

Supplementary Information for

Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer.

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Supplementary Note

Description of prostate tumor cohorts

Clinically localized primary prostate cancers were selected for exome- and transcriptome-sequencing from two cohorts: Weill Cornell Medical College (WCMC; New York, NY) (Esgueva *et al.*, in press) and Uropath (Perth, Australia), a commercial supplier of banked urological tissues. Participating patients had received no prior treatment for prostate cancer. We selected tumors based on suitability for sequencing, choosing specimens with cancerous foci relatively free from admixed normal tissue that yielded high-quality DNA.

Tumors from the WCMC cohort were collected by the Institutional Biobank from patients undergoing radical prostatectomy by one surgeon (A.K.T.) for clinically localized prostate cancer. Patient-matched normal DNA was obtained from whole blood samples for this cohort.

Tumors from the Uropath cohort were obtained from men undergoing radical prostatectomy for clinically localized prostate cancer across multiple medical centers in Western Australia between 2000 and 2010. Samples from both cohorts were stored at -80°C. Paired normal DNA was sequenced from benign prostate tissue with no histological evidence of neoplasia.

For both cohorts, Hematoxylin and Eosin (H&E)-stained tissue sections were centrally reviewed by J-M. M., K.P. and M.A.R. to verify Gleason score and to determine the percentage of Gleason pattern 4 and 5 histology at the site selected for DNA extraction. To characterize the ethnic composition of the cohorts, we analyzed high-density SNP array data by principal component analysis in combination with data from cohorts of known ethnicity from the HapMap database (CEU, YRI, CHB/JPT; <http://hapmap.ncbi.nlm.nih.gov/>) (Supplementary Fig. 16). All but five individuals chosen for exome sequencing clustered with CEU HapMap samples, indicating that patients were predominantly of European descent. Four samples showed mixed or undetermined ethnicity and one clustered clearly with CHB/JPT (Han Chinese in Beijing/Japanese in Tokyo) HapMap samples.

Prostate tumor cohorts from University of Michigan (UM), University of Washington (UW) and University Hospital Zurich (UZH) were used for extension screening for SPOP mutation. Prostate samples from the UM cohort were obtained from the radical prostatectomy series at the University of Michigan and from the Rapid Autopsy Program⁵⁴, University of Michigan Prostate Cancer Specialized Program of Research Excellence Tissue Core (Ann Arbor, MI). Tumors from the UW cohort were obtained from the Rapid Autopsy Program, University of Washington and Fred Hutchison Cancer Research Center

University (Seattle, WA). Samples from the UHZ cohort included a series of radical prostatectomy specimens, metastases, and benign prostatic hyperplasia samples. H&E–stained slides of all specimens were reevaluated by two experienced pathologists (P.J.W., H.M.) to identify representative areas. Tumor stage and Gleason score of the Zurich cohort were assigned according to the International Union Against Cancer and World Health Organization/International Society of Urological Pathology criteria.

All samples were collected with informed consent of the patients and prior approval of the institutional review boards (IRB) of respective institutions. Additionally, the sequencing and data release of all exome- and transcriptome-sequenced samples was reviewed and approved by local IRB.

Correlation of mutation rates and pathological features

We investigated whether pathological features corresponded to different mutational spectra. Pathologic stage pT3 tumors contained more mutations than pT2 tumors ($P = 0.0012$, rank sum test) despite equivalent tumor purity between these classes (Supplementary Figs. 17, 18). Substitutions in *PTEN* and *PIK3CA* were enriched in pT3 tumors ($P = 0.011$, Fisher's exact test) (Supplementary Table 11), suggesting that these mutations may play a role in disease progression. Consistent with this possibility, activation of the PI3-Kinase pathway in mouse models accelerates the progression of prostate cancer^{61,62}. This finding will need to be extended to larger panels of tumors due to the relatively small number of *PTEN* and *PIK3CA* mutations reported here. Interestingly, the base mutation rate showed no correlation with Gleason score (a histological measure of disease risk) (Supplementary Fig. 18), indicating that mutational burden does not track uniformly with disease aggressiveness.

Distinct mutational characteristics of *TMPRSS2-ERG* fusion-positive tumors

We determined whether the mutational spectrum varied between prostate tumors harboring the *TMPRSS2-ERG* fusion and fusion-negative tumors. *TMPRSS2-ERG* fusion-positive tumors showed an increased proportion of CpG to T transitions ($P = 0.0002$, Supplementary Fig. 18) but did not harbor more mutations overall. Since CpG to T transitions can arise from deamination of methylcytosine in cancer, this trend may reflect the differential methylation of DNA between ETS fusion-positive and fusion-negative tumors that was recently reported⁶³ or may indicate a distinct mutagenic process in fusion-positive tumors.

Distinct genomic lesions co-occurred with the *TMPRSS2-ERG* fusion. *PTEN* mutation or deletion was significantly associated with *ERG* rearrangement ($P = 0.00042$) (Fig. 4), consistent with reports of collaboration between these two oncogenic events in mouse models^{64,65}. Tumors with *ERG* rearrangement were also enriched for p53 lesions ($P = 0.000025$) (Fig. 4). This relationship is consistent with other primary prostate cancer datasets ($P = 0.0044$)^{12,33}.

Somatic copy number alteration at the *SPOP* locus

Previous reports indicate that *SPOP* may function as an oncogene based on genomic amplifications in other cancers¹⁷ and protein overexpression in clear cell renal cell carcinomas²⁹. However, minimal somatic copy number aberrations are seen in the *SPOP* locus in primary prostate cancers from multiple cohorts, with no evidence of deletions in tumors with *SPOP* mutations (Fig. 1, Supplementary Fig 10)¹². In addition, *SPOP* mRNA expression was not up-regulated in prostate cancer samples from multiple cohorts, but was more frequently down-regulated compared to benign controls (Supplementary Fig. 11). We therefore conclude that the biological effect of *SPOP* mutations is not recapitulated by somatic copy number alterations in primary prostate cancer.

Mutual exclusivity of *SPOP* and *ERG* alteration within a single prostate

A single tumor was originally classified as positive for both *SPOP* mutation and *TMPRSS2-ERG* fusion. Interestingly, re-analysis of this case revealed two morphologically distinct tumors: one focus with wild-type *SPOP* and *ERG* rearrangement, and a second with the *SPOP*^{F133V} mutation and no *ERG* rearrangement (Supplementary Fig. 13). Therefore, the mutual exclusivity of these events is recapitulated in two tumor foci from a single prostate.

Low-frequency mutations in cancer-associated genes

Multiple genes with established roles in other cancers were mutated at low frequency, including *IDH1*, *AKT1* and *HRAS* (Supplementary Table 4). An analysis of predicted “damaging” mutations (nonsense substitutions, frame-shift indels and splice site alterations) in genes expressed in prostate tumors identified mutations in *APC*, *PIK3R1* and *EPHA7* (Supplementary Table 4). In addition, several chromatin-modifying enzymes harbored low-frequency damaging mutations, including *MLL1*, *MLL2*, *MLL3*, *ARID1A*, *NCOR1* and the histone demethylase gene *KDM6A* (*UTX*). Two *KDM6A* mutations involved residues situated within the catalytic Jumonji domain (I1209 and G1212), while a third introduced a frame-shift deletion directly N-terminal to this region (Supplementary Table 4). These findings underscore the emerging importance of chromatin-modifying genes in prostate cancer⁶⁶. Notably, *AR* was not mutated in any primary tumor analyzed, consistent with prior studies suggesting that mutations in this gene are restricted to metastatic or castration-resistant disease^{7,12}.

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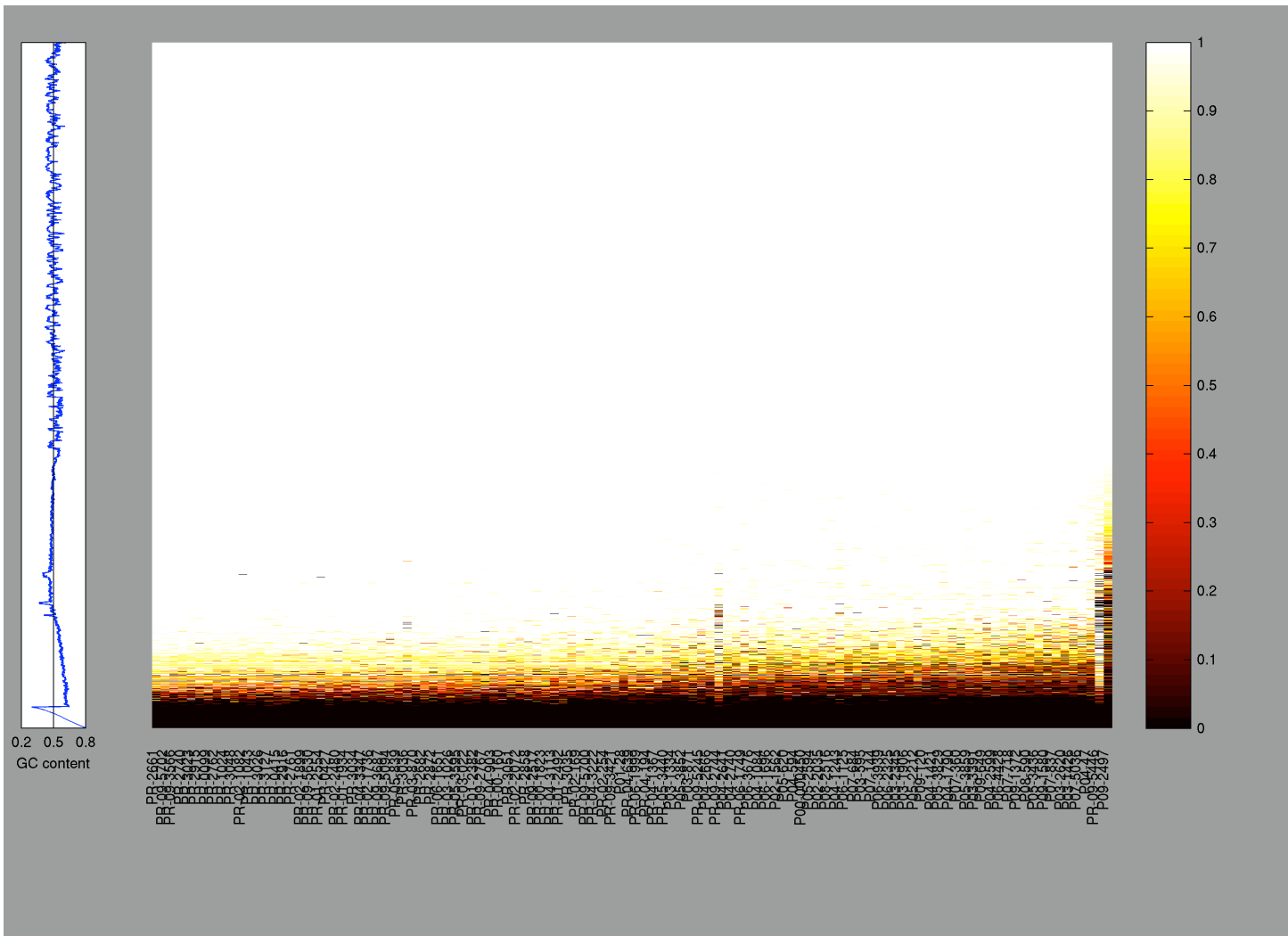
Supplementary Table 8. Somatic Copy Number Alterations Associated with *SPOP* Mutation

Supplementary Table 9. Primer Sequences

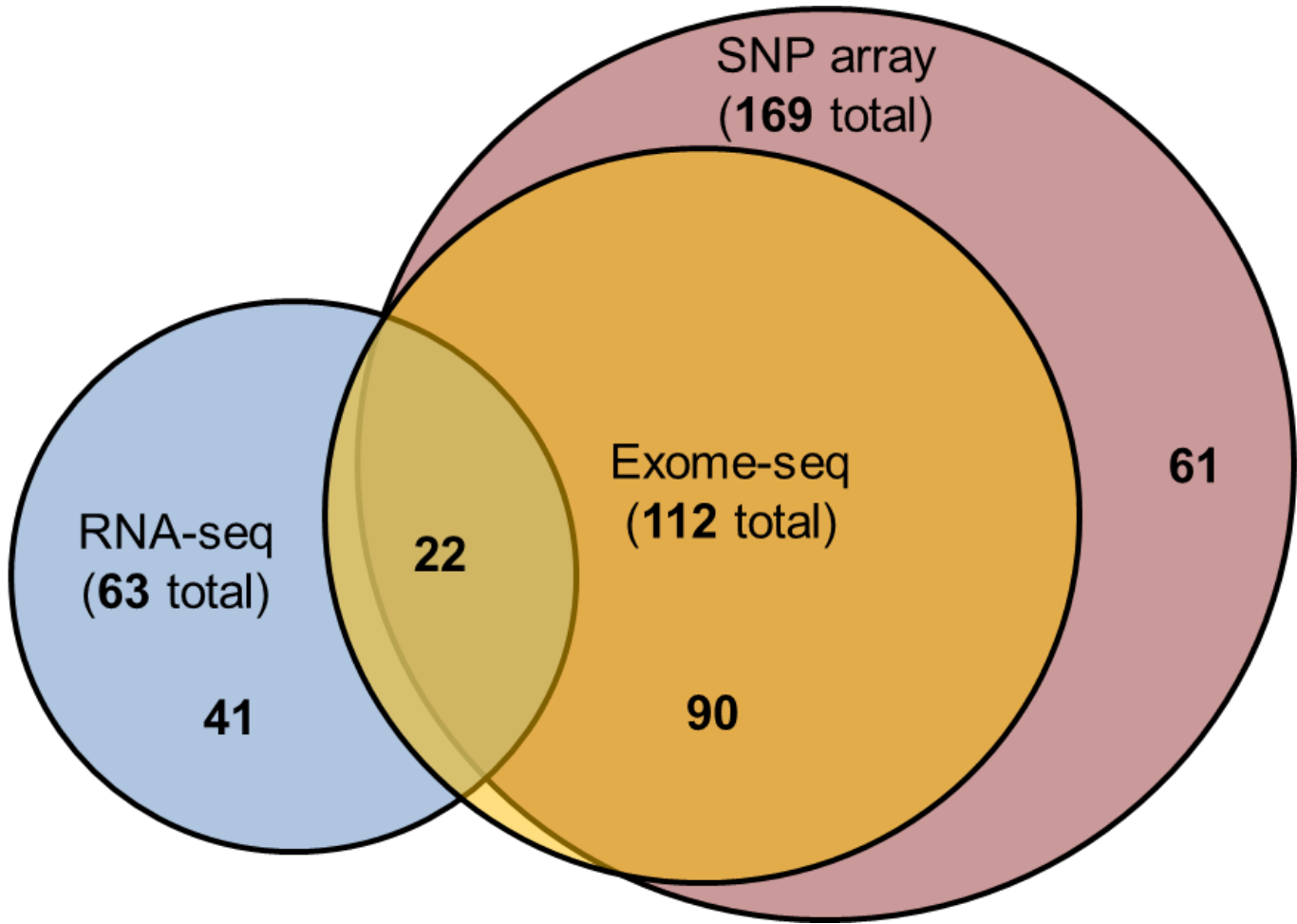
Supplementary Table 10. BAC Probes Used for FISH

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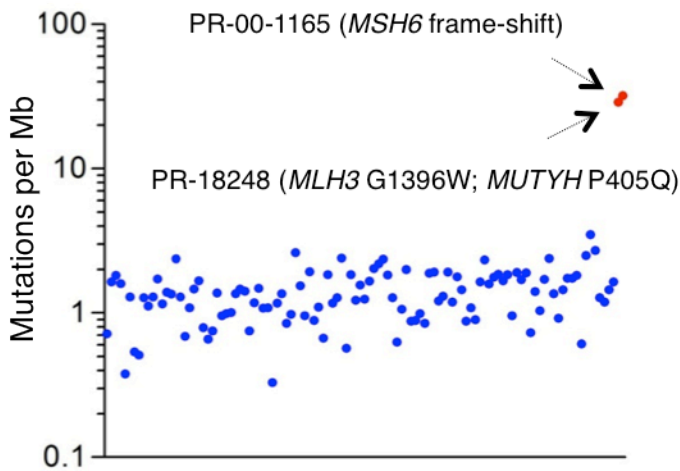
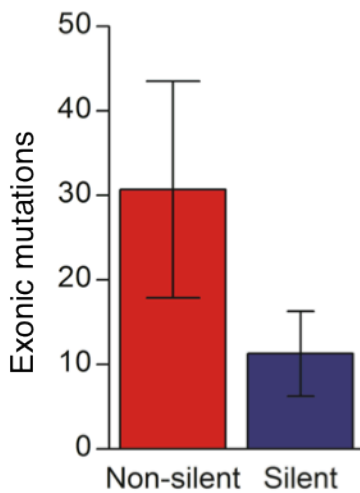
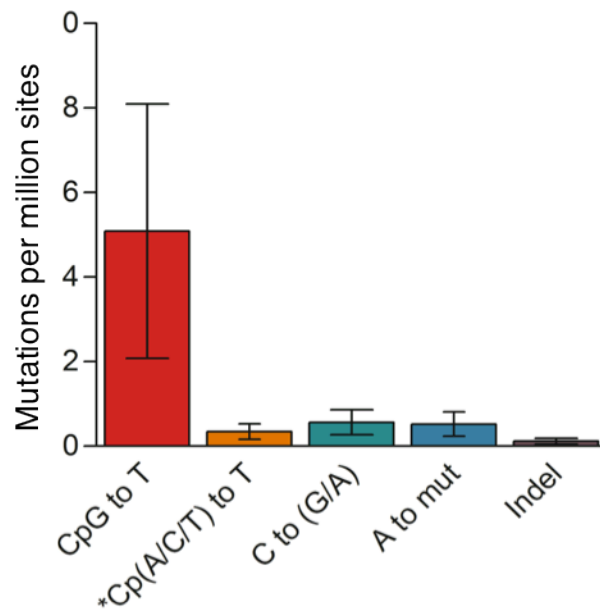
Supplementary Table 12. Copy Number Alterations in 112 Exome-Sequenced Prostate Tumors (seg file format)



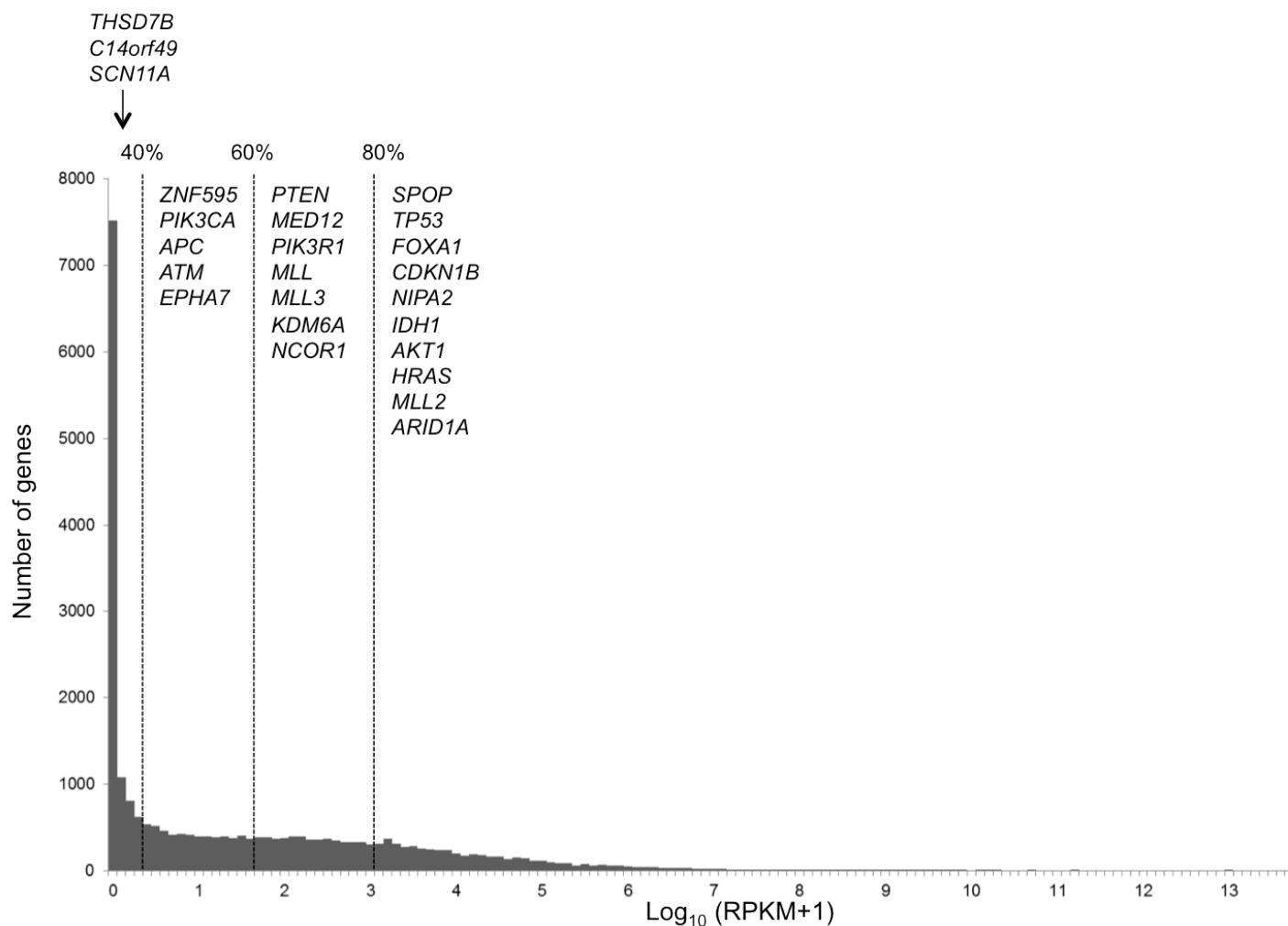
Supplementary Figure 1. Depth and Breadth of Exome Sequencing Coverage. (Center) Sequencing coverage across all sites targeted by hybrid capture. Each row represents a targeted exonic site; each column represents a tumor-normal pair. Coloring reflects the depth of sequencing coverage. White coloring indicates a minimum of 14 reads in the tumor and 8 reads in the normal. (Left) GC content across targeted sites (GC content is equal to the number of C or G nucleotides divided by the total number of nucleotides).



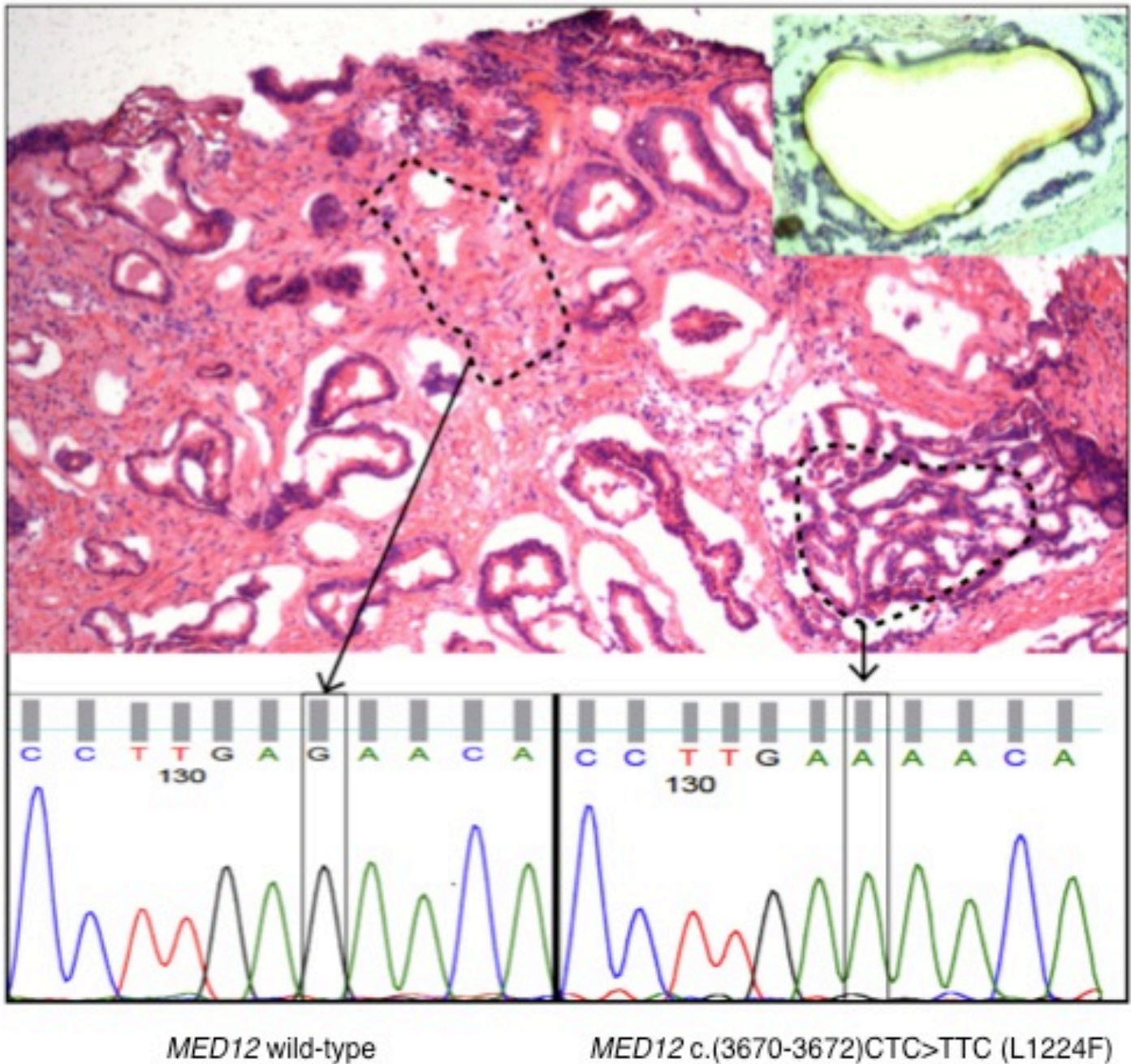
Supplementary Figure 2. Overlap of sample profiling across platforms. Exome sequencing was conducted on 112 tumor-normal pairs. A single highly-mutated tumor (PR-00-1165) was excluded from subsequent analyses, except where otherwise indicated, leaving 111 pairs. RNA-sequencing was performed on 22 of the exome-sequenced tumors and 41 independent tumors. All but four of the 112 exome-sequenced tumors, plus an additional 61 tumors, were analyzed for copy number alteration by high-density SNP array (169 total).

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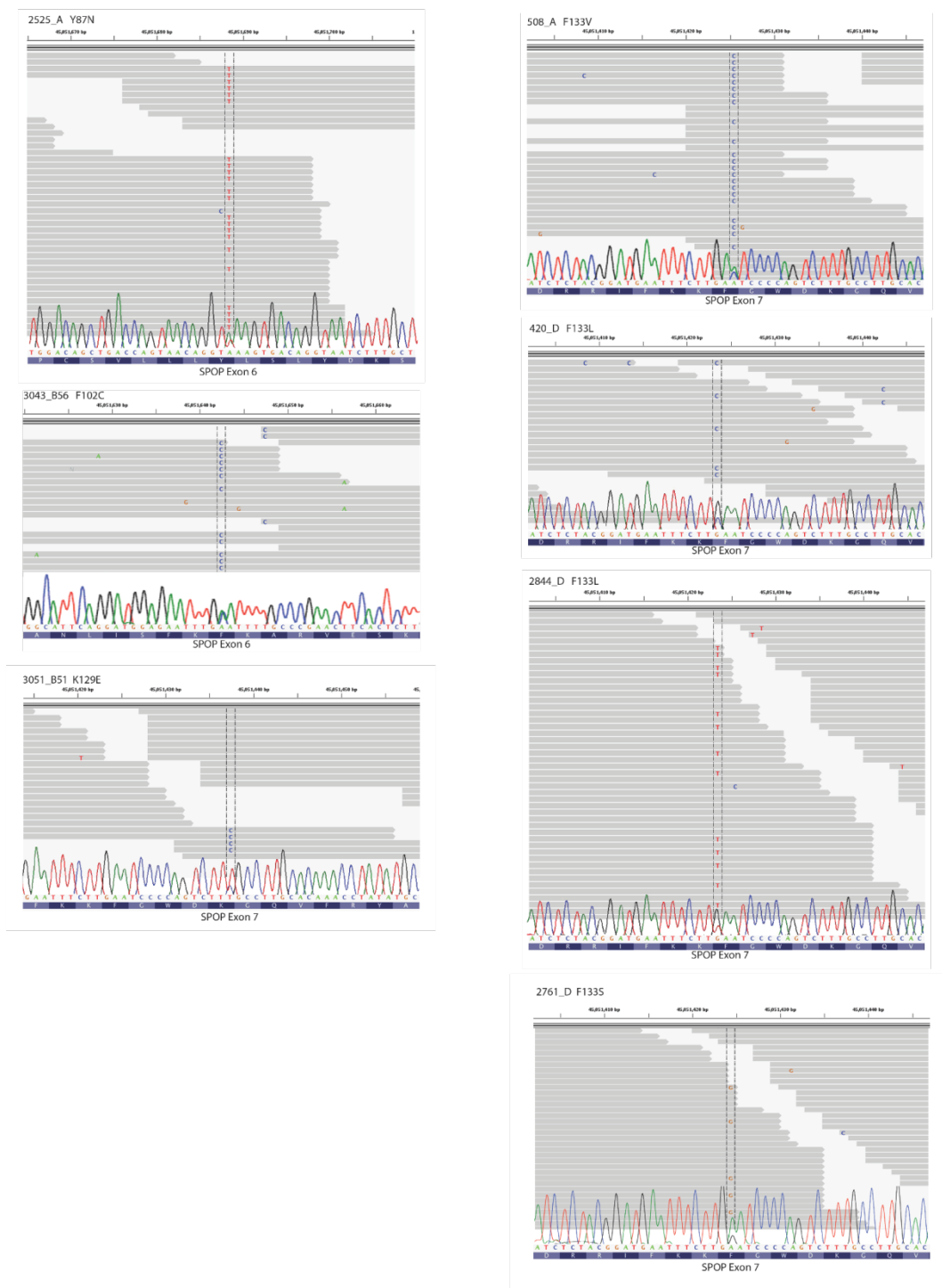
Supplementary Figure 3. Rates of somatic substitutions in prostate exomes. (A) Number of somatic mutations per Mb sequenced across the cohort of tumors. A single primary tumor (PR-00-1165) harbored a large excess of mutations compared to other tumors (32.1 per Mb versus 1.4 per Mb median in the remaining primary tumors, red). A prostate cancer metastasis sequenced but not reported here showed a similar extent of mutation (PR-18248; 29.0 mutations per Mb, red). The two highly mutated tumors contained the indicated alterations in DNA mismatch repair genes. (B) Median number of non-synonymous and synonymous mutations across 111 exomes (the single hyper-mutated primary tumor PR-00-1165, with 997 mutations, is excluded). (C) Mutations per million sites sequenced for the most frequent mutation categories in the dataset. *CpG to T, C to T transversion at a CpG dinucleotide; *CpG to A/C/T, C to T transversion not in the context of a CpG dinucleotide; C to (G/A), mutation of C to G or A; A to mut, mutation of A; Indel, small insertion or deletion. Error bars indicate standard deviation.



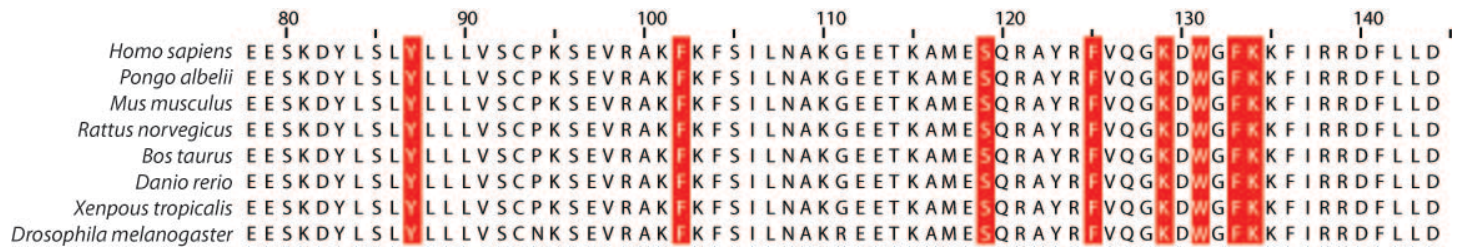
Supplementary Figure 4. Expression levels of select mutated genes. Significantly-mutated genes and selected genes listed in Supplementary Table 4 were analyzed for level of transcript expression in the RNA-seq dataset. The histogram shows the number of transcripts with a given value of $\log_{10}(\text{RPKM}+1)$ (where RPKM is the number of reads per kilobase of exon per million mapped sequence reads), binned by increments of 0.1. The RPKM provides an estimate of the relative expression of transcripts. Vertical lines indicate the percentile of $\log_{10}(\text{RPKM}+1)$ among all transcripts. Listed genes are grouped based on their percentile of $\log_{10}(\text{RPKM}+1)$ value: <40%, 40-60%, 60-80% and >80%. Values and percentiles are listed in Supplementary Table 4.



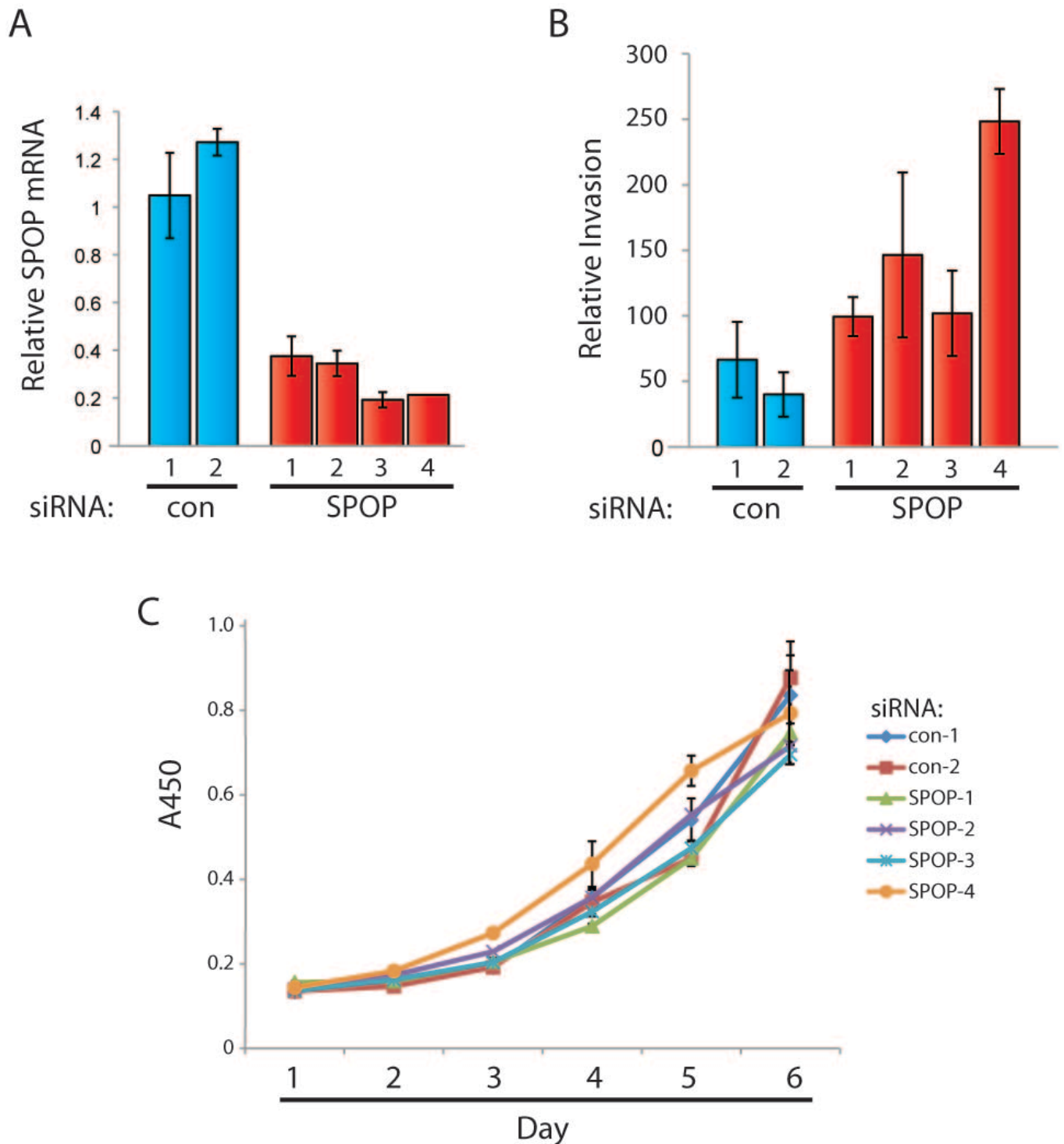
Supplementary Figure 5. Laser capture microdissection and sequencing of *MED12*. (Top) Laser capture micro-dissection and Sanger sequencing was performed on *MED12*-mutant tumors to determine whether the mutations were present in epithelial or stromal cells. H&E slide of frozen tissue from a *MED12*-mutant tumor (PR-3026) showing adenocarcinoma and surrounding mixed stroma. Exome sequence reads demonstrated an L1224F mutation in exon 26 of *MED12*. Laser capture micro-dissection was performed to separate epithelium from stroma (inset). (Bottom) The selected stromal area (dashed line, left) demonstrates wild-type *MED12* sequence by Sanger sequencing, while the dissected tumor gland (dashed line, right) exhibits the L1224F mutation.



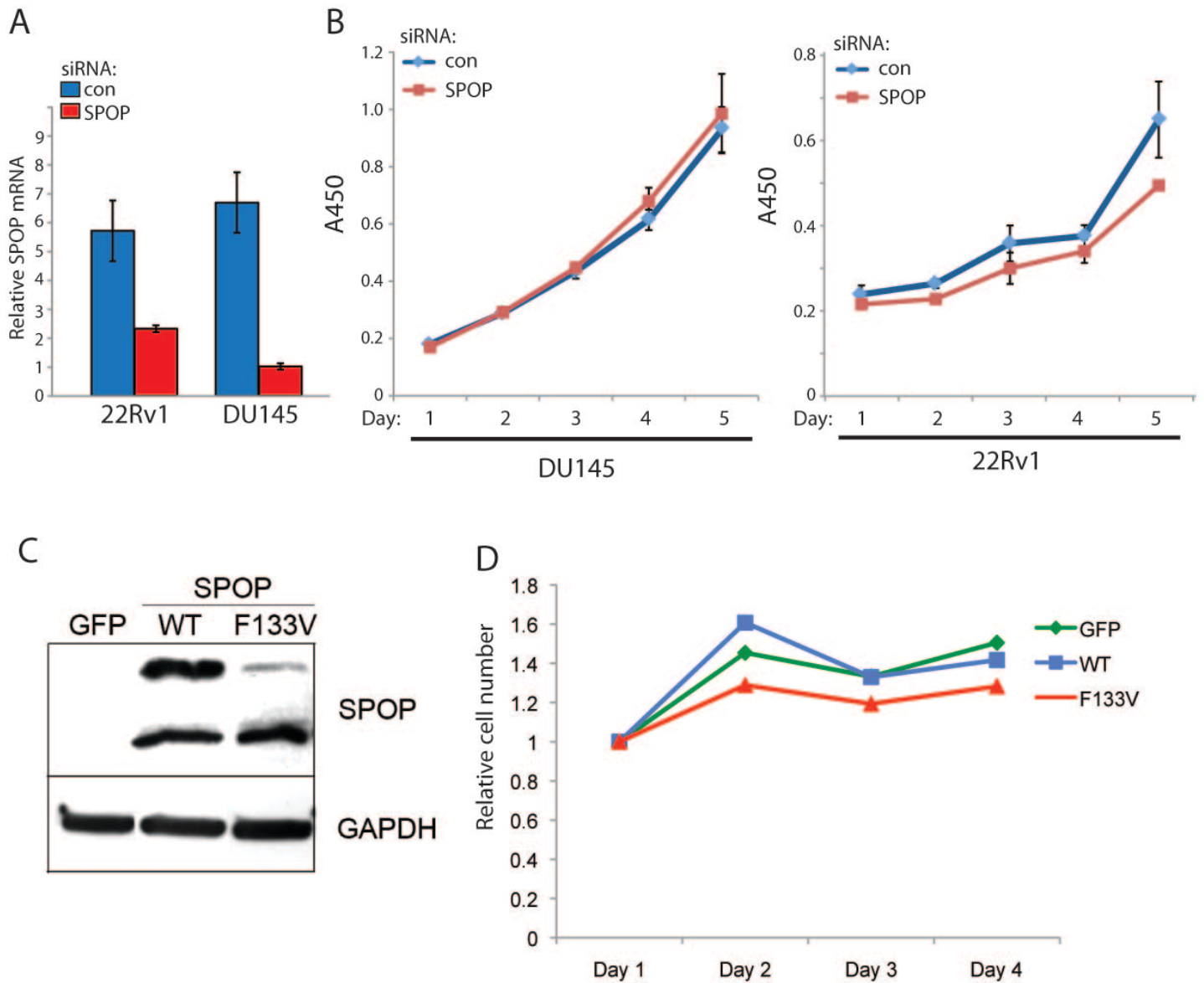
Supplementary Figure 6. Mutations in *SPOP* in RNA-seq data and Sanger sequencing of genomic tumor DNA. In each panel, RNA-seq reads mapping to *SPOP* Exon 6 or 7 from the indicated sample are shown. Coordinates (hg18) on chromosome 17 are at the top of each panel, and the reference genome (hg18) and wild-type *SPOP* amino acids are displayed at the bottom. Each horizontal gray bar represents one read. Nucleotide mismatches with respect to the reference genome in each read are highlighted by displaying the mismatched base. The Sanger tracing of genomic DNA from the same tumor focus is overlaid below the RNA-seq reads.



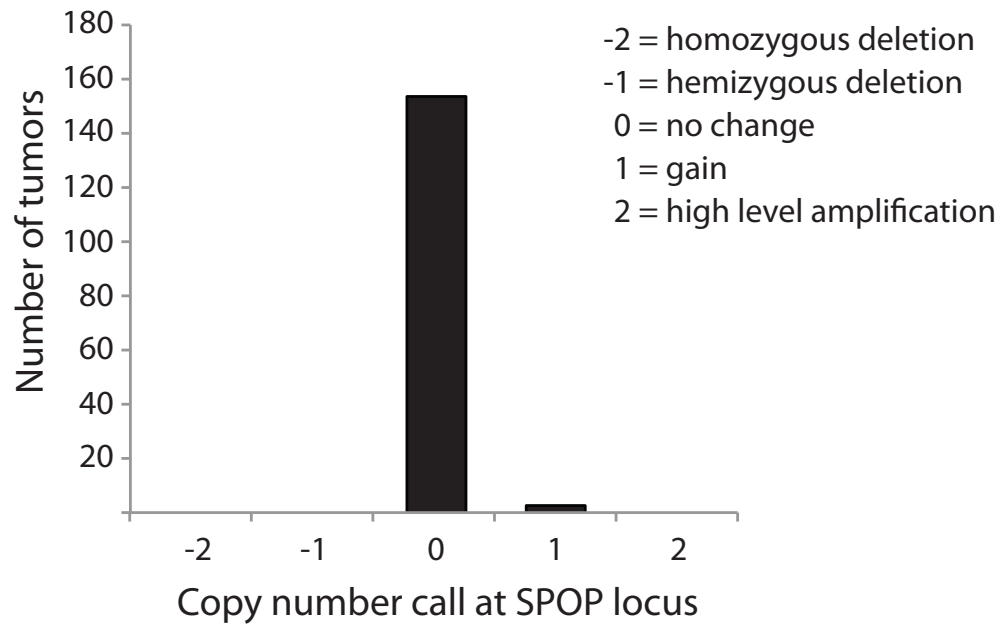
Supplementary Figure 7. Multiple Sequence Alignment of the MATH domain of SPOP across species. Multiple sequence alignment was performed with ClustalW2 and visualized using Jalview. Residues mutated in prostate cancer (Y87, F102, S119, F125, K129, W131, F133, K134) are highlighted.



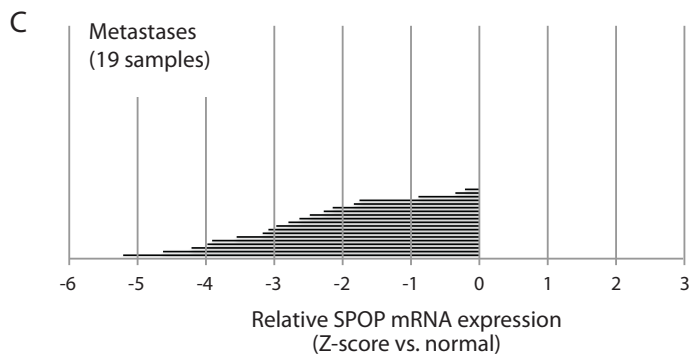
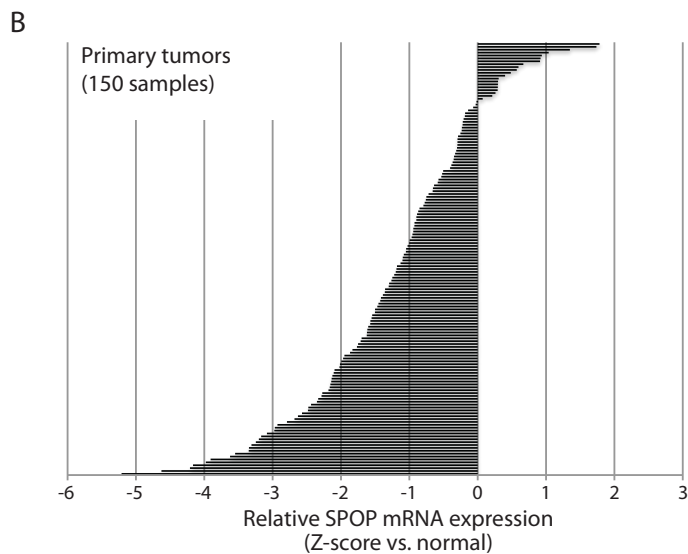
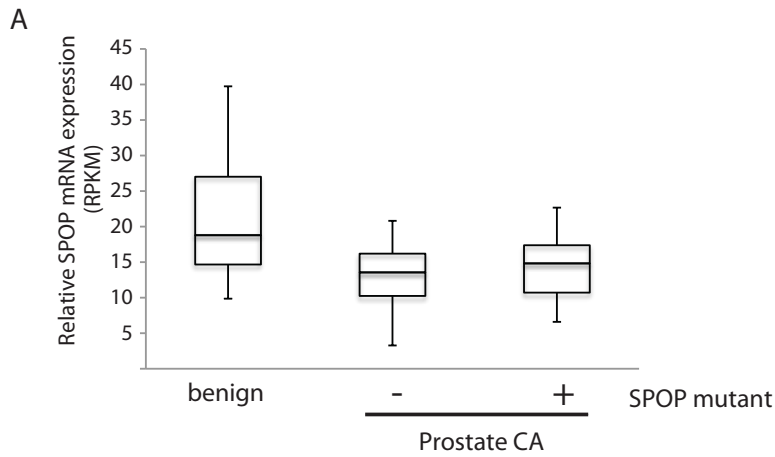
Supplementary Figure 8. Multiple independent siRNAs targeting *SPOP* have similar effects in prostate cell lines. (A) Expression of *SPOP* mRNA in DU145 cells transfected with 2 different control siRNAs and 4 different *SPOP* siRNAs, normalized to *GAPDH* expression, by real-time RT-PCR. (B) Quantitation of invaded DU145 cells transfected with control and *SPOP* siRNAs in Matrigel invasion assays. (C). Growth curves of DU145 cells transfected with control and *SPOP* siRNAs, measured with WST-1 assay.



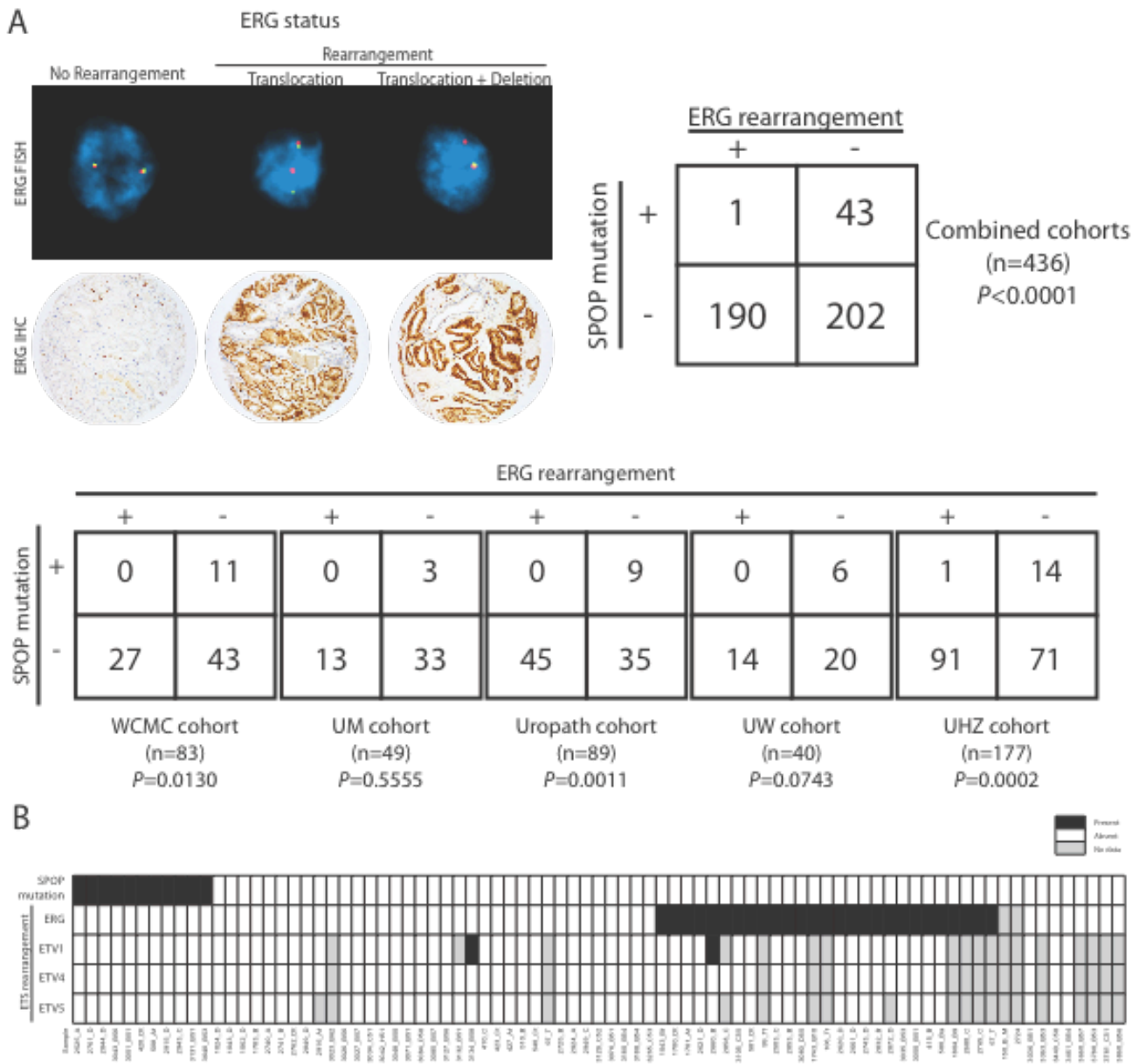
Supplementary Figure 9. Transfection with SPOP siRNA or SPOP mutant does not affect cell growth or viability. (A) Expression of SPOP mRNA in 22Rv1 and DU145 cells transfected with control and SPOP siRNA, normalized to GAPDH expression, by real-time RT-PCR. (B) Growth curves of 22Rv1 and DU145 cells transfected with control and SPOP siRNA, measured with WST-1 assay. (C) Western blot showing SPOP expression in DU145 cells transfected with SPOP wt and F133V. (D) Growth curves of DU145 cells transfected with SPOP wt and F133V, measured with WST-1 assay.



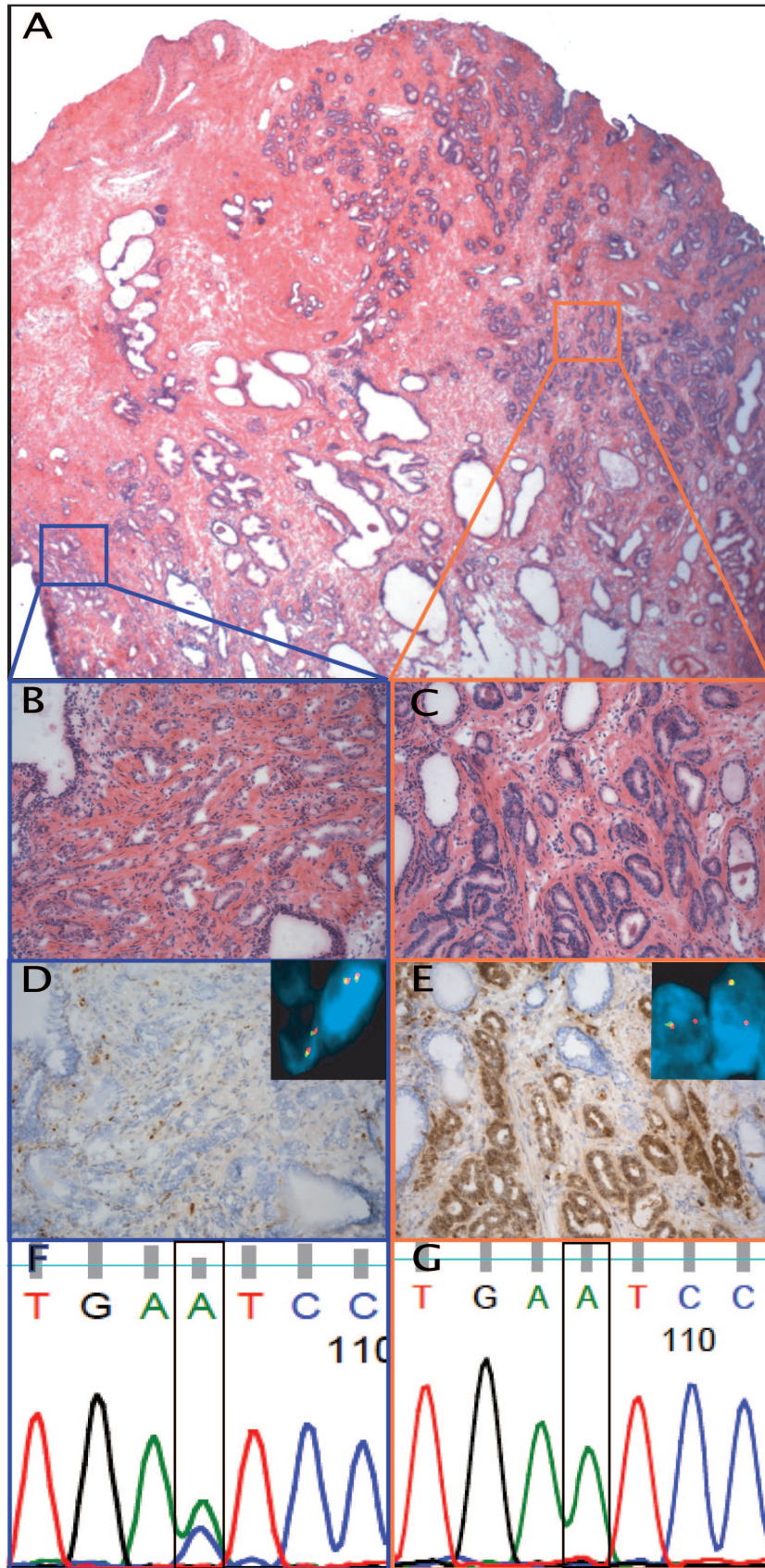
Supplementary Figure 10. Minimal changes in *SPOP* copy number in primary prostate cancer. Discretized copy number calls at SPOP locus in 157 primary prostate cancers, from a publicly available dataset (www.cbioportal.org/cgx/).¹²



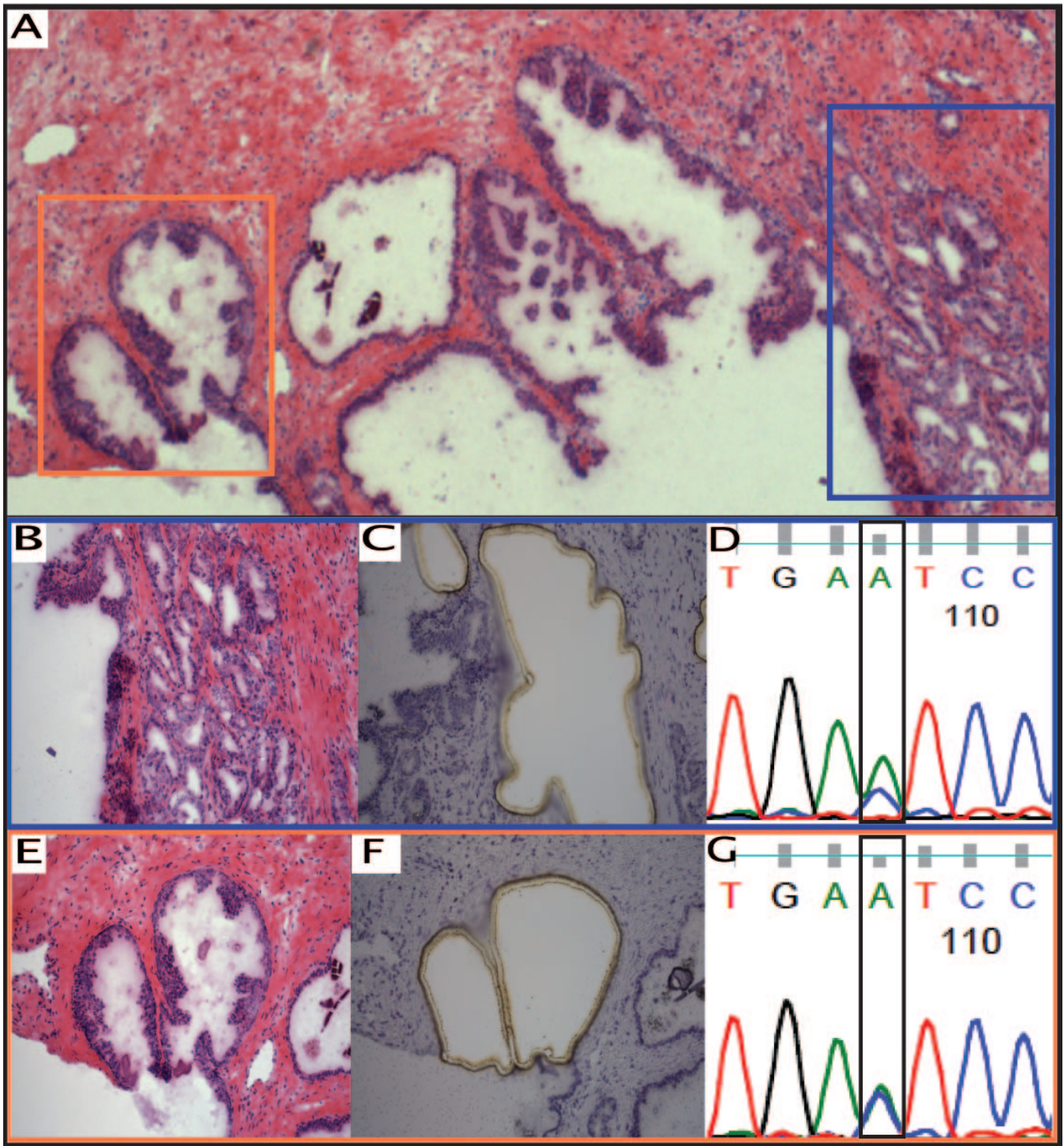
Supplementary Figure 11. *SPOP* is not upregulated in prostate cancer. (A) *SPOP* mRNA expression measured by RNA-seq in 6 benign prostate samples and 53 prostate cancers (7 *SPOP* mutant, 46 *SPOP* wt). Relative expression is displayed as reads Per kilobase per million mapped reads (RPKM). (B,C) *SPOP* mRNA expression from a publicly available dataset (www.ncbi.nlm.nih.gov/geo/)¹² in 150 primary prostate cancers (B) and 19 metastases (C). Relative expression is displayed as Z-score versus matched normal; positive = increased expression, negative = decreased expression.



Supplementary Figure 12. Tumors with *SPOP* mutation lack *ETS* rearrangements. (A) Relationship of *SPOP* mutation and *ERG* rearrangement. *ERG* rearrangement was determined by FISH and IHC. (B) Heatmap showing *SPOP* mutation status and rearrangement status of *ETS* genes in WCMC cohort.

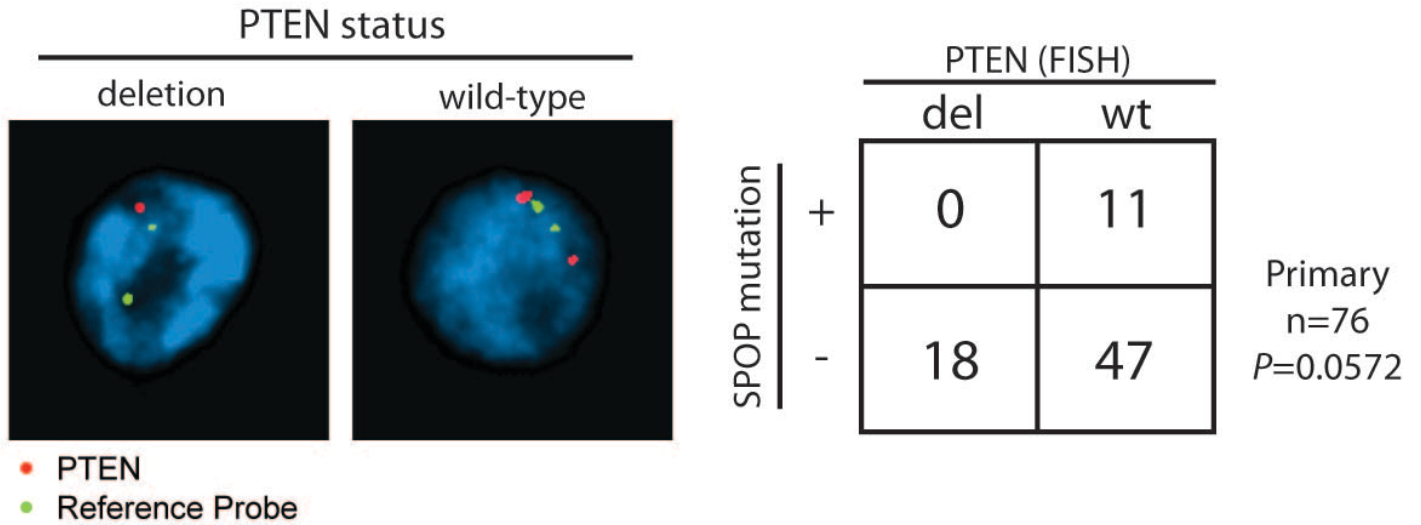


Supplementary Figure 13. Separate foci of prostate adenocarcinoma with mutually exclusive ERG-rearranged and SPOP-mutated status. (A) Low power view of two distinct foci of prostate adenocarcinoma in a prostatectomy specimen (H&E stained slide of frozen tissue, original magnification 2x). (B) The tumor on the left side (blue box) has Gleason score 3+4=7, is ERG-negative by immunohistochemistry (D) without *ERG* rearrangement by FISH (inset), and demonstrates F133V *SPOP*-mutation (F). (C) The tumor on the right side (orange box) has Gleason score 3+3=6, is ERG-positive by immunohistochemistry (E) with *ERG*-rearrangement by FISH (inset), and demonstrates *SPOP* wild-type sequence (F). H&E and immunohistochemistry for ERG in slides of frozen tissue, original magnification 20x; *ERG* break-apart fluorescent *in situ* hybridization assay, and *SPOP* DNA sequence by Sanger sequencing.

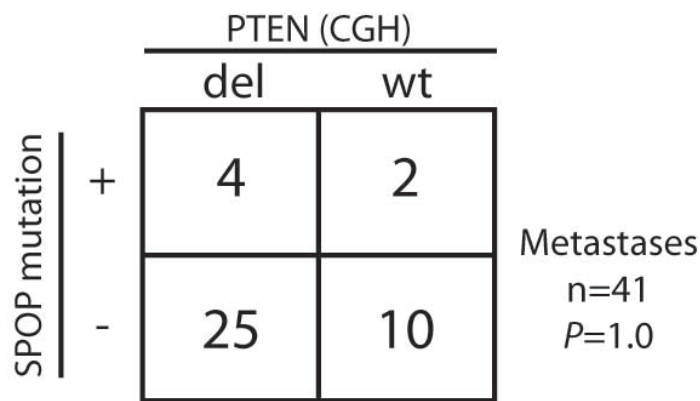


Supplementary Figure 14. Detection of *SPOP* mutation in high-grade prostatic intraepithelial neoplasia (HGPIN). (A) Low power view of prostate adenocarcinoma (blue box) and HGPIN (orange box) in a prostatectomy specimen. Cancer area before (B) and after (C) Laser Capture Microdissection (LCM). Images of HGPIN before (E) and after (F) LCM. DNA sequence demonstrates F133V *SPOP*-mutation in both adenocarcinoma (D) and HGPIN (G). H&E stained slide of frozen tissue, original magnification 10x; *SPOP* DNA sequence by Sanger sequencing.

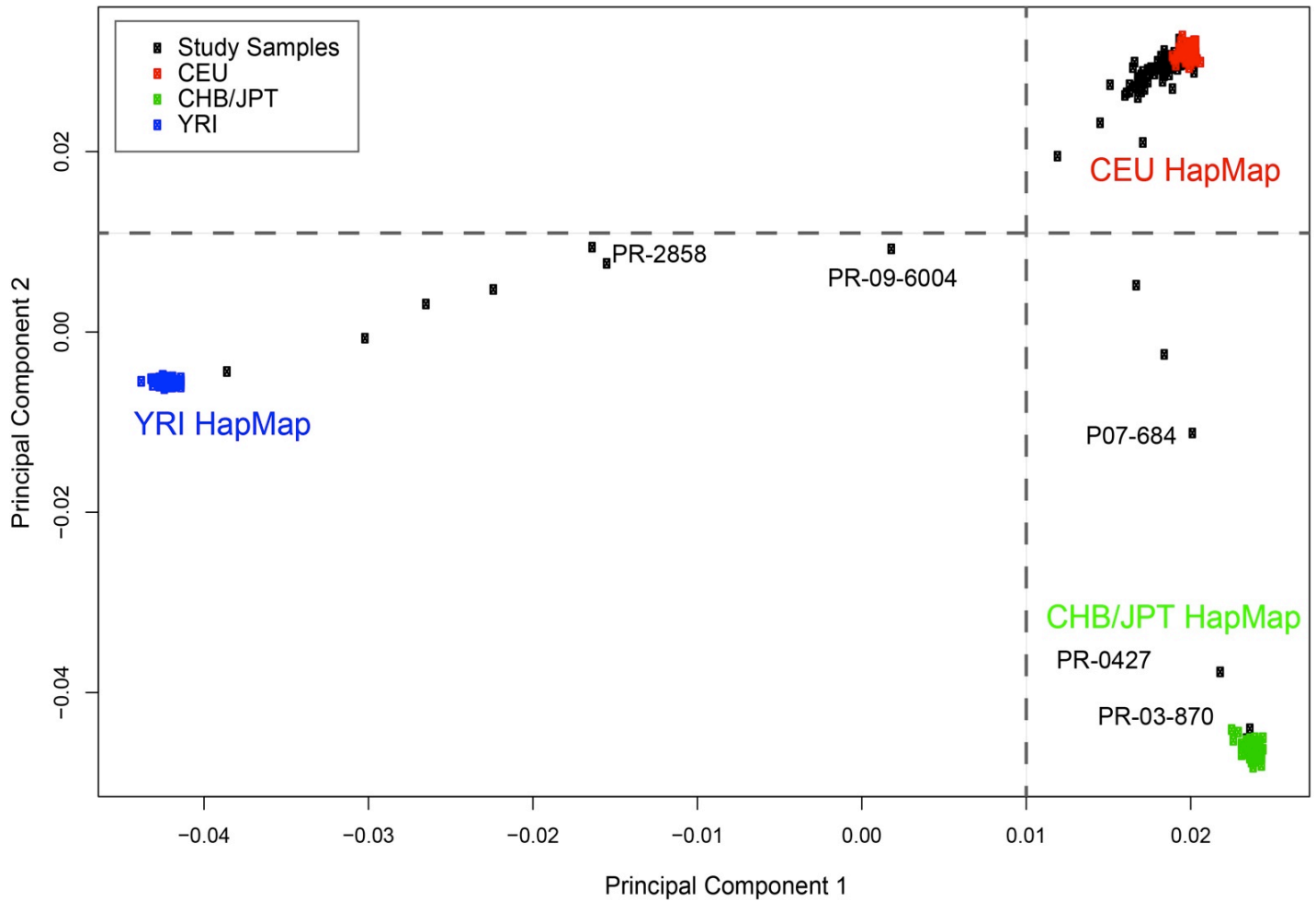
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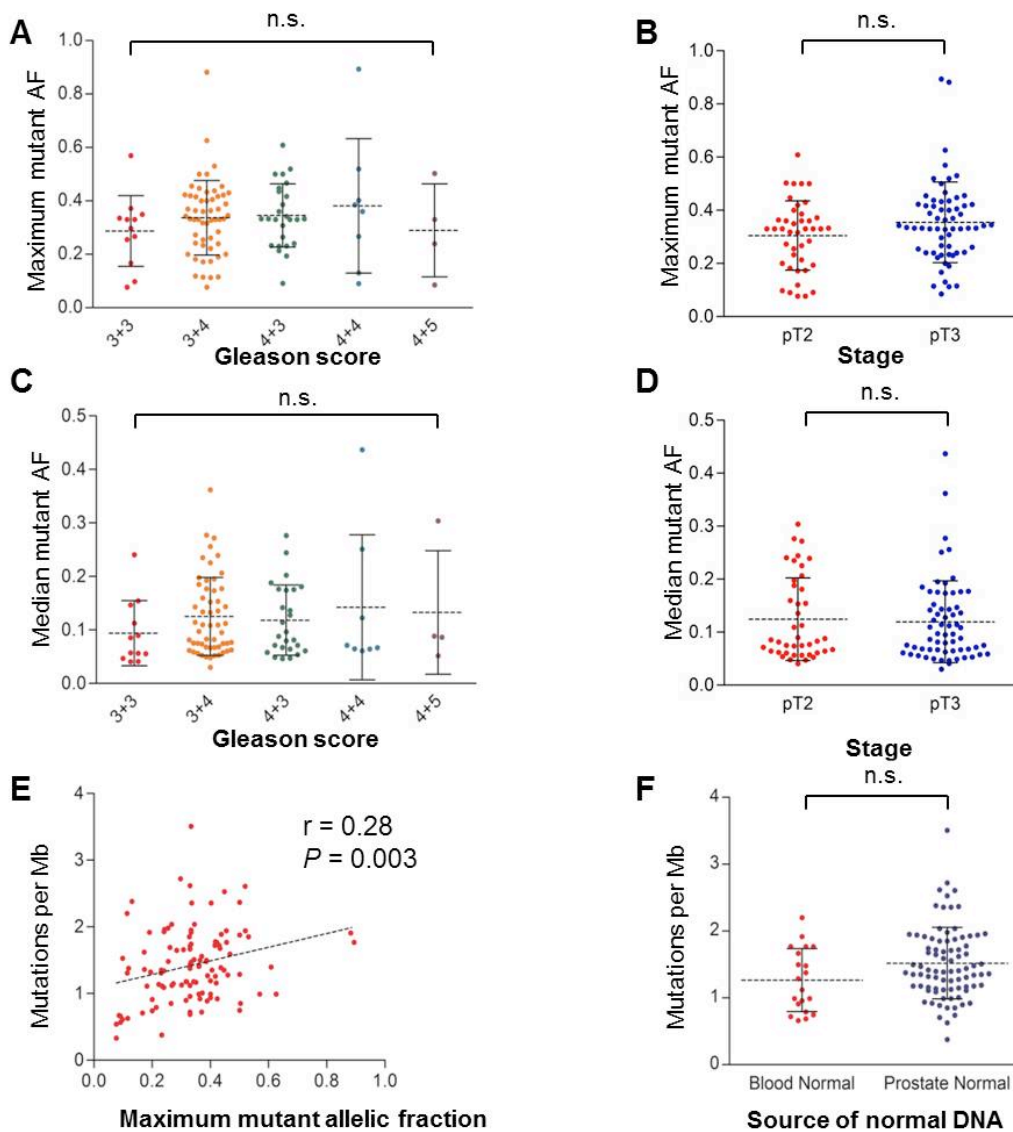
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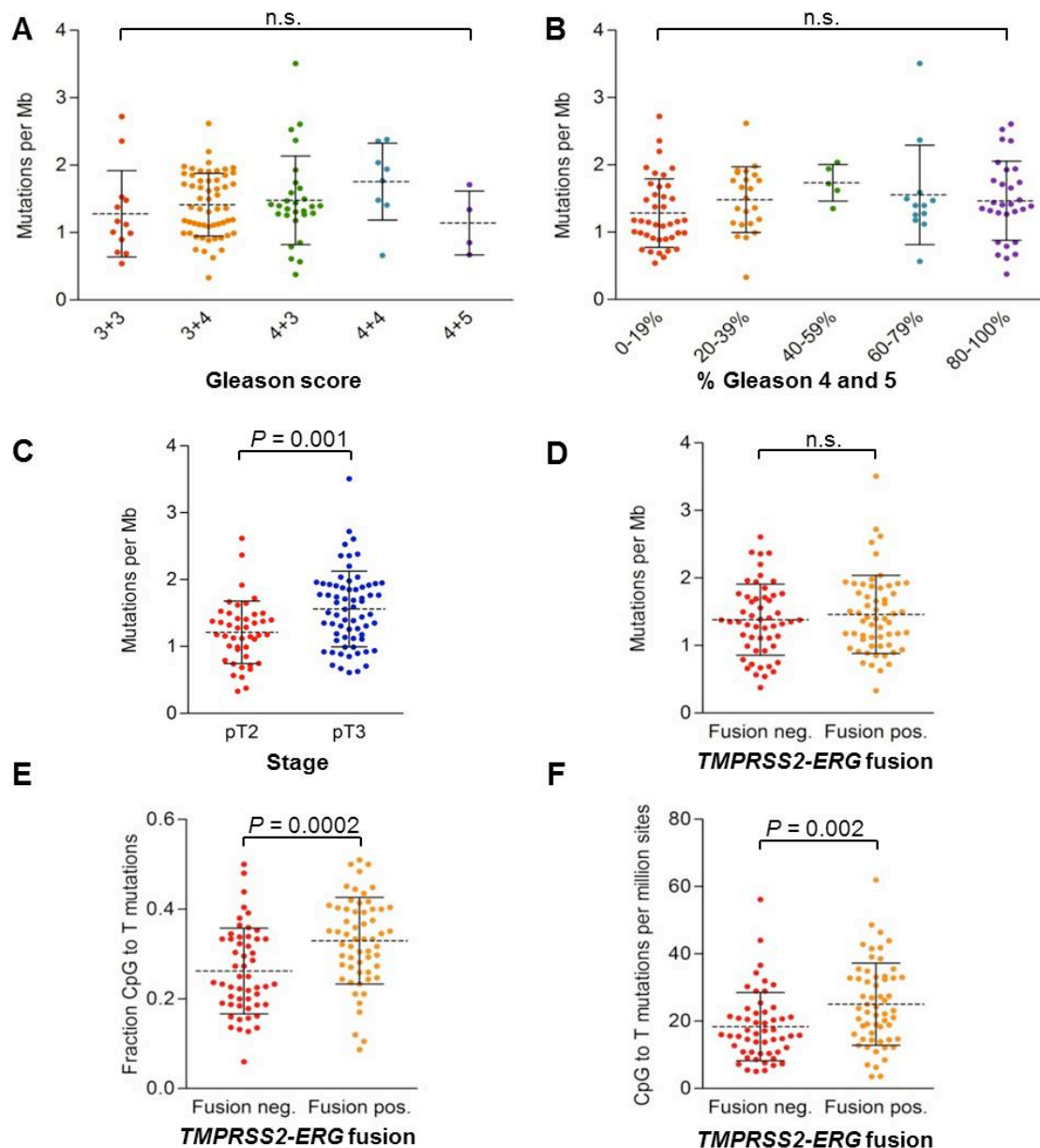
Supplementary Figure 15. Tumors with SPOP mutation lack PTEN deletion in primary but not metastatic prostate cancer. (A) Relationship of *SPOP* mutation and *PTEN* deletion determined by FISH in primary prostate cancers from the WCMC cohort. (B) Relationship of *SPOP* mutation and *PTEN* deletion determined by CGH in prostate cancer metastases from the UW cohort.



Supplementary Figure 16. Ethnicity analysis of exome-sequenced DNA. Principal component analysis was performed to assess the origin of the study individuals using SNP array data. HapMap Phase II samples representing three distinct populations, European (CEU) (red), Yoruban (YRI) (blue) and Chinese/Japanese (CHB/JPT) (green), were included in the analysis. The study identifiers of the exome-sequenced individuals whose genetic profiles deviate from the CEU pattern are shown.



Supplementary Figure 17. Relative ability to detect mutations in subgroups of tumors. (A-D) The allelic fraction (AF) values of mutations were used to characterize the relative purity of cancer DNA in each tumor. (A) and (B), maximum mutant AF observed in each tumor, grouped by Gleason score and stage. The top fifth percentile of AF values was removed in each tumor to exclude values that were elevated due to copy number variation at mutated sites. (C) and (D), as in (A) and (B), but showing median AF values across all mutations for each tumor. The relative purity of cancer DNA as assessed by AF did not vary by pathological stage or Gleason score. (E) Maximum mutant AF correlated only moderately with the number of mutations detected, implying that detection of mutations in most tumors was not limited by admixture of normal DNA. (F) Mutations per Mb sequenced in tumors grouped by source of paired normal DNA: peripheral blood (n=22) or non-cancerous prostate (n=89). No difference in mutation rates was observed between the two groups. Two-tailed p values were calculated using the Mann Whitney (two groups) or Kruskal-Wallis (multiple groups) tests and the Spearman test for correlation. Error bars indicate standard deviation. n.s., not significant. Statistical analysis was performed using GraphPad Prism.



Supplementary Figure 18. Mutational landscape across a spectrum of primary prostate cancers. (A-D) Mutations per Mb of covered DNA sequence for 111 primary prostatic adenocarcinomas grouped by clinical parameters. The horizontal axes denote: (A) Gleason score (major pattern + minor pattern); (B) Percent of cancer with Gleason pattern 4 or 5 histology; (C) Pathological tumor stage, where T3 indicates extra-prostatic extension; (D) Presence or absence of the *TMPRSS2-ERG* fusion based on fluorescence in-situ hybridization (FISH). Mutation rates are higher in pT3 tumors but do not vary by Gleason pattern or *TMPRSS2-ERG* fusion status. (E) Fraction of mutations in each tumor that are C to T transitions at CpG sites. (F) Number of CpG to T transitions per million CpG sites. Both the number and proportion of CpG to T mutations is increased in *TMPRSS2-ERG* fusion positive tumors. Two-tailed p values from the Mann Whitney test (two groups) or Kruskal-Wallis test (multiple groups) are indicated for each comparison. Statistical analysis was performed using GraphPad Prism. Error bars indicate standard deviation. n.s., not significant.

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Supplementary Table S1A. Clinical Characteristics of Exome-Sequenced Primary Prostate Cancers

Summary of exome-sequenced patient cohort

Characteristic	Whole exome-sequenced tumors	
Age, years		
Median (range)	63	(34 – 77)
Pre-operative Serum PSA (ng/μL)		
Median (range)	7.8	(2.7 – 31.5)
Pathologic Stage, N %		
Stage pT2 Total	44	39%
Stage pT2a	4	4%
Stage pT2b	1	1%
Stage pT2c	39	35%
Stage pT3 Total	68	61%
Stage pT3a	49	44%
Stage pT3b	19	17%
Gleason Pattern (major + minor), N %*		
Gleason 3+3	13	12%
Gleason 3+4	58	52%
Gleason 4+3	29	26%
Gleason 4+4	8	7%
Gleason 4+5	4	4%
Percentage of Gleason Pattern 4 and 5, N %*		
0-19%	40	36%
20-39%	23	12%
40-59%	5	5%
60-79%	12	11%
80-100%	29	26%
TMPRSS2-ERG Fusion Status, N % †		
Fusion-negative	53	48%
Fusion with interstitial deletion	34	31%
Fusion without interstitial deletion	24	22%

* Gleason scores based on review of hematoxylin and eosin slides from site of tumor chosen for DNA extraction and sequencing

† *TMPRSS2-ERG* fusion status assessed by fluorescence *in situ* hybridization (FISH)

Supplementary Table S1B. Clinical Characteristics of Exome-Sequenced Primary Prostate Cancers

Clinical information for individual patients

Patient	Age	Gleason Score	GS % 4 and 5	Stage	Serum PSA		TMPRSS2-ERG Fusion Status	mod CAPRA-S *
					(ng/mL)			
P00-000450	59	4+3	80	pT3a	8.3		Positive with interstitial deletion	4
P01-28	61	4+4	100	pT3a	14		Negative	6
P02-1562	67	4+3	95	pT3b	5.4		Negative	5
P02-2035	63	3+4	20	pT2c	5.8		Positive	1
P03-1334	69	3+4	2	pT3a	5.6		Negative	2
P03-1426	69	4+3	60	pT2c	13.9		Positive	4
P03-1906	75	3+4	30	pT3b	12.2		Positive	6
P03-2345	56	3+4	10	pT2c	7		Positive with interstitial deletion	2
P03-2620	68	3+4	20	pT3a	12.5		Negative	4
P03-3391	62	3+3	0	pT3b	18.6		Positive	5
P03-595	58	4+5	100	pT3a	24		Positive with interstitial deletion	7
P03-871	59	4+3:5	60	pT2c	6.5		Positive	3
P04-1084	65	3+4	40	pT3a	22		Negative	5
P04-1243	63	3+4	30	pT3a	7.3		Positive with interstitial deletion	3
P04-1421	54	3+4	20	pT2a	8.4		Positive	2
P04-1790	56	4+3:5	75	pT3a	9		Positive with interstitial deletion	4
P04-2599	68	4+3	60	pT2c	6.9		Negative	3
P04-2641	56	3+4	20	pT3a	6		Negative	3
P04-2666	58	4+5:3	95	pT3a	5		Negative	4
P04-2740	71	4+3	70	pT3a	11		Positive	5
P04-47	55	4+3:5	82	pT2b	14.6		Negative	4
P04-594	66	3+4	5	pT3a	8.5		Negative	3
P05-2212	66	3+4	30	pT3a	9.3		Positive with interstitial deletion	3
P05-2594	60	3+4	20	pT3b	13.5		Negative	6
P05-3436	62	4+3	80	pT2c	5.3		Positive	2
P05-3829	59	3+3	0	pT2c	10.8		Negative	2
P05-3852	62	4+3:5	60	pT3a	12.5		Negative	5
P05-3859	68	4+3	80	pT3a	8.2		Negative	4
P05-620	59	4+3	90	pT3a	5.8		Negative	3
P06-1125	66	3+4	30	pT3a	5.6		Positive with interstitial deletion	2
P06-1696	64	4+3:5	80	pT3a	4.6		Negative	4
P06-2325	62	4+3	70	pT2c	6.8		Negative	3
P06-3676	59	3+4	15	pT3a	4.8		Positive	2
P06-3939	71	4+5	100	pT3bN1	8.3		Negative	8
P06-4428	47	3+4	20	pT3a	31.5		Positive with interstitial deletion	5
P07-144	65	3+4	30	pT3a	2.7		Negative	2
P07-360	57	4+4	100	pT2c	16.7		Negative	5
P07-5036	52	3+4:5	60	pT3a	5.2		Positive	2
P07-684	58	4+3	90	pT3b	9.4		Negative	6
P07-718	57	3+4	5	pT2c	6.4		Negative	2
P07-837	69	4+3	80	pT3a	7.8		Negative	4
P08-2516	75	4+4	100	pT3b	9		Negative	7
P08-590	65	3+4	5	pT2c	9.2		Positive with interstitial deletion	2
P09-120	61	3+4	5	pT2c	6		Positive	1
P09-1372	73	3+4	5	pT3a	6.2		Positive with interstitial deletion	3
P09-1580	62	3+4:5	50	pT3a	5.2		Positive	2
P09-2497	62	3+3	0	pT3a	8		Positive with interstitial deletion	2
P09-649	71	4+3	80	pT2c	8.1		Negative	3
PR-00-1165	54	3+4	5	pT3b	6.7		Negative	5
PR-00-160	60	3+4	40	pT2c	9.4		Negative	2
PR-00-1823	60	3+4:5	12	pT3a	6.9		Positive with interstitial deletion	3
PR-0099	70	3+4	10	pT3a	10.3		Positive	4
PR-01-1934	59	3+4	20	pT3b	6.7		Negative	4
PR-01-2382	50	3+4	10	pT3a	6.2		Positive	4
PR-01-2492	67	3+4	10	pT3a	Not known		Negative	NA
PR-01-2554	69	4+3	90	pT3b	8.9		Positive	5
PR-02-1082	61	3+4	30	pT3b	11.5		Positive with interstitial deletion	6
PR-02-169	65	3+4	20	pT2c	10.6		Positive with interstitial deletion	3
PR-02-1736	58	3+4	5	pT3b	13		Negative	5
PR-02-1899	66	3+3	0	pT3a	13.4		Positive with interstitial deletion	3
PR-02-2072	54	4+3:5	62	pT3a	9		Positive with interstitial deletion	4
PR-02-2480	69	3+4	20	pT3b	10.4		Positive with interstitial deletion	5
PR-02-254	74	4+3	70	pT3b	17.6		Positive	7
PR-03-322	70	3+4	10	pT3a	9.7		Positive	3
PR-03-1026	56	4+3:5	85	pT3a	12.8		Positive	3
PR-03-870	52	4+3	90	pT3a	5.3		Negative	5
PR-04-1367	53	3+3	0	pT2c	9.9		Positive with interstitial deletion	1
PR-0415	71	4+4	100	pT3a	7.6		Positive with interstitial deletion	5
PR-04-194	65	3+4	30	pT2c	7.1		Positive with interstitial deletion	2
PR-0427	69	4+3		pT2a	5.1		Negative	1
PR-04-3113	66	3+3	0	pT3b	12		Positive	4
PR-04-3222	66	3+4	5	pT2c	5.2		Negative	1
PR-04-3347	59	3+3	0	pT2c	7.5		Negative	1
PR-04-639	61	3+4	5	pT3a	5.1		Negative	2
PR-04-903	68	3+4	5	pT3a	7.8		Positive with interstitial deletion	3
PR-05-3440	63	4+3	80	pT3a	8.5		Positive with interstitial deletion	4
PR-05-3595	64	3+4	50	pT2c	7.2		Negative	2
PR-05-839	69	3+4	30	pT2a	9		Negative	2
PR-06-1651	60	4+5:3	95	pT2c	5.8		Positive with interstitial deletion	3
PR-06-1749	60	4+3	60	pT2c	6.7		Negative	3
PR-06-1999	56	3+4	40	pT2a	30		Positive	4
PR-09-2517	64	3+4	5	pT3a	14		Positive with interstitial deletion	4
PR-09-2744	48	3+3	0	pT3a	4.4		Positive with interstitial deletion	1
PR-09-2767	58	4+4	100	pT3a	4.6		Positive with interstitial deletion	4
PR-09-3421	65	3+4	10	pT3a	4.8		Positive with interstitial deletion	2
PR-09-3586	65	4+3	80	pT3a	5		Positive with interstitial deletion	3
PR-09-3687	73	3+4	5	pT2c	7.8		Positive	2
PR-09-5094	77	4+4	100	pT3b	17		Negative	7
PR-09-5245	72	4+4	100	pT3a	6.1		Negative	5
PR-09-5446	67	3+3	0	pT2c	13		Positive with interstitial deletion	2
PR-09-5630	64	4+3	60	pT3b	14		Positive with interstitial deletion	7
PR-09-5700	60	3+4	20	pT3a	14		Positive	4
PR-09-5702	68	3+4	20	pT2c	8.2		Positive with interstitial deletion	2
PR-1024	73	3+4:5	22	pT3b	7.2		Negative	4
PR-1043	59	3+3	0	pT2c	4.9		Positive	0
PR-2661	59	3+3	0	pT2c	4.5		Positive with interstitial deletion	0
PR-2682	71	4+3	80	pT2c	7.6		Negative	3
PR-2740	58	3+3	0	pT2c	2.8		Negative	0
PR-2761	56	3+3	0	pT2c	4.3		Negative	0
PR-2762	68	3+4	5	pT2c	5.5		Negative	1
PR-2858	52	3+4	N/A	pT3a	5.3		Positive	2
PR-2872	62	3+4	5	pT3a	6		Positive with interstitial deletion	2
PR-2915	66	3+4	30	pT2c	3.6		Negative	1
PR-2916	65	3+4	2	pT3a	9.1		Negative	3
PR-3023	67	4+3	95	pT2c	5		Negative	2
PR-3026	56	3+4	20	pT3b	24.2		Negative	7
PR-3034	70	4+4	100	pT2c	14.6		Negative	5
PR-3035	34	3+4	5	pT2c	4.2		Positive with interstitial deletion	1
PR-3036	47	3+4	5	pT2c	5.1		Negative	1
PR-3048	64	3+4	10	pT2c	3.5		Negative	1
PR-3051	60	3+4	20	pT2c	20		Negative	3
PR-3127	55	3+4	2	pT2c	4.2		Negative	1

* Modified CAPRA-S score, derived from CAPRA-S score (61) without including surgical margins

Supplementary Table S4. Mutations in Significantly-Mutated Genes and other Selected Genes

Selected mutations detected by exome sequencing are annotated based on the indicated UCSC Genome Browser identifier. Additional mutations detected by Sanger sequencing or transcriptome sequencing of independent tumors are also listed. Mutations are grouped by three categories: (1) Mutations in significantly mutated genes. (2) Mutations previously reported in cancer. (3) Selected genes with damaging mutations (splice site-alteration, truncation or frame-shift) and are inactivated by similar mutations in other cancer types. References are listed that support the role of either the particular mutation (for missense mutations) or the type of mutation (for nonsense, frame-shift or splice site mutations) in promoting cancer or other neoplasms. "RNA" denotes the median log₂(RPKM+1) value for each transcript across 63 tumors that underwent transcriptome sequencing. fs, frame-shift mutation; del, deletion; CV, coefficient of variation.

Category	Gene symbol (Annotation transcript)	Patient	Genomic coordinates (hg19)	Variant type	Codon change	Protein change	Median RNA	Mean RNA	RNA CV	RNA Percentile	References				
1. Significantly mutated genes (q-value < 0.1)	SPOP (uc010dbk.2)	P04-2599	g.chr17:47696425A>G	Missense	c.(397-399)TTC>TCC	p.F133S	3.88	3.78	0.13	88%					
		P07-684	g.chr17:47696448A>T	Missense	c.(373-375)TTT>TTA	p.F126L									
		P07-837	g.chr17:47696424C>G	Missense	c.(397-399)TTC>TTG	p.F133L									
		P06-649	g.chr17:47696467C>T	Missense	c.(355-357)AGT>AAT	p.S119N									
		PR-00-160	g.chr17:47696421C>G	Missense	c.(400-402)AAG>AAC	p.K134N									
		PR-01-2492	g.chr17:47696425A>C	Missense	c.(397-399)TTC>TGC	p.F133C									
		PR-04-3222	g.chr17:47696424G>T	Missense	c.(397-399)TTC>TTA	p.F133L									
		PR-05-839	g.chr17:47696689A>T	Missense	c.(259-261)TAC>AAC	p.Y87N									
		PR-09-5245	g.chr17:47696432A>C	Missense	c.(391-393)TGG>GGG	p.W131G									
		PR-2961	g.chr17:47696426A>C	Missense	c.(397-399)TTC>GTC	p.F133V									
		PR-2740	g.chr17:47696424C>T	Missense	c.(397-399)TTC>TTA	p.F133L									
		PR-2761	g.chr17:47696425A>G	Missense	c.(397-399)TTC>TCC	p.F133S									
		PR-2915	g.chr17:47696426A>C	Missense	c.(397-399)TTC>GTC	p.F133V									
		PR-3051	g.chr17:47696438T>C	Missense	c.(385-387)AAA>GAA	p.K129E									
		STD0000000420_D (RNA-seq)	g.chr17:47696424G>C	Missense	c.(397-399)TTC>TTG	p.F133L									
		STD0000000508_A (RNA-seq)	g.chr17:47696426A>C	Missense	c.(397-399)TTC>GTC	p.F133V									
		STD00000002525_A (RNA-seq)	g.chr17:47696689A>T	Missense	c.(259-261)TAC>AAC	p.Y87N									
		STD00000002844_D (RNA-seq)	g.chr17:47696424G>T	Missense	c.(397-399)TTC>TTA	p.F133L									
		STD00000002943_C (Sanger seq)	g.chr17:47696688T>C	Missense	c.(259-261)TAC>TGC	p.Y87C									
		STD00000003043_B56 (Sanger seq)	g.chr17:47696643A>C	Missense	c.(304-306)TTC>TGC	p.F102C									
		STD00000003131_B51 (Sanger seq)	g.chr17:47696425A>C	Missense	c.(397-399)TTC>TGC	p.F133C									
		STD00000003640_B53 (RNA-seq)	g.chr17:47696688T>C	Missense	c.(259-261)TAC>TGC	p.Y87C									
		TP53 (uc002gjm.2)	P03-871	P03-871	g.chr17:7579417-7579426(del)	Frame shift deletion	c.(259-270)fs	p.P87fs	3.99	3.95	0.16	89%			
				P04-1243	g.chr17:7577121G>A	Missense	c.(817-819)CGT>TGT	p.R273C							
				P06-4428	g.chr17:7577547C>T	Missense	c.(733-735)GGG>GAC	p.G245D							
				P06-4428	g.chr17:7578457C>T	Missense	c.(472-474)CGG>CAC	p.R158H							
				P07-5036	g.chr17:7577538C>T	Missense	c.(742-744)CGG>CAG	p.R248Q							
				PR-02-254	g.chr17:7578542G>A	Missense	c.(388-390)CTC>TTC	p.L130F							
				PR-0415	g.chr17:7575259A>T	Missense	c.(751-753)ATC>AAC	p.I251N							
				PR-05-1999	g.chr17:7573593G>A	Missense	c.(328-330)CGT>TGT	p.R110C							
				PTEN (uc001kb.2)	P04-1790	P04-1790	g.chr10:89692900C>C	Missense	c.(382-384)AAG>AAC	p.K128N	2.78	2.55	0.29	75%	
						P06-3676	g.chr10:89692905G>A	Missense	c.(388-390)CGA>CAA	p.R130Q					
						PR-02-254	g.chr10:89720857C>G	Nonsense	c.(1006-1008)TAC>TAG	p.Y336*					
						PR-02-254	g.chr10:89692901G>C	Missense	c.(385-387)GGA>CGA	p.G129R					
				FOXK1 (uc001wuf.2)	P05-620	P05-620	g.chr10:89711874G>A	Splice site mutation	N/A	p.G165_splice	6.92	6.94	0.09	99%	
PR-04-1367	g.chr14:38081294G>A	Missense	c.(694-696)GCA>GTA			p.A232V									
PR-0427	g.chr14:38081231A>C	Missense	c.(757-759)ATG>AGG			p.M253R									
PR-09-3687	g.chr14:38081313C>T	Missense	c.(676-678)GAC>AAC			p.D226N									
STD00000003071_B51 (RNA-seq)	g.chr14:38081231A>C	Missense	c.(757-759)ATG>AGG			p.M253R									
STD0000000423_C1 (RNA-seq)	g.chr14:38081231A>T	Missense	c.(757-759)ATG>AAG			p.M253K									
STD00000016350_B53T (RNA-seq)	g.chr14:38081313C>T	Missense	c.(676-678)GAC>AAC			p.D226N									
STD00000002832_C (RNA-seq)	g.chr14:38081313C>A	Missense	c.(676-678)GAC>TAC			p.D226Y									
CDKN1B (uc001rat.2)	P00-000450	P00-000450	g.chr12:12870910A>G	Missense	c.(136-138)GAG>GGG	p.E46G	3.90	3.72	0.15	88%					
		P03-1426	g.chr12:12871788-12871788(del)	Frame shift deletion	c.(505-507)fs	p.R169fs									
		PR-00-160	g.chr12:12871228-12871228(del)	Frame shift deletion	c.(454-456)fs	p.R152fs									
		P03-1334	g.chr4:59330T>C	Missense	c.(10-12)GTA>GCA	p.V4A	1.23	1.25	0.36	53%					
		P04-594	g.chr4:59357T>A	Missense	c.(37-39)TTC>TAC	p.F13Y									
		PR-09-3687	g.chr4:65889A>T	Missense	c.(493-495)ATA>TTA	p.I165L									
THSD7B (uc002va.1)	PR-01-1934	P01-1934	g.chr2:138163182G>C	Splice site mutation	N/A	p.A803_splice	0.07	0.13	1.41	27%					
		PR-03-1026	g.chr2:137988713C>T	Missense	c.(1729-1731)ACC>ATG	p.T577M									
		PR-05-839	g.chr2:138000058C>T	Missense	c.(2089-2091)CGC>TGC	p.R697C									
		PR-09-2767	g.chr2:138373832C>G	Missense	c.(3424-3426)GTG>CTG	p.V1142L									
		PR-09-2702	g.chr2:138033591C>A	Missense	c.(2401-2403)ACA>AAA	p.T801K									
MED12 (uc004dy.2)	P04-594	P04-594	g.chrX:70349258C>T	Missense	c.(1627-1629)CGC>TGC	p.R543C									
		P07-684	g.chrX:70349258C>T	Missense	c.(3670-3672)CTC>TTC	p.L1224F	2.92	2.93	0.12	77%					
		PR-00-160	g.chrX:70344951C>A	Missense	c.(3928-3930)CCA>CAA	p.P1310Q									
		PR-3026	g.chrX:70349258C>T	Missense	c.(2179-2181)GAC>GAA	p.D727E									
		PR-3026	g.chrX:70349258C>T	Missense	c.(3670-3672)CTC>TTC	p.L1224F									
		PR-3026	g.chrX:70349258C>T	Missense	c.(3670-3672)CTC>TTC	p.L1224F									
MIPAA (uc001yux.2)	P03-1906	P03-1906	g.chr15:23012398A>C	Missense	c.(328-330)CCT>CGT	p.L110R	3.58	3.61	0.17	85%					
		P07-837	g.chr15:23006670G>A	Missense	c.(634-636)GTG>TTG	p.V212L									
		PR-09-3421	g.chr15:23014493C>A	Missense	c.(232-234)CGG>TCG	p.A78S									
		PR-04-639	g.chr3:178952085A>G	Missense	c.(3139-3141)CAT>CGT	p.H1047R	1.39	1.31	0.28	55%					
		PR-09-2517	g.chr3:178917478G>A	Missense	c.(352-354)AGT>GAT	p.G118D									
		PR-09-2744	g.chr3:178936095A>C	Missense	c.(1636-1638)CAG>CCG	p.Q546P									
C14orf49 (uc001yei.3)	P00-000450	P00-000450	g.chr3:178952006T>C	Missense	c.(3061-3063)TAC>CAC	p.Y1021H									
		P07-718	g.chr14:95884213C>T	Missense	c.(2878-2880)GCC>ACC	p.A060T	0.38	0.42	0.57	38%					
		PR-09-2700	g.chr14:95932380C>T	Missense	c.(514-516)CGG>CAG	p.R172Q									
		PR-2916	g.chr14:95899740C>A	Nonsense	c.(2545-2547)GAG>TAG	p.E849*									
		PR-3023	g.chr14:95906394G>A	Missense	c.(1087-1089)GCC>GTG	p.A363V									
		PR-3023	g.chr14:95906394G>A	Missense	c.(1930-1932)CGG>TGG	p.R644W									
SCN11A (uc011ays.1)	P07-144	P07-144	g.chr3:38926826A>T	Nonsense	c.(3016-3018)TTA>TAA	p.L1006*	0.02	0.03	1.15	24%					
		PR-00-1823	g.chr3:38938566G>A	Missense	c.(2173-2175)CGT>TGT	p.R725C									
		PR-01-2554	g.chr3:38938608C>A	Nonsense	c.(2791-2793)GAA>TAA	p.E931*									
		PR-06-1999	g.chr3:389388451C>T	Missense	c.(2287-2289)CGC>CAC	p.R763H									
		PR-09-2517	g.chr3:389388451C>T	Missense	c.(2287-2289)CGC>CAC	p.R763H									
2. Mutations previously reported in cancer	IDH1 (uc002vcs.2)	P06-2325	P06-2325	g.chr2:209113112C>T	Missense	c.(394-396)CGT>CAT	p.R132H	6.00	5.94	0.14	98%	(62),(63)			
			P06-1125	g.chr14:105246551C>T	Missense	c.(49-51)GAA>AAG	p.E17K	5.51	5.51	0.06	97%	(64)			
			PR-09-5702	g.chr11:533874T>C	Missense	c.(181-183)CAG>CGG	p.O617R	4.98	4.94	0.08	95%	(65),(66)			
			P00-000450	g.chr11:108218029G>T	Missense	c.(8608-8610)GAT>TAT	p.D2870Y	1.33	1.30	0.30	54%	(67-69)			
			PR-09-2767	g.chr11:108218537C>T	Missense	c.(8465-8467)CCA>CTA	p.P2829L								
			PR-2662	g.chr11:108201148-108201148(del)	Frame shift deletion	c.(7513-7515)fs	p.K2505fs								
			P05-620	g.chr5:112173635A>T	Nonsense	c.(2344-2346)AAG>TAG	p.K782*	1.11	1.18	0.34	51%	(70),(71)			
3. Genes with a damaging mutation that undergo damaging mutations in other cancer types	APC (uc010by.2)	PR-00-160	P00-160	g.chr5:112175799-112175799(del)	Frame shift deletion	c.(4687-4689)fs	p.L1563fs								
			PR-09-3566	g.chr5:67591248A>G	Splice site mutation	N/A	p.M582_splice	2.29	2.22	0.23	69%	(72)			
			PR-2961	g.chr12:49438067G>A	Nonsense	c.(5104-5106)CGA>TGA	p.R1702*	3.26	3.13	0.17	81%	(40),(73),(74)			
			PR-3048	g.chr7:151859711C>A	Nonsense	c.(10851-10853)GAG>TAG	p.E3651*	2.25	2.22	0.21	68%	(40),(74)			
			P07-360	g.chrX:44941855-44941872(del)	Frame shift deletion	c.(3343-3354)fs	p.S1115fs	2.00	2.01	0.18	64%	(75)			
			PR-2916	g.chrX:44945155G>T	Missense	c.(3634-3636)GGC>GTC	p.G1212V								
			PR-3036	g.chrX:44945146T>A	Missense	c.(3625-3627)ATA>AAA	p.I1209K								
			P04-2599	g.chr1:27101666C>T	Nonsense	c.(4948-4950)CAG>TAG	p.Q1650*	4.03	3.98	0.09	89%	(76),(77)			
			PR-09-3421	g.chr1:27098957A>T	Missense	c.(3736-3738)ATG>TTG	p.M1246L								
			EPHA7 (uc003poe.2)	P04-1084	g.chr6:93967815A>C	Splice site mutation	N/A	p.G704_splice	0.92	0.95	0.47	48%	(78),(79)		
			NCOR1 (uc002ppo.2)	P05-620	g.chr17:16012098A>C	Splice site mutation	N/A	p.V728_splice	3.07	3.10	0.13	79%	(76)		
			PR-2490	g.chr17:15935762G>A	Missense	c.(7171-7173)CGG>TTG	p.R2391W								
PR-2682	g.chr11:11835577G>T	Nonsense	c.(4219-4221)GAG>TAG	p.E1407*	2.35	2.31	0.16	69%	(76)						

Supplementary Table S5. Significantly Mutated Gene Sets

Curated gene sets representing canonical pathways were analyzed for enrichment of mutations in their constituent genes (Supplementary Methods). Sets that are significantly mutated above a q-value of 0.05 (Benjamini-Hochberg adjustment) are listed.

Gene Set	No. mutations	No. tumors with a mutation	B-H q-value	Genes in set mutated (no. tumors affected)
SA_G1_AND_S_PHASES	11	10	0.00033	CDKN1B(3), TP53(8)
RBPATWAY	14	13	0.00033	ATM(3), CDC25A(1), MYT1(1), TP53(8), WEE1(1)
P53HYPOXIAPATHWAY	14	13	0.0048	ABCB1(2), AKT1(1), ATM(3), TP53(8)
TERTPATHWAY	9	8	0.0048	SP1(1), TP53(8)
PLK3PATHWAY	11	10	0.0062	ATM(3), TP53(8)
IGF1MTORPATHWAY	14	13	0.0072	AKT1(1), GSK3B(1), IGF1R(1), INPPL1(1), PIK3CA(4), PIK3R1(1), PTEN(5)
ARFPATHWAY	13	12	0.008	PIK3CA(4), PIK3R1(1), TP53(8)
G1PATHWAY	16	14	0.008	ATM(3), CDC25A(1), CDKN1B(3), GSK3B(1), TP53(8)
G2PATHWAY	17	16	0.011	ATM(3), CDC25A(1), EP300(1), MYT1(1), PRKDC(1), RPS6KA1(1), TP53(8), WEE1(1)
CHEMICALPATHWAY	16	14	0.011	AKT1(1), ATM(3), CASP3(1), CASP9(1), TLN1(2), TP53(8)
PTENPATHWAY	17	16	0.011	AKT1(1), BCAR1(1), CDKN1B(3), ILK(1), PIK3CA(4), PIK3R1(1), PTEN(5), SHC1(1)
RNAPATHWAY	9	8	0.011	DNAJC3(1), TP53(8)
P53PATHWAY	11	10	0.017	ATM(3), TP53(8)
COMPLEMENT_ACTIVATION_CLASSICAL	10	10	0.017	C1QB(1), C1S(1), C3(3), C6(1), C8A(1), C9(2), MASP1(1)
CLASSICPATHWAY	9	9	0.018	C1QB(1), C1S(1), C3(3), C6(1), C8A(1), C9(2)
COMPPATHWAY	10	10	0.018	C1QB(1), C1S(1), C3(3), C6(1), C8A(1), C9(2), MASP1(1)
HCMVPATHWAY	10	10	0.018	AKT1(1), CREB1(1), MAP2K6(1), MAPK14(1), PIK3CA(4), PIK3R1(1), SP1(1)
TELPATHWAY	14	13	0.025	AKT1(1), EGFR(1), IGF1R(1), POLR2A(1), TEP1(1), TP53(8), XRCC5(1)
ALTERNATIVEPATHWAY	7	7	0.027	C3(3), C6(1), C8A(1), C9(2)
CDC42RACPATHWAY	8	7	0.027	ACTR2(1), PDGFRA(2), PIK3CA(4), PIK3R1(1)
SA_PTEN_PATHWAY	12	11	0.04	AKT1(1), ILK(1), PIK3CA(4), PTEN(5), SHC1(1)
RACCYCDPATHWAY	11	11	0.04	AKT1(1), CDKN1B(3), HRAS(1), PIK3CA(4), PIK3R1(1), RAF1(1)
CELL_CYCLE_KEGG	29	28	0.042	ATM(3), BUB3(1), CDC20(1), CDC25A(1), CDC6(1), CDH1(1), EP300(1), ESPL1(1), GSK3B(1), HDAC2(2), HDAC3(1), HDAC5(2), MAD1L1(1), MCM4(1), PRKDC(1), SMAD4(1), TP53(8), WEE1(1)
IGF1RPATHWAY	11	11	0.042	AKT1(1), HRAS(1), IGF1R(1), IRS1(1), PIK3CA(4), PIK3R1(1), RAF1(1), SHC1(1)

Supplementary Table S6. Systematic Sequencing Studies Including SPOP

Cancer type	Samples (N)	SPOP mutations	Reference	Approach
Prostate	7	2: F102C, F133V	(14)	Paired-end whole-genome sequencing
Prostate	58	2: F125V, F133V	(17)	Mismatch repair detection (MRD)
Lung	134	2: N169S, L190F	(17)	Mismatch repair detection (MRD)
Ovarian	316	1: E249*	(43)	Whole exome sequencing
Ovarian	8	1: E47K	(80)	Whole exome sequencing
Ovarian	58	None	(17)	Mismatch repair detection (MRD)
HNSCC	76	None	(39)	Whole exome and paired-end whole-genome sequencing
RCC	101	None	(81)	PCR-based exon resequencing
RCC	7	None	(82)	Whole exome sequencing
Pancreatic	8	None	(17)	Mismatch repair detection (MRD)
Pancreatic	24	None	(83)	Whole exome sequencing
Breast	183	None	(17)	Mismatch repair detection (MRD)
Breast	11	None	(84)	Whole exome sequencing
Breast	24	None	(85)	Paired-end whole-genome sequencing
Medulloblastoma	22	None	(74)	Whole exome sequencing
GBM	22	None	(63)	Whole exome sequencing
Colorectal	11	None	(84)	Whole exome sequencing
AML	1	None	(86)	Single-end whole-genome sequencing
AML	1	None	(87)	Paired-end whole-genome sequencing
Melanoma	1	None	(88)	Paired-end whole-genome sequencing
SCLC	1	None	(89)	Paired-end whole-genome sequencing

Supplementary Table S7. SPOP Mutations in Multiple Cohorts

Cohort	SPOP mutation prevalence	Technology	Mutated residues
WCMC	13.3 % (11/83)	WES, RNA-seq, Sanger	Y87N, Y87C, F102C, K129E, F133V, F133S, F133L, F133C
Uropath	10.1% (9/89)	WES	Y87N, S119N, F125L, W131G, F133S, F133L, F133C, K134N
UM	6.1% (3/49)	RNA-seq	F102C, F133L, F133V
UHZ	8.3% (16/193)	Sanger	Y87N, F102C, F102S, W131C, F133V, F133L
UW	14.5% (6/39)	Sanger	F102C, F102D, W131G, F133V

Supplementary Table S8. Somatic Copy Number Alterations Associated with SPOP Mutation

Type	Cytoband	Area Coordinates (hg18)	Peak Coordinates (hg18)	q.values	Association with SPOP mutation:					
					Discovery set		Validation set		Combined	
					P-value	Odds ratio	P-value	Odds ratio	P-value	Odds ratio
Del	6q15	chr6:84993071-90693659	chr6:89844069-89854517	7.60E-14	0	49.0936	0.0494	4.6152	0	14.9182
Del	5q14.3	chr5:86736848-92949317	chr5:90698354-90718446	4.76E-06	0	28.9982	0.0023	10.5654	0	18.5816
Del	6q21	chr6:102623781-118338352	chr6:107452805-107484929	2.72E-12	0	30.1055	0.0003	14.5979	0	23.0189
Del	5q21.1	chr5:96540739-99903527	chr5:98211239-98298151	6.24E-17	0	26.1837	0	23.6731	0	26.5255
Del	5q21.1	chr5:98288994-100177733	chr5:99892827-99956161	1.12E-11	0.0001	13.0406	0.0006	11.2936	0	12.8012
Del	6q14.1	chr6:79992719-89377471	chr6:82929067-83023893	3.50E-10	0.0001	10.9692	0.0427	4.4101	0	7.6868
Del	5q21.3	chr5:100266177-130524037	chr5:108694667-108775892	2.46E-06	0.0012	7.412	0.0001	20.5115	0	10.8653
Del	6q13	chr6:71718724-79638241	chr6:74188319-74228252	6.62E-05	0.0017	6.8441	0.2702	2.4264	0.0015	4.4487
Del	21q22.3	chr21:41569692-42033506	chr21:41754509-41805040	3.20E-13	0.0043	0	0.1836	0	0.0004	0
Del	5q11.2	chr5:54500232-57785740	chr5:55428291-55450265	3.84E-08	0.0044	5.4502	0.0107	7.6381	0.0004	4.9509
Amp	8q24.3	chr8:142130394-143948850	chr8:143649163-143686346	0.005918	0.0104	4.7801	0.4292	0.3188	0.1905	1.9013
Del	17p13.1	chr17:7099211-7923319	chr17:7723851-7759444	2.24E-07	0.0157	0.1101	0.6699	0.409	0.0089	0.1662
Del	2q21.1	chr2:114430192-140707944	chr2:131673017-131764875	0.0027275	0.0179	5.1272	0.0191	6.0238	0.0007	5.6709
Del	3p13	chr3:70098695-73514455	chr3:71884403-71891255	2.46E-06	0.0188	0	0.6731	0.4708	0.0179	0.1198
Amp	21q21.1	chr21:18980067-18997817	chr21:18980170-18994744	0.024377	0.0279	7.9899	1	0	0.144	2.9523
Amp	3q22.3	chr3:138503860-138525897	chr3:138503876-138514781	0.0046072	0.0284	3.8842	1	1.0223	0.1342	2.3893
Amp	7q11.23	chr7:71946015-71962929	chr7:71948417-71962425	0.076891	0.0333	4.1148	0.6154	1.7416	0.0554	2.9006
Amp	7q21.2	chr7:91212265-91215184	chr7:91212282-91215163	0.059607	0.0333	4.1148	0.6154	1.7416	0.0554	2.9006
Del	2q23.3	chr2:151939541-152050232	chr2:151970139-152045679	0.074786	0.0349	4.8599	0.65	1.4242	0.0829	2.9745
Del	17q21.31	chr17:39091591-40194083	chr17:39820952-39945190	1.17E-05	0.0366	0	0.1836	0	0.0026	0
Amp	7p15.2	chr7:26355665-26394413	chr7:26356137-26393733	0.059607	0.0435	3.7332	0.5879	2.2144	0.0554	2.9006

Supplementary Table S9. Primer Sequences

Amplification	sense (5' --> 3')	antisense (5' --> 3')
<i>SPOP</i>		
Exons 6 and 7	TTCTATGGGGCCTGCATTT	CTCCACTTGGGGCTTTTTCT
Sequencing		
<i>SPOP</i>		
Exon 6	TTTTCTATCTGTTTTGGACAGG	CAAAGCCACAACCTTGTCAGTG
Exon 7	TTTGCGAGTAAACCCCAAAG	CTCATCAGATCTGGGAACTGC
qPCR		
<i>SPOP</i>		
GAPDH	CTTCTGCGAGGTGAGTGTTG	TCCCACAGTCCTCCTAACTCA
	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

Supplementary Table S10. BAC Probes Used for FISH

Assay	BAC
<i>ERG</i> break-apart	
5' probe	RP11-372O17
3' probe	RP11-24A11
<i>ETV1</i> break-apart	
5' probe	RP11-661L15
3' probe	RP11-79G16
<i>ETV4</i> break-apart	
5' probe	RP11-147C10
3' probe	CTD-3215I16
<i>ETV5</i> break-apart	
5' probe	RP11-822O23
3' probe	RP11-480B15
<i>PTEN</i> deletion	
PTEN probe	CTD-2047N14
Reference probe	RP11-431P18

Supplementary Table S11. Mutation of *PIK3CA* and *PTEN* is Enriched in Locally Advanced Tumors

(A) Tumors are grouped by stage and mutational status of *PIK3CA* or *PTEN*. Only stage pT3 tumors displayed mutations in either gene (two-sided $p = 0.011$, Fisher's exact test).

(B) Mutations in *PTEN* or *PIK3CA* detected by exome sequencing. The hyper-mutated tumor, PR-00-1165, was included in this analysis and contained two canonical mutations in *PTEN*. Amino acids are numbered based on RefSeq protein ID NP_000305 for *PTEN* and NP_006209 for *PIK3CA*. All substitutions have been documented previously in prostate cancer or other cancer types in the Cosmic database (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>). *, nonsense mutation.

A

Genotype:	Stage pT2	Stage pT3	Total
PIK3CA/PTEN mutant:	0	9	9
PIK3CA/PTEN wild-type:	44	59	103
Total:	44	68	112

B

PTEN mutations:	Reported in Cosmic?
K128N	Yes
R130Q	Yes
Y336*	Yes
G129R	Yes
R173H, R233*	Yes, Yes

PIK3CA mutations:	Reported in Cosmic?
p.H1047R	Yes
p.G118D	Yes
p.Q546P	Yes
p.Y1021H	Yes