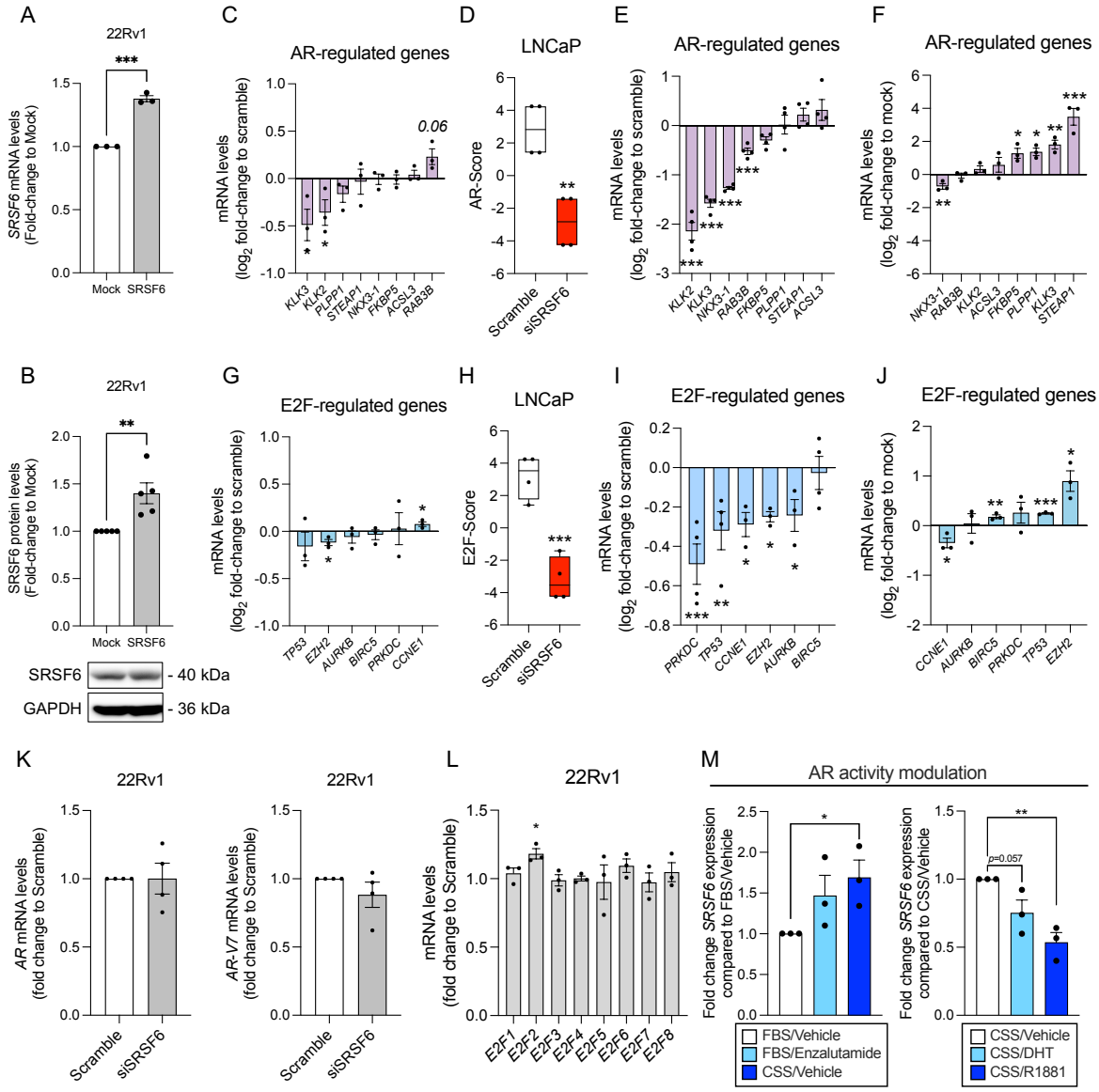
Expression of SR-proteins in response to the modulation of *SRSF6*. Expression of genes encoding SR-proteins in response to SRSF6 silencing in 22Rv1 cells. Data are normalized by control cells (mean ± SEM). Asterisks (*p<0.05) indicate statistically significant differences between groups.

Fig. S6.


A
22Rv1
***
SRSF6 signature score
Scramble
siSRSF6
B
TCGA cohort
SRSF6 signature score
r = 0.490
p < 0.001
SRSF6 mRNA levels
[log2(FPKM+1)]
C
TCGA cohort
SRSF6 signature score
r = 0.366
p < 0.001
AR-Score
D
SU2C cohort
SRSF6 mRNA levels
[log2(FPKM+1)]
r = 0.248
p < 0.001
AR-Score
TCGA cohort
r = 0.169
p < 0.001
AR-Score
E
TCGA cohort
SRSF6 signature score
r = 0.366
p < 0.001
E2F-Score
F
SU2C cohort
SRSF6 mRNA levels
[log2(FPKM+1)]
r = 0.085
p > 0.05
E2F-Score
TCGA cohort
r = 0.060
p > 0.05
E2F-Score
G
TCGA cohort
SRSF6 mRNA levels
[log2 (FPKM+1)]
r = 0.112
p = 0.01
Whitfield cell cycle signature score
SU2C cohort
r = 0.211
p < 0.001
Whitfield cell cycle signature score
H
TCGA cohort
SRSF6 signature score
r = 0.521
p < 0.001
Whitfield cell cycle signature score
SU2C cohort
r = 0.462
p < 0.001
Whitfield cell cycle signature score
I
22Rv1
*
Whitfield cell cycle signature score
Scramble
siSRSF6

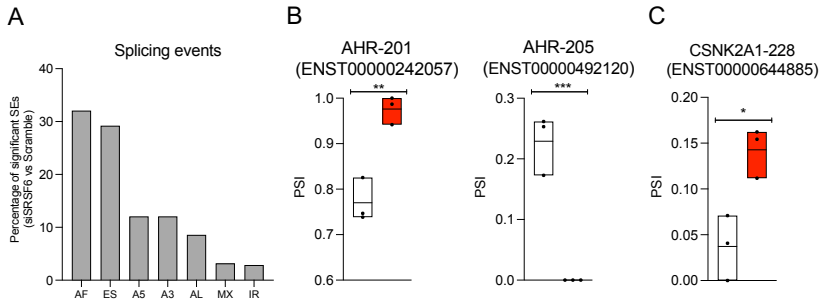
Association of SRSF6 with AR, E2F, and proliferation-related molecular signatures. (A) Effect of SRSF6 silencing on SRSF6 activity (SRSF6 signature score) in 22Rv1 cells. (B) Correlation between *SRSF6* mRNA and SRSF6 signature score in TCGA cohort. (C) Correlation between SRSF6 activity and AR-Score in TCGA cohort. (D) Correlation between *SRSF6* mRNA levels and AR-Score in SU2C and TCGA cohort. (E) Correlation between SRSF6 activity and E2F-Score in TCGA cohort. (F) Correlation between *SRSF6* mRNA levels and E2F-Score in SU2C and TCGA cohort. (G-H) Correlation of *SRSF6* mRNA levels (G) and activity (H) with Whitfield cell cycle signature score in TCGA and SU2C cohort. (I) Effect of *SRSF6* silencing on Whitfield cell cycle signature score in 22Rv1 cells. Asterisks (*$p<0.05$; ***$p<0.001$) indicate statistically significant differences between groups.

Fig. S7.



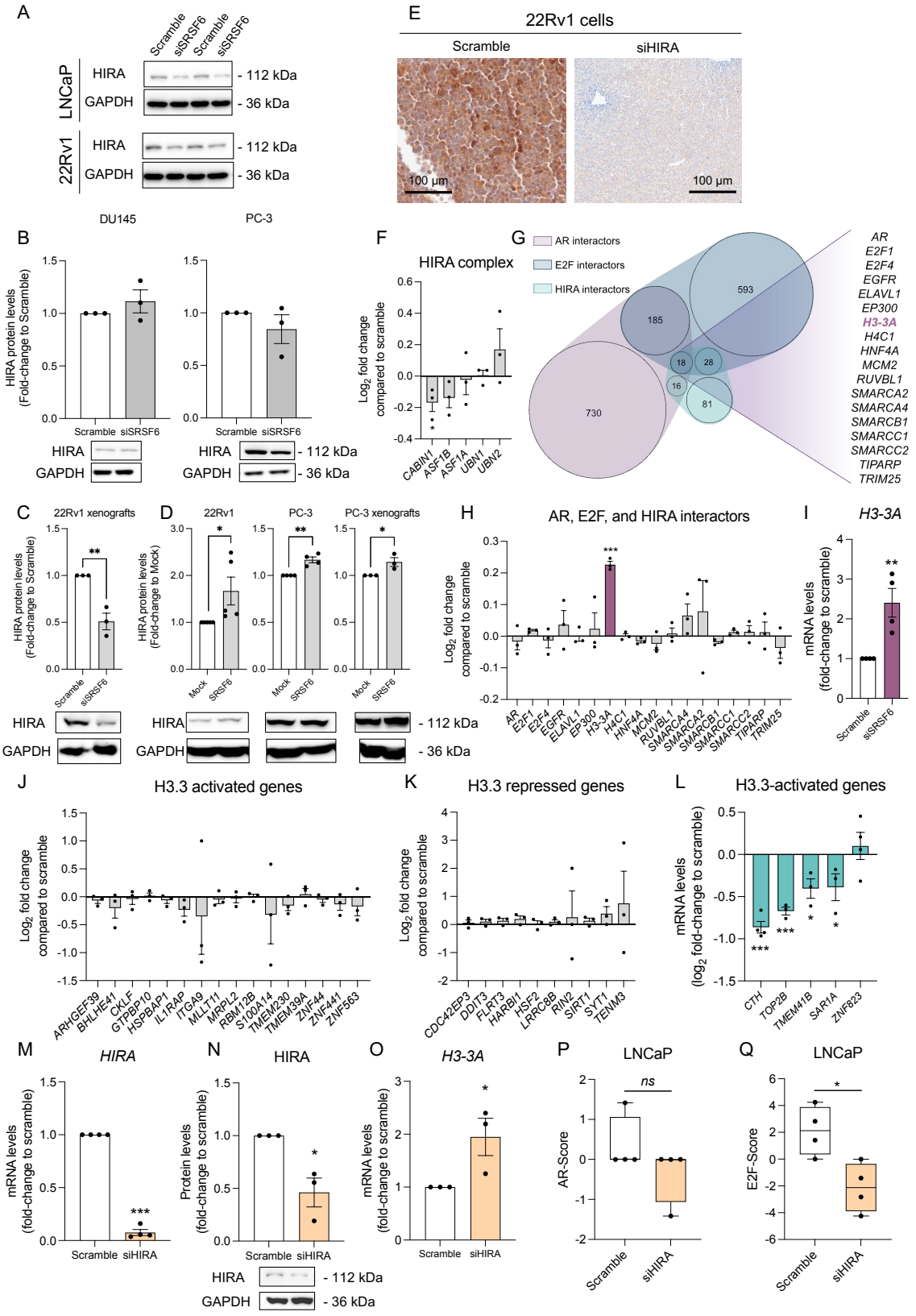
Expression of *SRSF6*, AR-, and E2F-related genes in response to the modulation of SRSF6 and AR. (A-B) Validation by qPCR (A) and Western blot (B) of SRSF6 overexpression in 22Rv1 cells. Representative images of Western blot are depicted on bottom panels. (C-E) Expression of AR-Score genes in response to SRSF6 silencing in 22Rv1 by RNA-Seq (C) and LNCaP cells by qPCR (D-E). (F) Expression of AR-Score genes in response to SRSF6 overexpression in 22Rv1 cells by qPCR. (G-I) Expression of E2F-Score genes in response to SRSF6 silencing in 22Rv1 by RNA-Seq (G) and LNCaP cells by qPCR (H-I). (J) Expression of E2F-Score genes in response to SRSF6 overexpression in 22Rv1 cells by qPCR. (K-L) Expression of *AR* (K; left panel), *AR-V7* (K; right panel) by qPCR and *E2F1-8* (L) by RNA-Seq in response to *SRSF6* silencing in 22Rv1 cells. (M) *SRSF6* expression profile in response to inhibition (left panel) and activation (right panel) of AR activity in LNCaP cells by qPCR. Data are normalized by control cells (mean ± SEM). Asterisks (*p<0.05; **p<0.01; ***p<0.001) indicate statistically significant differences between groups.

Fig. S8.



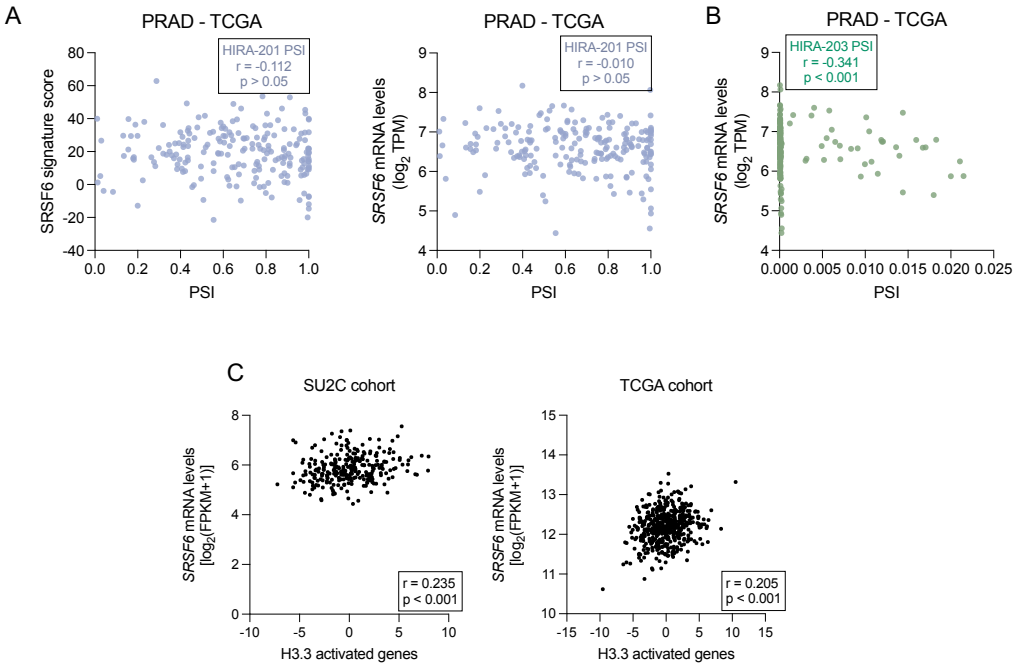
Splicing alteration in response to SRSF6 silencing. (A) Percentage of significantly altered splicing events according on its type. (B-C) Effect of SRSF6 silencing on the splicing of *AHR* (B) and *CSNK2A1* (C) pre-mRNA. Data are normalized by control cells (mean ± SEM). AF: alternative first exon, ES: exon skipping, A5: alternative 5' splice site, A3: alternative 3' splice site, AL: alternative last exon, MX: mutually exclusive exons, IR: intron retention, PSI: percent-spliced-in. Asterisks (*p<0.05; **p<0.01; ***p<0.001) indicate statistically significant differences between groups.

Fig. S9.


A
Scramble
siSRSF6
Scramble
siSRSF6
LNCaP
HIRA
GAPDH
- 112 kDa
- 36 kDa
22Rv1
HIRA
GAPDH
- 112 kDa
- 36 kDa
E
22Rv1 cells
Scramble
siHIRA
100 µm
100 µm
DU145
PC-3
B
HIRA protein levels (Fold-change to Scramble)
Scramble
siSRSF6
HIRA
GAPDH
- 112 kDa
- 36 kDa
F
HIRA complex
Log2 fold change compared to scramble
CABIN1
ASF1B
ASF1A
UBN1
UBN2
G
AR interactors
E2F interactors
HIRA interactors
593
185
730
18
28
16
81
AR
E2F1
E2F4
EGFR
ELAVL1
EP300
H3-3A
H4C1
HNF4A
MCM2
RUVBL1
SMARCA2
SMARCA4
SMARCB1
SMARCC1
SMARCC2
TIPARP
TRIM25
C
22Rv1 xenografts
HIRA protein levels (Fold-change to Scramble)
Scramble
siSRSF6
D
22Rv1
PC-3
PC-3 xenografts
HIRA protein levels (Fold-change to Mock)
Mock
SRSF6
HIRA
GAPDH
- 112 kDa
- 36 kDa
H
AR, E2F, and HIRA interactors
Log2 fold change compared to scramble
I
H3-3A
mRNA levels (fold-change to scramble)
Scramble
siSRSF6
J
H3.3 activated genes
Log2 fold change compared to scramble
ARHGEF39
BHLHE41
CKLF
GTPBP10
HSPBAP1
IL1RAP
ITGA9
MLLT11
MRPL2
RBM12B
S100A14
TMEM230
TMEM39A
ZNF44
ZNF441
ZNF563
K
H3.3 repressed genes
Log2 fold change compared to scramble
CDC42EP3
DDIT3
FLRT3
HARBI1
HSF2
LRRC8B
RIN2
SIRT1
SYT1
TENM3
L
H3.3-activated genes
mRNA levels (log2 fold-change to scramble)
CTH
TOP2B
TMEM41B
SAR1A
ZNF823
M
HIRA
mRNA levels (fold-change to scramble)
Scramble
siHIRA
N
HIRA
Protein levels (fold-change to scramble)
Scramble
siHIRA
HIRA
GAPDH
- 112 kDa
- 36 kDa
O
H3-3A
mRNA levels (fold-change to scramble)
Scramble
siHIRA
P
LNCaP
ns
AR-Score
Scramble
siHIRA
Q
LNCaP
E2F-Score
Scramble
siHIRA

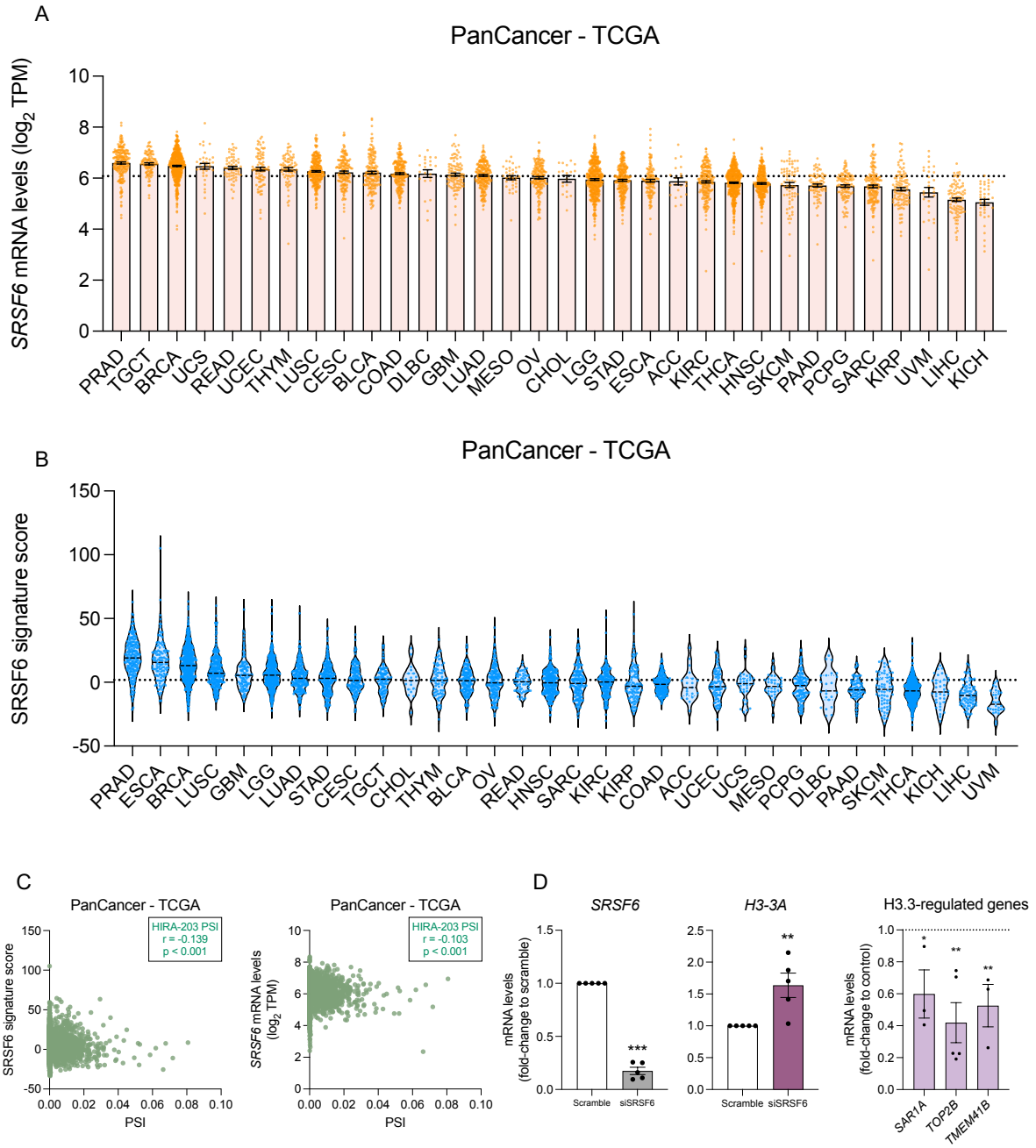
Impact of SRSF6 and HIRA silencing on HIRA-, AR-, and E2F-regulated pathway in PCa cells. (A-B) Western blot of HIRA and GAPDH protein levels in response to SRSF6 silencing in LNCaP (A; upper panel), 22Rv1 cells (A; bottom panel), DU145 (B; left panel), and PC-3 cells (B; right panel). (C) Western blot of HIRA and GAPDH protein levels in response to SRSF6 silencing in 22Rv1 xenografts. (D) Western blot of HIRA and GAPDH protein levels in response to SRSF6 overexpression in 22Rv1 (left panel) and PC-3 cells (central panel) and PC-3 xenografts (right panel). Representative images of Western blot are depicted. (E) Antibody validation by IHC in response to HIRA siRNA. (F) Expression by RNA-Seq of components of the HIRA complex in response to SRSF6 silencing in 22Rv1 cells. (G) Venn diagram representing common AR, E2F, and HIRA protein interactors. (H) Expression by RNA-Seq of common AR, E2F, and HIRA interactors in response to SRSF6 silencing in 22Rv1 cells. (I) Expression of *H3-3A* in response to SRSF6 silencing in LNCaP cells. (J-K) Expression by RNA-Seq of non-significantly dysregulated H3.3 activated (J) and H3.3 repressed (K) genes in response to SRSF6 silencing in 22Rv1 cells. (L) Expression by qPCR of H3.3-activated genes in response to SRSF6 silencing in LNCaP cells. (M-O) Expression of HIRA by qPCR (M) and Western blot (N) and *H3-3A* by qPCR (O) in response to HIRA silencing in LNCaP cells. HIRA protein levels were adjusted by GAPDH. Representative images of Western blot are depicted. (P-Q) Expression by qPCR of AR (P) and E2F (Q) activity in response to HIRA silencing in LNCaP cells. Data are normalized by controls cells (mean ± SEM). Asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.001$) indicate statistically significant differences between groups.

Fig. S10.



Association between SRSF6 and HIRA-201 PSI, HIRA-203 PSI, and H3.3 activity in human samples. (A) Correlation between HIRA-201 PSI and SRSF6 signature score (left panel) and mRNA levels (right panel) in PRAD-TCGA cohort. (B) Correlation between HIRA-203 PSI and *SRSF6* mRNA levels in PRAD-TCGA cohort. (C) Correlation between H3.3 activity and *SRSF6* mRNA levels in SU2C (left panel) and TCGA cohort (right panel). PSI: percent-spliced-in.

Fig. S11.



Pan-cancer association of SRSF6 and H3.3 pathway. (A-B) Expression of *SRSF6* mRNA levels (mean ± SEM; A) and SRSF6 signature score value (min to max boxplot, with median; B) in pan-cancer TCGA cohort. Median value of *SRSF6* mRNA levels and SRSF6 signature score is depicted by a dotted line in each graph. (C) Correlation between HIRA-203 PSI and SRSF6 signature score (left panel) and mRNA levels (right panel) in PanCancer TCGA cohort. (D) Expression by qPCR of *SRSF6* (left panel), *H3-3A* (central panel), and H3.3-regulated genes (right panel) in response to SRSF6 silencing in BT-549 cells. Data are normalized by control cells (mean ± SEM). ACC: Adrenocortical carcinoma, BLCA: Bladder Urothelial Carcinoma, BRCA: Breast invasive carcinoma, CESC: Cervical squamous cell carcinoma and endocervical

adenocarcinoma, CHOL: Cholangiocarcinoma, COAD: Colon adenocarcinoma, DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, ESCA: Esophageal carcinoma, GBM: Glioblastoma multiforme, HNSC: Head and Neck squamous cell carcinoma, KICH: Kidney Chromophobe, KIRC: Kidney renal clear cell carcinoma, KIRP: Kidney renal papillary cell carcinoma, LGG: Brain Lower Grade Glioma, LIHC: Liver hepatocellular carcinoma, LUAD: Lung adenocarcinoma, LUSC: Lung squamous cell carcinoma, MESO: Mesothelioma, OV: Ovarian serous cystadenocarcinoma, PAAD: Pancreatic adenocarcinoma, PCPG: Pheochromocytoma and Paraganglioma, PRAD: Prostate adenocarcinoma, READ: Rectum adenocarcinoma, SARC: Sarcoma, SKCM: Skin Cutaneous Melanoma, STAD: Stomach adenocarcinoma, TGCT: Testicular Germ Cell Tumors, THYM: Thymoma, THCA: Thyroid carcinoma, UCS: Uterine Carcinosarcoma, UCEC: Uterine Corpus Endometrial Carcinoma, UVM: Uveal Melanoma, PSI: percent-spliced-in.

Table S1.

Patients [*n*]	84
Age, years [median (IQR)]	61 (57-66)
PSA levels, ng/mL [median (IQR)]	5.2 (4.2-8.0)
Gleason score ≥ 7 [*n* (%)]	76 (90.5%)
pT ≥ 3a [*n* (%)]	59 (70.2%)
Perineural infiltration [*n* (%)]	72 (85.7%)
Lymphovascular invasion [*n* (%)]	8 (9.52%)
Recurrence [*n* (%)]	35 (41.7%)
Metastasis [*n* (%)]	0 (0%)

Demographic, biochemical, and clinical parameters of PCa patients from cohort 1. PSA: Prostate specific antigen; pT: Pathological tumor staging; PI: Perineural invasion.

Table S2.

Control (non-prostate cancer)	
Patients [*n*]	9
Age, years [median (IQR)]	67 (51-83)
Prostate cancer	
Patients [*n*]	42
Age, years [median (IQR)]	75 (69-81)
PSA levels, ng/mL [median (IQR)]	62.0 (36.2-254.5)
Gleason score ≥ 7 [*n* (%)]	42 (100%)
Metastasis [*n* (%)]	28 (66.7%)

Demographic, biochemical, and clinical parameters of PCa patients from cohort 2. PSA: Prostate specific antigen.

Table S3.

Gene	Ensembl ref.	Sense	Antisense	Product size (bp)
SRSF6	ENSG00000124193	AGACCTCAAAAATGGGTACGG	CTTGCCGTTCAGCTCGTAA	82
ACTB	ENSG00000075624	ACTCTTCCAGCCTTCCTTCC	CAGTGATCTCCTTCTGCATC	176
GAPDH	ENSG00000111640	GCCTCAAGATCATCAGCAATG	CTTCCACGATACCAAAGTTGT	90
PCA3	ENSG00000225937	CAGAGGGGAGATTTGTGTGG	TGTCATCTTGCTGTTTCTAGTGATG	172
SST_5TMD4	-	TACCTGCAACCGTCTGCC	AGCCTGGGCCTTTCTCCT	98
In1-Ghrelin	-	TCTGGGCTTCAGTCTTCTCC	GTTCATCCTCTGCCCCTTCT	215
AR	ENSG00000169083	GCAGGAAGCAGTATCCGAAG	GTTGTCAGAAATGGTCGAAGTG	112
AR-V7	ENST00000504326	CAGGGATGACTCTGGGAAAA	TGAGGCAAGTCAGCCTTTCT	87
ESRP1	ENSG00000104413	TTTTGGGATCACTGCTGGGG	TGTCCCACCTTCTTGTTGGC	108
MYC	ENSG00000136997	TTCGGGTAGTGGAAAACCAG	CCTCGTCGCAGTAGAAATACG	117
ACSL3	ENSG00000123983	TTCCAGAACTAGGAGAGGAAGATG	CATCCGTGAGAAAGACAGACAA	97
FKBP5	ENSG00000096060	CGGAGAACCAAACGGAAA	TCAAACATCCTTCCACCACA	98
KLK2	ENSG00000167751	TGATTCTGGGGGTCCACTT	GTACACAGCAGGCTTTTCAGG	97
KLK3	ENSG00000142515	AGAGGAGTTCTTGACCCCAAA	CCTTCTGAGGGTGAACTTGC	89
NKX3-1	ENSG00000167034	TCAGGTGATCGAGTTGGAGAG	TCCGTGAGCTTGAGGTTCTT	93
PLPP1	ENSG00000067113	CCTCACTTCTTGGATGTTTGTG	GACAACCTGCCTTCCTTAACTCT	116
RAB3B	ENSG00000169213	TCCTCTTCCGCTATGCTGAT	AGTTTCACCCGCTTCTCGT	106
STEAP1	ENSG00000164647	TGGCAATACTGGCTCTGTTG	GGGAAACAATTCCTAGCTTGC	105
AURKB	ENSG00000178999	AGAGTGCATCACACAACGAGAC	CCTGAGCAGTTTGGAGATGAG	107
BIRC5	ENSG00000089685	GGACCACCGCATCTCTACATT	GTCTGGCTCGTTCTCAGTGG	115
CCNE1	ENSG00000105173	GCCTTGGGACAATAATGCAG	TGCACGTTGAGTTTGGGTAA	98
EZH2	ENSG00000106462	CACTCCTTTCATACGCTTTTCTG	TGTTTCTGTGTTCTTCCGCTTA	120
PRKDC	ENSG00000253729	GGGACGAGGTGGATAACAAA	ACGCCTTTTCTGGCATACAT	118
TP53	ENSG00000141510	AAGGAAATTTGCGTGTGGAG	CCAGTGTGATGATGGTGAGG	180
HIRA	ENSG00000100084	GAGGTCATTCTGGCTTGGTC	CTTTAGGCTGCGGTCATCA	86
H3-3A	ENSG00000163041	GAGAAGGGGGTAAGGAGGTCT	TTGCTTCCTGGGTGCTTTAC	88
CTH	ENSG00000116761	CACTGTCCACCACGTTCAAG	TGCCACTGCTTTTTCAAGG	104
TMEM41B	ENSG00000166471	GGATCAGCAAGAATGTCACTCC	CTCTGGGAACCTTCATATTCACTC	130

Specific primers for human genes and splicing variants used in this study. Official name of the genes, Ensembl accession number, primers sequences, and product sizes of the amplification products are included.

Data S1. (separate file)
Differential gene expression in response to SRSF6 silencing in 22Rv1 cells (siSRSF6 vs. scramble).

Data S2. (separate file)
Differential expression of transcripts in response to SRSF6 silencing in 22Rv1 cells (siSRSF6 vs. scramble).

Data S3. (separate file)
List of AR, E2F, and HIRA interactors.

Data S4. (separate file)
Gene sets used to infer proliferative state and MYC, AR, E2F, SRSF6, and H3.3 activity.