

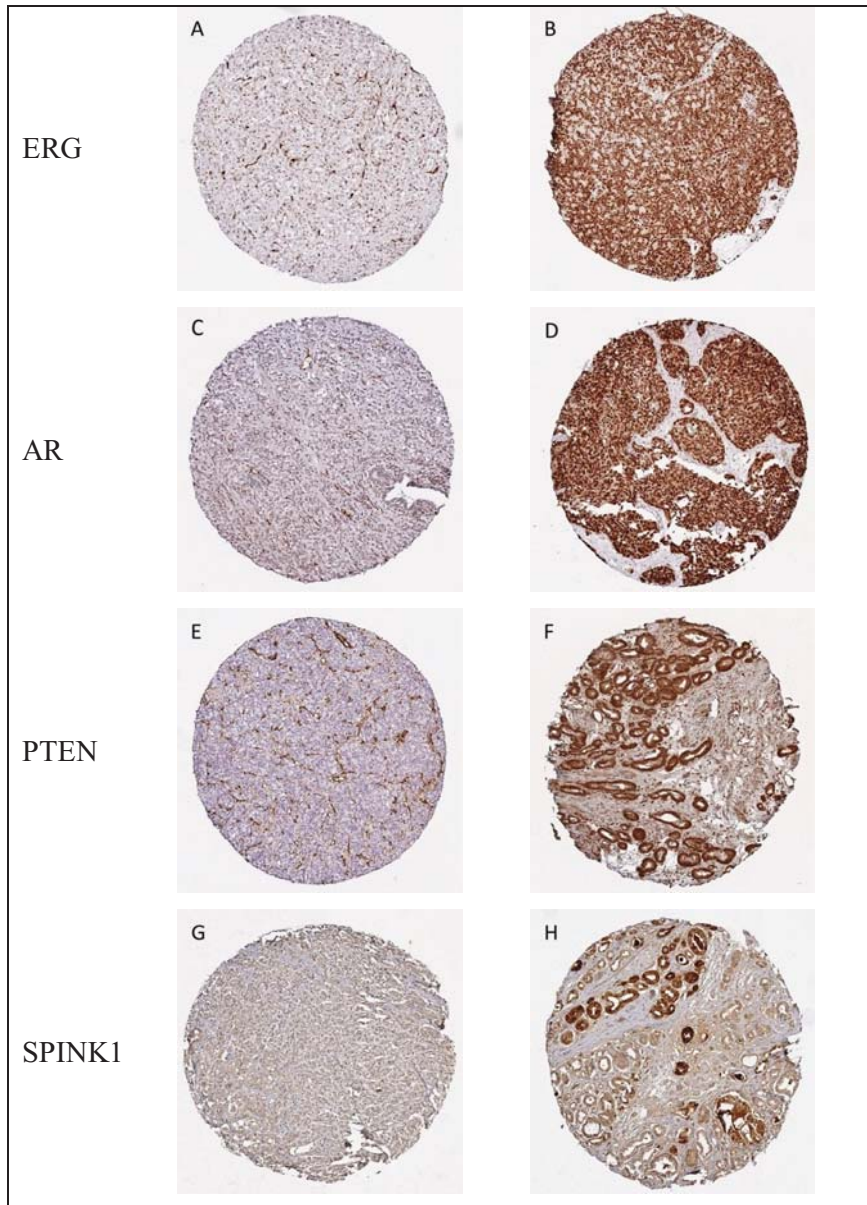
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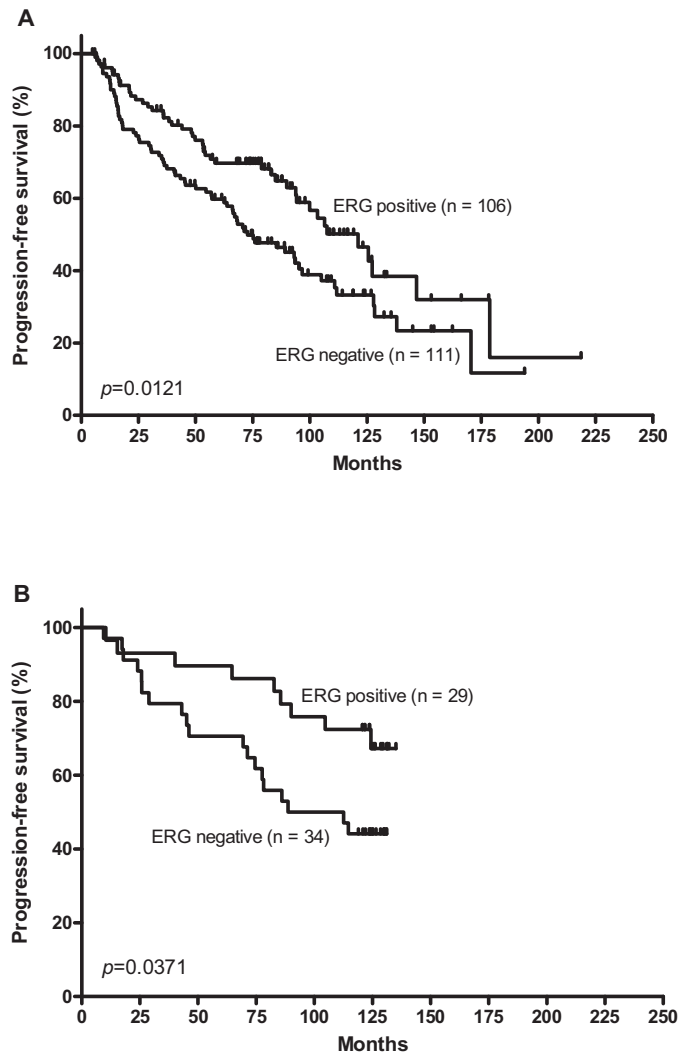
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Loss of PTEN is associated with aggressive behavior in ERG positive prostate cancerKatri A. Leinonen *et al.*

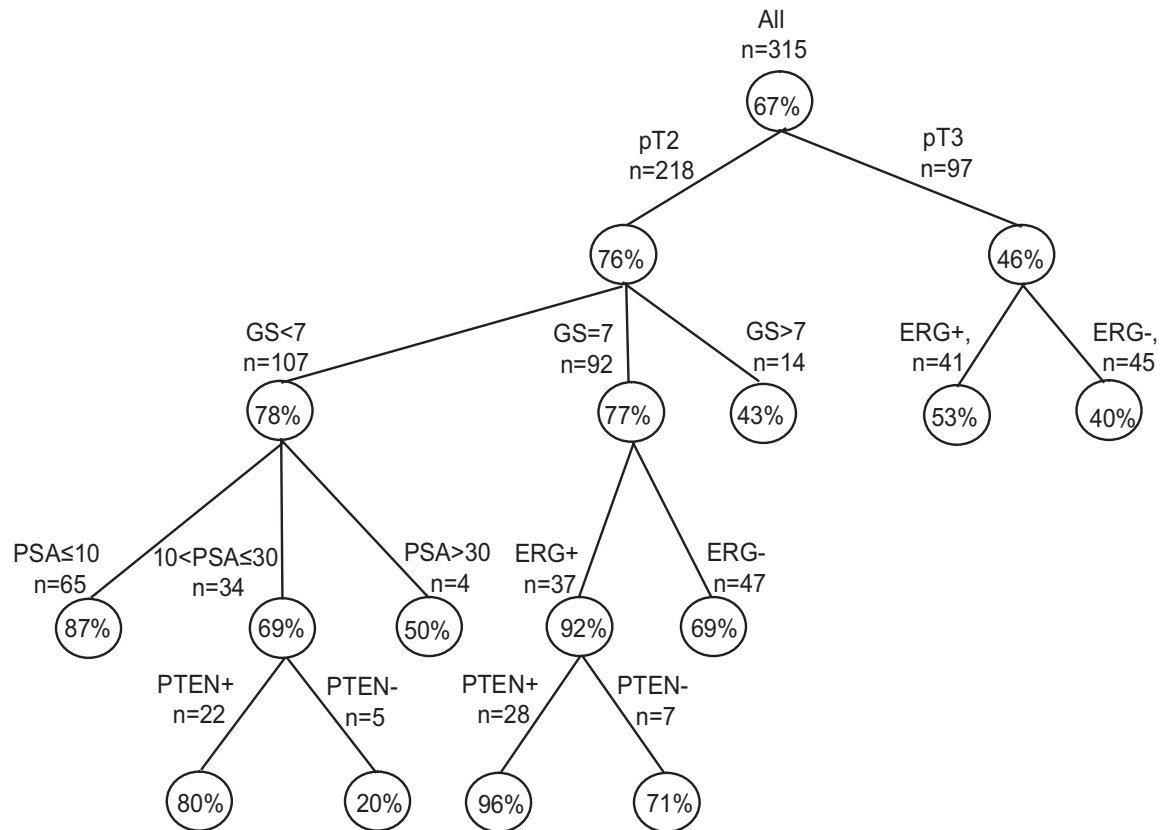
SUPPLEMENTARY FIGURES

Supplementary Figure 1. Representative (A, C, E and G) negative and (B, D, F and H) positive IHC images of ERG, AR, PTEN and SPINK1 staining.

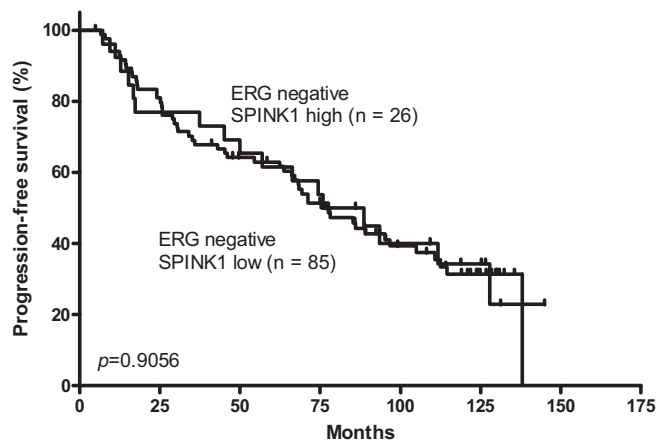
Supplementary Figure 2. Kaplan-Meier analysis of progression-free survival of prostatectomy-treated patients. (A) Previously used (Saramäki *et al.*, 2008) prostatectomy material, and (B) new prostatectomy samples.



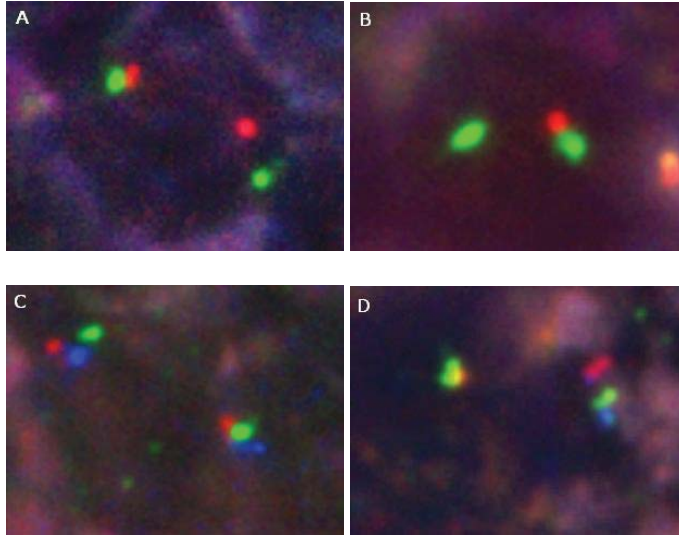
Supplementary Figure 3. Regression-tree analysis of prognostic markers in the prostatectomy cohort. The factor used in each level splits the patients into distinct survival groups according to a Cox-regression model. The number of patients in each group are indicated. The circles show the 5-year progression-free survival fraction for each group. Sample numbers in each subgroup represent informative cases where no clinical data was missing.



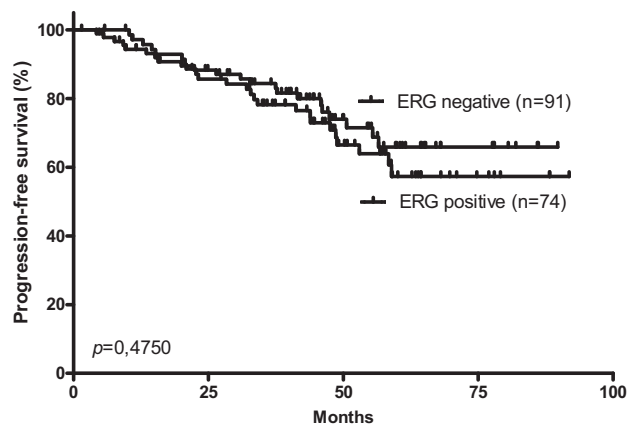
Supplementary Figure 4. Kaplan-Meier analysis of progression-free survival of prostatectomy-treated patients according to SPINK1 expression in ERG negative patients.



Supplementary Figure 5. FISH analyses of (A-B) *TP53* and (C-D) 3p14 deletion. (A-B) The signals are red for *TP53* and green for centromere 17. (A) Normal *TP53* locus, (B) deletion of *TP53*. (C-D) The signals are red (telomeric) and green (centromeric) for regions around the 3p14 region, and blue for 3p14 region. (C) Normal 3p14 locus, (D) deletion of 3p14 region.



Supplementary Figure 6. Kaplan-Meier analysis of progression-free survival of endocrine-treated patients according to ERG expression.



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SUPPLEMENTARY TABLES

Supplementary Table 1. The clinicopathological description of the prostatectomy, needle biopsy and locally recurrent CRPC samples.

<u>Prostatectomy specimens, n</u>	326
Gleason score, n (%)	
<7	126 (40)
7	152 (48)
>7	39 (12)
pT stage, n(%)	
pT2	221 (69)
pT3	98 (31)
Mean age at diagnosis	63.0 years (median 63.0, range 44.0-74.0 yrs)
Median follow-up time	76.1 months (range 4.9-218.7 mo)
<u>Needle biopsy specimens, n</u>	166
Gleason score, n (%)	
<7	52 (28)
7	55 (29)
>7	81 (43)
T-stage, n (%)	
T1-2	116 (59)
T3-4	80 (41)
M-stage, n (%)	
MX	101 (52)
M0	69 (35)
M1	26 (13)
Mean age at diagnosis	73.6 years (median 74.3, range 52.7-88.8 yrs)
<u>Locally recurrent CRPCs, n</u>	177
Mean time from the beginning of treatment to the TURP	46.7 months (range 1.0-187.0 mo)
Endocrine treatment used:	
Orchiectomy	80 cases
Luteinizing hormone-releasing hormone (LHRH) analogue	51 cases
Estrogen	5 cases
Bicalutamide alone	1 case
Orchiectomy + estrogen	3 cases
Maximal androgen blockade	29 cases
Maximal androgen blockade + estramustine	1 case

Supplementary Table 2. List of metastasized CRPC samples studied in each study subject in IHC stainings.

PELICAN Autopsy Study Case Number (A)	Subject Race, Ethnicity	Sample Name
1	White, Nonhispanic	Subdural Met, Seminal vesicle Met, Cranial Bone Met
2	White, Nonhispanic	Liver Met, Spleen Met, Adrenal Met, Chest Wall Met
3	African American, Nonhispanic	Subdural Met, Bladder Met, Thoracic Paraaortic LN Met, R Rib Met, Pericardium Met
4	White, Nonhispanic	Liver Met, Rib Met, Abdominal Paraaortic LN Met, Pelvic LN Met
5	White, Nonhispanic	L Iliac LN Met, Manubrium Met, Pelvic LN Met, L Retro Paritoneal Mass Met
7	White, Nonhispanic	Paraaortic LN Met, Liver Met
8	White, Nonhispanic	Liver Met, R Rib 8 Met, R Inguinal LN Met, Pelvic LN Met
9	White, Nonhispanic	Periportal LN Met, Hilar LN Met, Lung Met, L Pelvic LN Met
10	African American, Nonhispanic	R Iliac LN Mets, Perigastric LN Met, Periportal LN Met
11	White, Nonhispanic	L Inguinal LN Met, R Pelvic LN Mets, Bladder Met, Mediastinal LN Met
12	African American, Nonhispanic	Paraaortic LN Met, Mediastinal LN Met, L Pelvic LN Met, Retroperitoneal LN Met
13	White, Nonhispanic	Paraaortic LN Met, L3 Met, T6 Met
14	White, Nonhispanic	Liver Met, Thoracic Paraaortic LN Met, R Axillary LN Met, L Subclavian LN Met, R Adrenal Met
15	White, Nonhispanic	Liver Met, R Adrenal Met, Humerus Marrow Met
16	White, Nonhispanic	Pericardial Mets, L Hilar LN Met, R Temporal Subdural Met, Abdominal Paraaortic LN Met

17	White, Nonhispanic	Abdominal Paraaortic LN Met, Subdural Met, R Femur Marrow Met, R Iliac LN Met, L Axillary LN Met
18	White, Nonhispanic	L Cervical LN Met, Supraclavicular LN Met
19	White, Nonhispanic	Paraaortic LN Met, L Axillary LN Met, Subdural Met
20	African American, Nonhispanic	Seminal Vesicle Met, Left Upper Lung Met
21	White, Nonhispanic	Multiple Liver Met, L Clavicular LN Met, Rib Met, Left Rib 5 Mass Met
22	White, Hispanic	R pelvic LN Mets, L Periaortic LN Met, R Adrenal Met, Seminal Vesicle Met
23	African American, Nonhispanic	Liver Met, Periaortic Soft Tissue Met, R Adrenal Met
24	White, Nonhispanic	R Axillary LN Met, Pericardial Met, R Diaphragmatic Mass Met
25	White, Nonhispanic	R Femur Met, L Seminal Vesicle Met
26	White, Nonhispanic	L Rib 5 Met
27	White, Nonhispanic	R Axillary LN Met, Seminal Vesicle Met, T7 Met
28	White, Nonhispanic	Bladder Met, R Lung Met, Anterior Mediastinal LN Met, Hilar LN Met
29	White, Nonhispanic	Cerebellum Met, R Superficial Inguinal LN Met
30	White, Nonhispanic	R Femur Marrow Met, R Humerus Marrow Met, L Lobe Liver Single Met, R Adrenal Met, Hilar LN Met
31	White, Nonhispanic	L Adrenal Met, R Subdural Met, L Inguinal LN Met, R Inguinal LN Met, Pelvic LN Met
32	White, Nonhispanic	L Subclavicular LN Met, R Rib 8 Soft Met, L Inferior Paraaortic LN Met, Superior Abdominal LN Met, R Subclavicular LN Met
33	White, Nonhispanic	L Axillary LN Met, L Subdural Met, L Periadrenal Met, Paratracheal LN Met, Perigastric LN Met

Supplementary Table 3. List of metastasized CRPC samples studied in each study subject in *TP53* mutation analysis.

PELICAN Autopsy Study Case Number (A)	Subject Race, Ethnicity	Cancerous Tissues Studied, Sample Name	Noncancerous tissue Studied
1	White, Nonhispanic	Bladder Met, L Seminal Vesicle Met, Epidural Met	Liver
2	White, Nonhispanic	Spleen Met, Single Liver Met C1, Liver Met, Rib 6 Met	Kidney
3	African American, Nonhispanic	Pelvic Paraaortic LN Met, Subdural Met, Vertebral Bone S2 Met	Lymphs
4	White, Nonhispanic	Vertebral Bone Met, Rib Met, Liver Met 8, Liver Met 17	Transformed Lymphs
5	White, Nonhispanic	L Iliac LN Met, L Humerus Met, Soft Manubrium Mass Met	Spleen
7	White, Nonhispanic	P meta-aortic LN Met, Prostate CA, R Posterior Subdural Met	Liver
8	White, Nonhispanic	Multiple Liver Mets, R Inguinal LN Met, R Adrenal Met	Kidney
9	White, Nonhispanic	L Supraclavicular LN Met, Pulmonary Hilar LN Met, Periportal LN Met	Liver
10	African American, Nonhispanic	R Iliac LN Met, Perigastric LN Met, Prostate CA	Liver
11	White, Nonhispanic	Bladder Neck Met, R Pelvic LN Met, L Inguinal LN Met	Liver
12	African American, Nonhispanic	R Pelvic LN Met, Paraaortic LN Met, Mediastinal LN Met	Liver
13	White, Nonhispanic	R Iliac LN Met, Paraaortic LN Met, Vertebral L4 Bone Met, Vertebral S2 Bone Met	Liver
14	White, Nonhispanic	R Axillary LN Met, Thoracic paraaortic LN Met, Liver Met	Liver
15	White, Nonhispanic	Liver Met, R Rib 10 Bone Met, R Adrenal Met	Liver
16	White, Nonhispanic	L Pulmonary Hilar LN Met, R Tempora Subdural Met, Pericardial Mets	Liver

17	White, Nonhispanic	Subdural Met Fossa C, Subdural Tumor met, R Femur Marrow Met, R Iliac LN Met, Paraaortic LN Met, R Supraclavicular LN Met, L Humerus Marrow Met, L Inguinal LN Met	Kidney
18	White, Nonhispanic	L Cervical LN Met 2, L Cervical LN Met 4, L Supraclavicular LN met	Kidney
19	White, Nonhispanic	L Axillary LN Met, L Pelvic LN Met, Paraaortic LN Met	Liver
20	African American, Nonhispanic	R Mid Prostate CA, Seminal Vesicle Met, L Upper Lung Met	Liver
21	White, Nonhispanic	L Clavicular LN Met, Multiple Liver Mets, Single Liver Met #2, Single Liver Met #4	Kidney
22	White, Hispanic	R Adrenal Met, R Pelvic LN Met, L Periaortic LN Met, L Humerus Bone Marrow Met, Tumor Around Seminal Vesicle #2 Met	Liver
23	African American, Nonhispanic	Single Liver Met, R Periaortic Met, R Adrenal Met, Vertebral T9 Bone Met	Spleen
24	White, Nonhispanic	R Axillary LN Met, R Diaphragmatic Met, Pericardial Met, Xiphoid Met	Spleen
25	White, Nonhispanic	Prostate CA, R Femur Marrow Met, R Femur Bone Met	Liver
26	White, Nonhispanic	Vertebral L4 Hemorrhagic Bone Met, Bladder Met, Vertebral T9 Bone Met, Peribronchial LN Met	Spleen
27	White, Nonhispanic	Prostate CA, R Axillary LN Met, L Clavicle Met	Liver
28	White, Nonhispanic	Posterior Bladder Polypoid Met, Anterior Mediastinal LN Met, R Lower Lung Met	Liver
29	White, Nonhispanic	R Inguinal LN Met, Prostate CA, Intraparenchymal Cerebellum Met	Liver
30	White, Nonhispanic	Liver Left Lobe Single Met, R Femur Marrow Met, R Humerus Marrow Met	Spleen
31	White, Nonhispanic	Prostate CA, L Adrenal Met, R Subdural Met	Liver
32	White, Nonhispanic	L Subclavicular LN Met, Prostate CA, R Rib 8 Bone Met	Spleen

33	White, Nonhispanic	L Axillary LN Met, L Subdural Met, L Periadrenal Met	Liver
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Supplementary Table 4. Criteria for IHC stainings. ERG, AR, PTEN and SPINK1 intensities were evaluated from 0 to 3.

	Negative	Low Expression	High Expression	Positive/Strong
ERG	0	-	-	1-3
AR	0	0-1	2-3	-
PTEN	0	0-1	-	1-3
SPINK1	0	0-2	-	3

Supplementary Table 5. Primers used for TP53 sequencing.

Primer	
Exon 4	5'-GGC TGA GGA CCT GGT CCT-3' 5'-ATA CGG CCA GGC ATT GAA GTC TC-3'
Exon 5-6	5'-TTC TTT GCT GCC GTG TTC C-3' 5'-ACA ACC ACC CTT AAC CCC TC-3'
Exon 7	5'-CCA CAG GTC TCC CCA AGG-3' 5'-AAA TCG GTA AGA GGT GGG C-3'
Exon 8-9	5'-TGG GAG TAG ATG GAG CCT GGT-3' 5'-GAG CCA TTG TCT TTG AGG CA-3'

Supplementary Table 6. Primers used for SSCP.

Primer	
exon 5	5'-AAT CAG TGA GGA ATC AGA GGC-3'
intron 5	5'-TTC TTT GCT GCC GTG TTC C-3' 5'-ACA ACC ACC CTT AAC CCC TC-3'
exon 6	5'-AGG CCT CTG ATT CCT CAC TG-3'
exon 8	5'-GTG AAT CTG AGG CAT AAC TGC-3'
exon 9	5'-CAG ATT CAC TTT TAT CAC C-3'

Supplementary Table 7. Association between ERG expression according to IHC and *TMPRSS2:ERG* fusion according to FISH in prostatectomy and locally recurrent CRPC samples.

<i>TMPRSS2:ERG</i> fusion	ERG expression	
	Negative, n (%)	Positive, n (%)
No	138 (98)	56 (39)
Yes	3 (2)	87 (61)

$p < 0.0001$, Fisher's exact test.

Supplementary Table 8. The effect of use of PSA on ERG positivity rate in prostatectomy samples.

Prostatectomy samples	ERG expression, n (%)	
	Negative	Positive
Before PSA-era	6 (55)	5 (45)
PSA tests done occasionally	24 (53)	21 (47)
Routine PSA use	120 (52)	111 (48)

Supplementary Table 9. Association between *TP53* deletion and PTEN expression in prostatectomy, locally recurrent and metastasized CRPC samples.

PTEN expression	<i>TP53</i> deletion		P^a
	No deletion, n (%)	Deletion, n (%)	
Prostatectomy samples:			
Negative	26 (13)	6 (35)	0.0281
Positive	167 (87)	11 (65)	
Locally recurrent CRPCs:			
Negative	38 (48)	8 (40)	0.6188
Positive	41 (52)	12 (60)	
Metastasized CRPCs:			
Negative	11 (69)	1 (50)	1.0000
Positive	5 (31)	1 (50)	

^aFisher's Exact Test

Supplementary Table 10. Details of *TP53* mutations found from metastasized CRPC samples. A total of 14 *TP53* mutations were identified. Identical *TP53* mutation was found in each metastatic cancer samples studied in 12 of 13 (92%) subjects. No mutation was found in noncancerous control samples studied in parallel from each subject. Substitution (Subst), deletion (del), insertion (ins), exon (ex), intron (intr), frame shift (fs), stop codon (X).

Case Number	Sample Name	Subject race, ethnicity	TP53 mutation			
			Type	Exon /intron	cDNA	Protein
A1	Bladder Met L Seminal Vesicle Met Epidural Met	White, Nonhispanic	Subst	Ex 8	818 G>A	R273H
A3	Pelvic Paraaortic LN Met Subdural Met Vertebral Bone S2 Met	African American, Nonhispanic	Subst	Ex 8	827 C>A	A276D
A4	Vertebral Bone Met Rib Met Liver Met 8 Liver Met 17	White, Nonhispanic	Subst	Ex 5	473 G>T	R158L
A5	L Humerus Met L Iliac LN Met Soft Manubrium Mass Met	White, Nonhispanic	No mut	-	-	-
			Del	Intr 5	-	-
A7	Paraortic LN Met Prostate CA R Posterior Subdural Met	White, Nonhispanic	Subst	Ex 4	314 G>T	G105V
			Ins	Ex 7	713- 714insT	C238ins.fs.
A12	R Pelvic LN Met Paraortic LN Met Mediastinal LN Met	African American, Nonhispanic	Ins	Ex 7	713- 714insT	C238ins.fs.
A14	R Axillary LN Met Thoracic paraortic LN Met Liver Met	White, Nonhispanic	Subst	Ex 6	574	Q192X
A16	L Pulmonary Hilar LN Met R Temporal Subdural Met Pericardial Mets	White, Nonhispanic	Del	Ex 6	575- 588del.	Q192- R196del.fs.
A18	L Cervical LN Met 2 L Cervical LN Met 4 L Supraclavicular LN Met	White, Nonhispanic	Subst	Ex 6	637 C>T	R213X
A24	R Axillary LN Met R Diaphragmatic Met Xiphoid Met	White, Nonhispanic	Del	Ex 6	625- 626del.	R209del. fs.
A28	Posterior Bladder Polypoid Met Anterior Mediastinal LN Met R Lower Lung Met	White, Nonhispanic	Subst	Ex 8	817 C>T	R273C
A30	Liver Left Lobe Single Met R Femur Marrow Met R Humerus Marrow Met	White, Nonhispanic	Subst	Ex 5	535 C>T	H179Y
A32	L Subclavicular LN Met	White,	Subst	Ex 5	396 G>T	K132N

R Rib8 Bone Met Prostate CA	Nonhispanic
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Supplementary Table 11. Association of ERG negative and positive subjects with mutation or deletion of *TP53* in CRPC metastases.

TP53 change (mutation or deletion)	ERG expression	
	Negative, n(%)	Positive, n(%)
No	13 (72)	5 (36)
Yes	5 (28)	9 (64)
<i>p</i> =0.0720, Fisher's Exact Test		