

## SUPPLEMENTARY MATERIALS

### Tracking the Clonal Origin of Lethal Prostate Cancer

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## SUPPLEMENTARY INFORMATION

### CASE REPORT

Here we present the clinical history of a man who was diagnosed with prostate cancer at age 47 years after presenting with a serum prostate-specific antigen (PSA) value >40 ng/mL (Figure S1) and no evidence for distant metastases. A radical retropubic prostatectomy revealed a Gleason 4 + 4 = 8 prostate adenocarcinoma with capsular penetration, with one of seventeen sampled pelvic lymph nodes harboring a microscopic metastatic deposit. Three months following surgery, the serum PSA was 0.13 ng/mL. Five years later, despite a lack of symptomatic or imaging evidence of progressive prostate cancer, the serum PSA rose to above 6 ng/mL, and treatment with an investigational prostate cancer vaccine (GVAX) was initiated, involving inoculation of lethally-irradiated allogeneic prostate cancer cells genetically-engineered to secrete granulocyte macrophage colony stimulating factor (GM-CSF) (1). Following treatment, the serum PSA fell from 6.4 ng/mL to 1.2 ng/mL. A subsequent rise in serum PSA prompted retreatment with the prostate cancer vaccine a little more than a year after the first course of vaccine immunotherapy. Again, the PSA fell to 1.6 ng/mL. About twelve and a half years after the initial presentation with clinically localized prostate cancer, metastatic deposits appeared in bones, lymph nodes, and the prostate bed, leading to initiation of goserelin acetate, bicalutamide, and zoledronic acid. Further treatment featured docetaxel, <sup>89</sup>Sr, mitoxantrone, and external beam radiation of a skull metastasis. These maneuvers arrested disease progression for nearly three years, when a work-up for a persistent cough revealed throat and lung metastases. As part of a clinical trial, abiraterone acetate was administered with an improvement in symptoms and a fall in the serum PSA to as low as 0.3 ng/mL. A little more than a year later, imaging studies disclosed vertebral and extensive liver metastases (Figure S1), which were treated systemically with etoposide and cisplatin, along with attempts at chemoembolization of specific liver lesions. More than seventeen years after his initial presentation, the patient died of metastatic castrate-resistant prostate cancer at age 64 years. Autopsy findings included an enlarged liver that was largely replaced with extensive metastatic carcinoma, involvement of the thoracic cavity with carcinoma, numerous lymph node metastases, and cancer present in thoracic vertebrae.

### METHODS

#### Sample collection

All patient samples were collected in accordance with protocols approved by the Johns Hopkins Medicine Institutional Review Board and written consent was obtained.

At the time of radical prostatectomy, the entire prostate specimen was cross sectioned at 11 levels and each of these levels were processed into 2 to 4 formalin fixed paraffin embedded (FFPE) tissue blocks, dividing the entire prostate into a total of 36 FFPE tissue blocks. For analysis, all of these archived FFPE blocks were retrieved and 5 micron sections of each block were stained with hematoxylin and eosin or with antibodies specific to PTEN. Pathological analysis revealed tumor glands in 31 out of the 36 blocks. Images from these blocks were then aligned to reconstruct each of the 11 levels of cross sectioning in order to thoroughly characterize the landscape of pathological and morphological alterations in the entire prostate. After the death of the patient, a rapid autopsy was performed under the guidance of two experienced pathologists (A.M.D. and M.K.H.) and sample material was obtained from all grossly identified metastases. Tumor tissues were either snap-frozen in liquid nitrogen or fixed for 24 h in 10% buffered-formalin and then further embedded in paraffin. For all tissues, for which snap frozen material were available, frozen tissue blocks embedded in tissue tek OCT (Sakura, Netherlands) were sectioned and stained with hematoxylin and eosin to evaluate

tissue integrity and tumor content. Where necessary, tissue blocks were trimmed to achieve a purity of >70% tumor cell content. DNA was extracted from 20 to 30 10 µm sections using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). With the exception of an increased proteinase K digestion step (the incubation was carried out for 20 h at 55°C), DNA extractions were performed according to the manufacturer's recommendations. DNA content was quantitated using the Qubit DNA quantification kit (Invitrogen, Carlsbad, CA). DNA integrity was assessed by gel electrophoresis.

### **Microdissection**

Tumor tissue samples for a lymph node metastasis resected at the time of radical prostatectomy, a lung metastasis obtained by biopsy during clinical management, as well as samples from the primary tumor were microdissected using a PALM MicroLaser System (Carl Zeiss, Germany). Seven µm thick sections from paraffin embedded archival tissue blocks were mounted onto PALM membrane slides (Carl Zeiss). In preparation for microdissection, slides were deparaffinized and stained with hematoxylin. Representative tumor areas were then microdissected using the PALM MicroLaser System. For DNA extraction, microdissected tissue material was digested overnight at 55°C in TE buffer supplemented with 1% Tween 20 and 10mAU/ml proteinase K (Qiagen). DNA was then recovered by phenol chloroform extraction followed by ethanol precipitation using the DrGentle DNA precipitation reagent (Clontech, Mountain View, CA) as a carrier.

### **Whole genome Sequencing**

Whole genome sequencing of DNA samples from 3 distant metastases (M5, M38, M40) and germline DNA from cardiac tissue was performed by Complete Genomics, Inc. (Mountain View, California) using a validated unchained combinatorial probe anchor ligation chemistry on self-assembling DNA nanoarrays as described previously.(2, 3) After alignment to the reference genome (UCSC Genome build hg19) variations were called using a local de-novo assembly algorithm as described previously.(2) SNVs and indels specific to the metastases samples were called using the calldiff function from cgatools (<http://cgatools.sourceforge.net/>). For high confidence SNVs, at least 7 reads needed to show the variation for homozygous calls and at least 3 for heterozygous calls. Structural variations were uncovered using the junctiondiff function of cgatools. High confidence structural rearrangements were called when the *de novo* assembly of the junction was based on >10 mate pair reads with no association to known repeat sequences. All Circos plots were generated using "CIRCOS" (<http://mkweb.bcgsc.ca/circos>).(4). Phylogenetic trees were built separately for SNV, CNV and SV data (Figure S2). Sequences used in parsimony analysis were generated for each sample using all observed variants in the specific variant class (SNV, CNV and SV). If a variant was present in the sample, the base 'C' was added to the sequence; if the sample matched reference, the base 'T' was added to the sequence. This was done for M5, M38, M40 and the germline control sample. The four assembled sequences (for M5, M38, M40 and germline control) were then analyzed using the DNAPARS algorithm (<http://cmgm.stanford.edu/phyliip/dnapars.html>), with default settings. The germline control sample was used as the outgroup when running DNAPARS. The distance between the three metastases corresponds to the number of divergent alterations.

### **PCR validation**

PCR were carried out in 40 µl reactions containing DNA, 1× Platinum *Taq* buffer (Invitrogen), 1.5 U Platinum *Taq* (Invitrogen), 250 µM each dNTPs, 1.5 mM MgCl<sub>2</sub>, 0.25 µg/µl BSA, 2 µl dimethyl sulfoxide, 400 nM forward primer and 400 nM reverse primer. Cycling conditions were 95°C for 3 min followed by 34 cycles of 95°C for 30 sec, depending on the primer used 55 or 58°C for 30 sec, 72°C for 30 sec and a final step for 3 min 72°C (Primers for all PCR reactions are listed in Table S5). For direct sequencing, PCR products were purified using a PCR

Purification kit from Qiagen and subjected to Sanger sequencing. For the sequencing of individually cloned amplicons, PCR products were resolved by gel electrophoresis, purified and sub-cloned into pCR<sup>®</sup>2.1-TOPO<sup>®</sup> vectors (Invitrogen). Individual bacterial colonies containing plasmids with PCR product inserts were then analyzed by Sanger sequencing. All sequences were evaluated using the 4Peaks software package (version 1.7.1; A. Griekspoor and Tom Groothuis, mekentosj.com).

### **Immunohistochemistry**

Pretreatment conditions and primary antibody concentrations are summarized in Table S5. The NKX3.1 specific antibody was a kind gift of Dr. Charles Bieberich (UMBC, Baltimore, MD) and was previously validated. (5) Immuno-complexes were detected using the PowerVision+<sup>™</sup> immunohistochemistry detection system from ImmunoVision Technologies Co (Norwell, MA, USA) with 3,3'-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. All slides were counterstained with hematoxylin.

### **Fluorescence In-Situ Hybridization (FISH) for the AR locus.**

The *AR* locus specific BAC clone (RP11-479J1) was purchased from CHORI (Children's Hospital Oakland Research Institute, Oakland, CA; <http://bacpac.chori.org>). Glycerol stocks were prepared from the original BAC clone and used for further amplification. BAC-DNA was amplified by isothermal strand displacement using GenomiPhi V2 DNA amplification kit from GEHealthcare (Pittsburgh, PA). Amplified DNA was labeled by incorporation of SpectrumOrange (Abbott, IL) directly-labeled d-UTP using the NickTranslation Kit from Abbott. Labeled probes were precipitated in 100% EtOH and resuspended in Hybridization Buffer (50% Formamide, 2xSSC, 10% Dextran Sulfate). X-chromosome centromere-specific probes were obtained from CymoGenDx (Irvine, CA). Slides were deparaffinized and pretreated in 0.01 N HCl supplemented with 750 U/ml pepsin (Sigma-Aldrich, cat#P6887) for 10 min at 37°C. Probes were applied on the slide, denatured for 5 min at 95°C, and then incubated at 37°C in a humid chamber (StatSpin ThermoBrite, IRISInc, MA). Slides were then washed in Wash buffer (2xSSC, 0.3 % NP-40) for 2 min at 71 °C, counterstained with DAPI and mounted with Prolong Gold (Invitrogen).

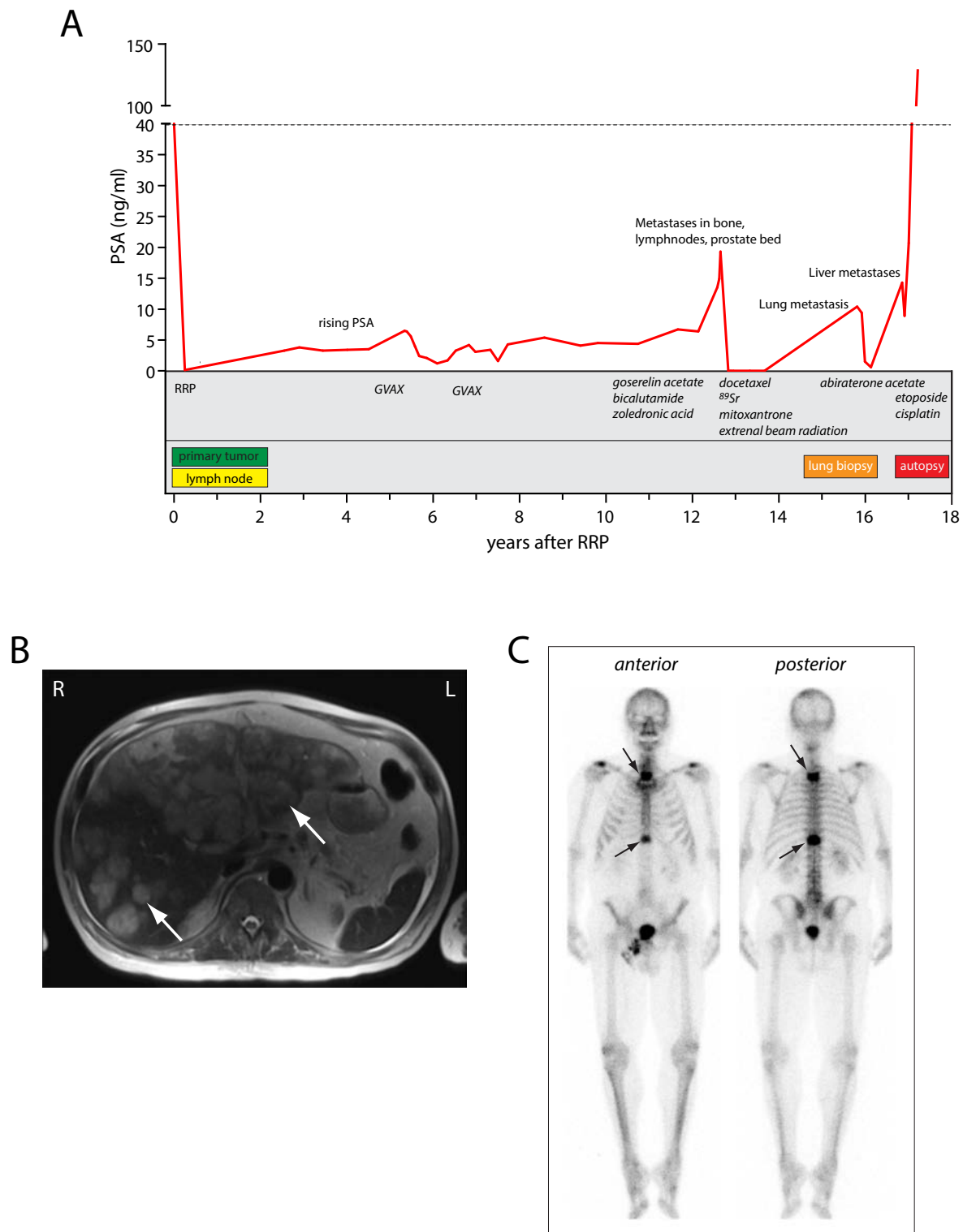
### **Telomere FISH**

Telomere FISH was performed as described previously. (6, 7) Briefly, slides were deparaffinized, steamed in citrate buffer (cat#S2003, Vector Laboratories) for 20 min and then hybridized with a Cy3-labeled peptide nucleic acid (PNA) probe complementary to the telomeric repeat sequence (CCCTAACCCTAACCTAA) followed by a nuclear counterstain with DAPI. Lesions were considered ALT-positive if >1% of neoplastic cells displayed ultra-bright intra-nuclear telomere FISH signal aggregates.(8)

All slides were imaged on a Nikon 50i microscope. Immunofluorescence images were captured using a CoolsnapEZ digital camera (Photometrics, Tucson, AZ) and the Nikon NIS-Elements (Nikon, Melville, NY) software package.

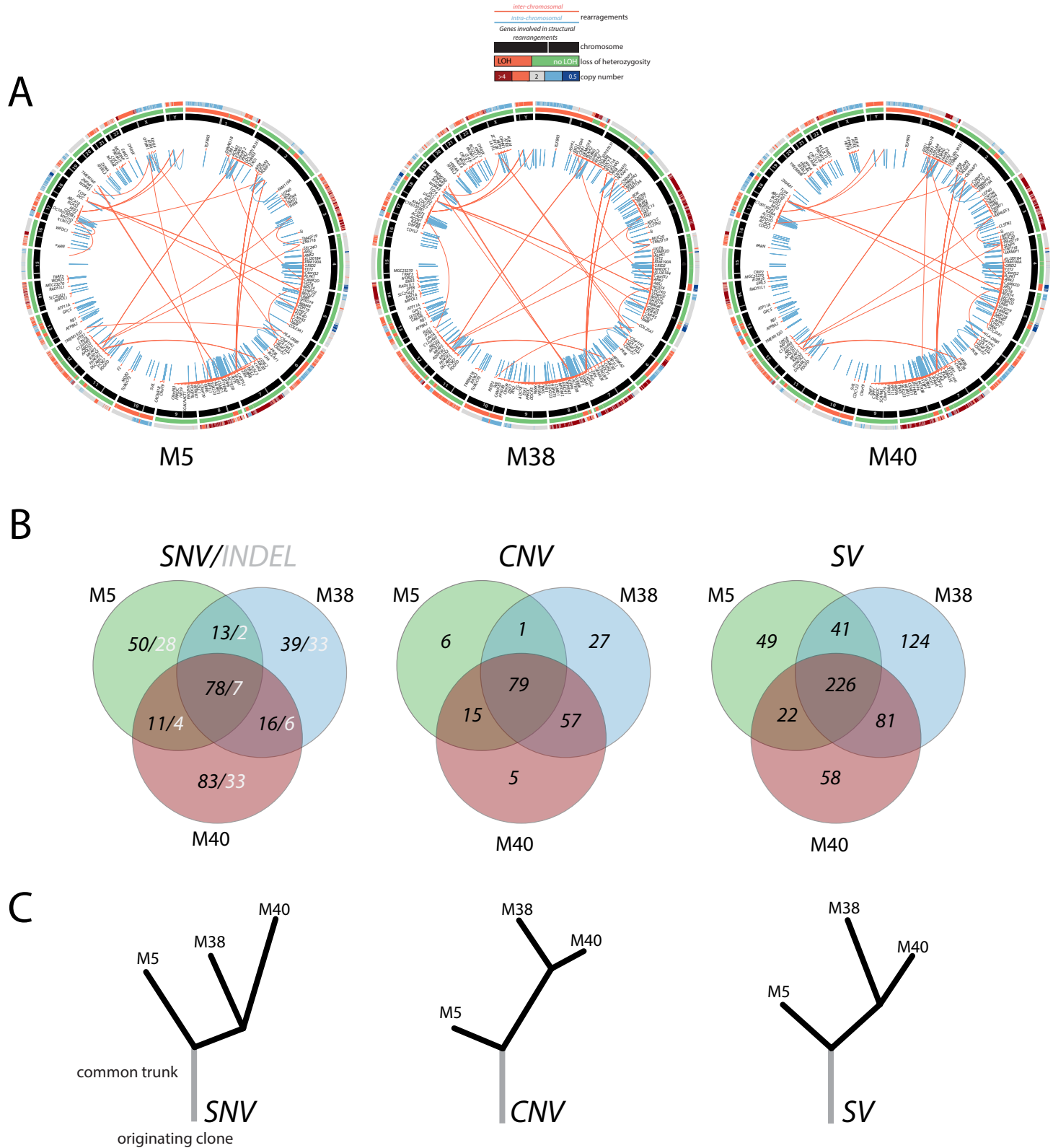
### **ADDITIONAL ACKNOWLEDGEMENTS**

The authors would like to thank Dr. Tamara Lotan and Dr. George Netto for helpful comments on the manuscript, Dr. Charles Bieberich for sharing reagents, Dr. Christina Iacobuzio-Donahue for assistance with the logistical planning of the rapid autopsy, Lillian Dasko-Vincent from the SKCCC Cell Imaging Core Facility for technical advice and the SKCCC Next Generation Sequencing Center for sequencing and analytical support.

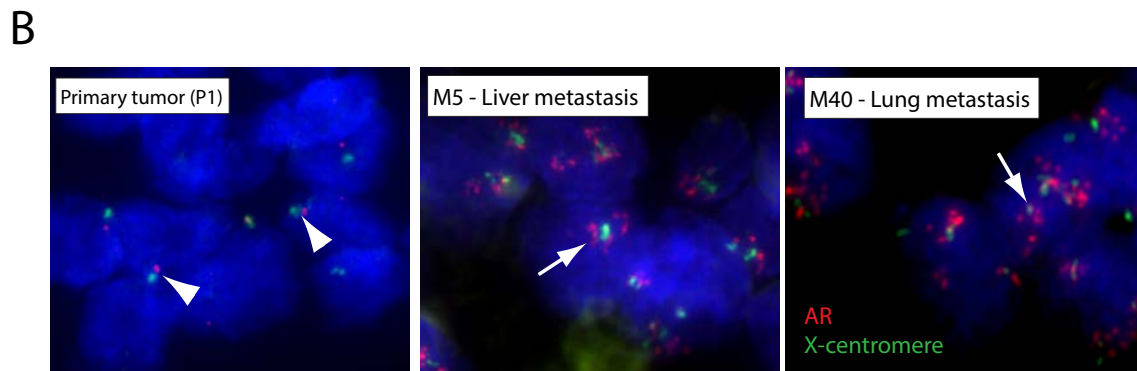
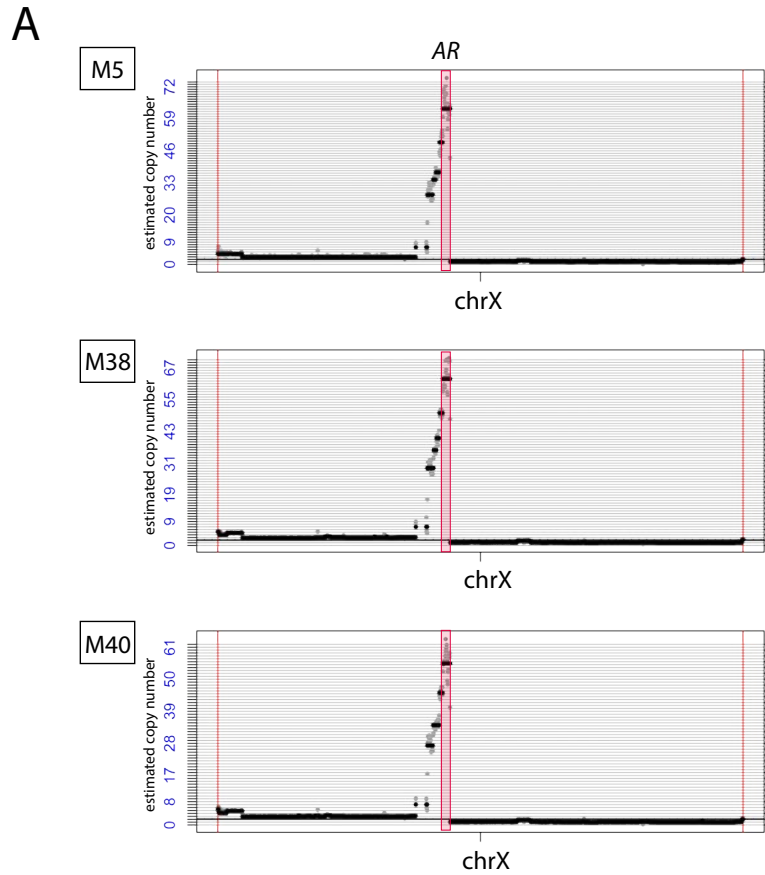


**Figure S1. Summary of clinical and radiographic findings.**

Disease manifestations, therapeutic interventions and longitudinal serum PSA values for the >17 year follow-up period after radical retropubic prostatectomy (RRP) (Panel A). Time points and anatomic site of sampled lesions analyzed in the study are indicated. Panel B. Magnetic resonance imaging several months before the autopsy reveals extensive metastatic lesions in the liver (arrows). Panel C. Bone scans show two major lesions in thoracic and cervical vertebrae (arrows).

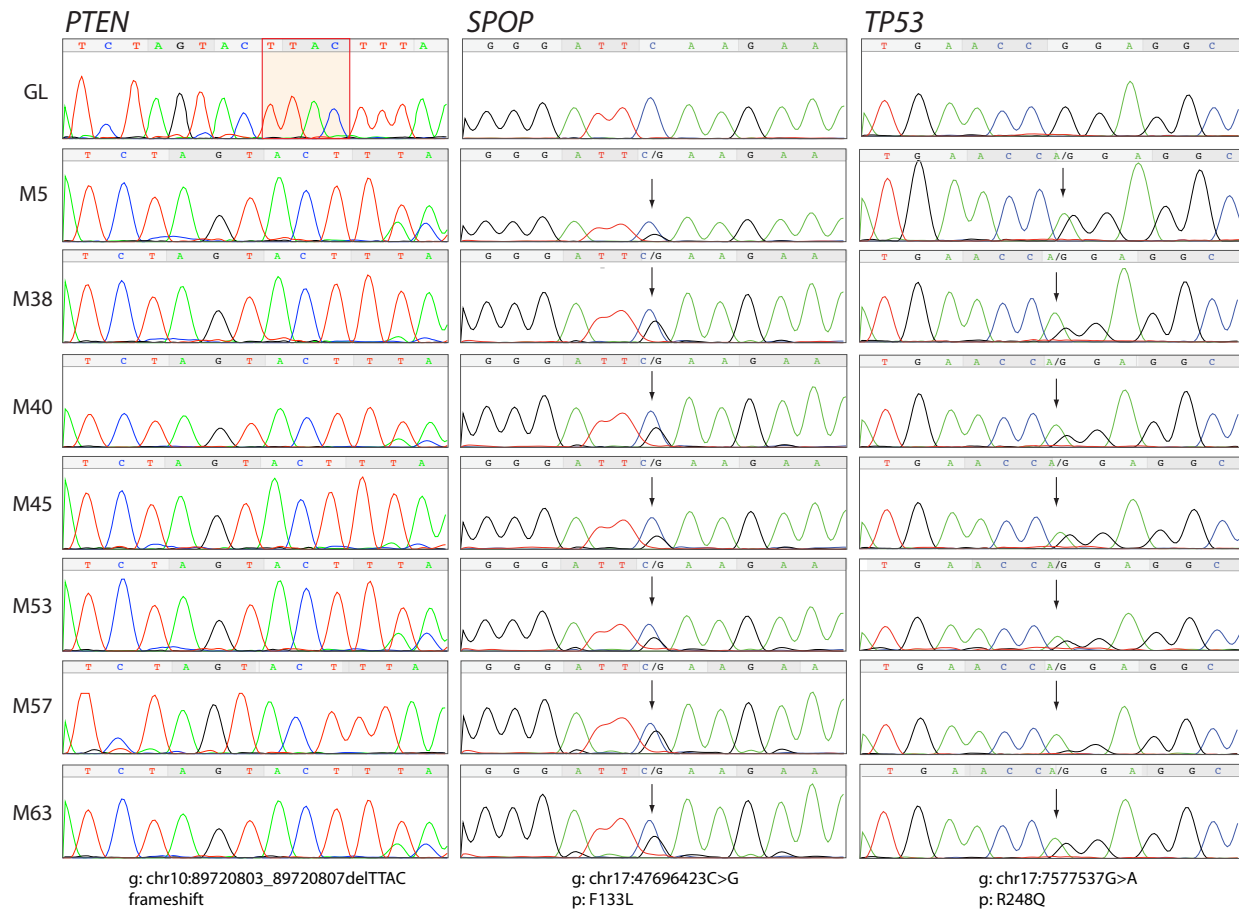


**Figure S2. Unique and shared alterations in 3 individual distant metastases analyzed by whole genome sequencing.** Panel A, Circos plots depict copy number variations (CNV), structural variations (SV) and loss of heterozygosity (LOH) based on whole genome sequencing of 3 metastases from distinct anatomic sites (M5 - liver, M38 - perigastric lymph node, M40 - lung). CNV and LOH changes are color-coded in the outer ring, SVs and associated gene names are indicated in the middle ring. Intra-chromosomal rearrangements are shown in blue; inter-chromosomal rearrangements are highlighted in orange. Venn diagrams in Panel B show the number of shared and unique SNVs in the coding region and CNVs and SVs in 3 distant metastases. Phylogenetic trees shown in Panel C were built based on SNV, CNV and SV alterations using the DNAPARS algorithm. For all analyses shown in Panel A and B only high level copy-number alterations ( $>4$ ,  $<0.5$ ) were considered.



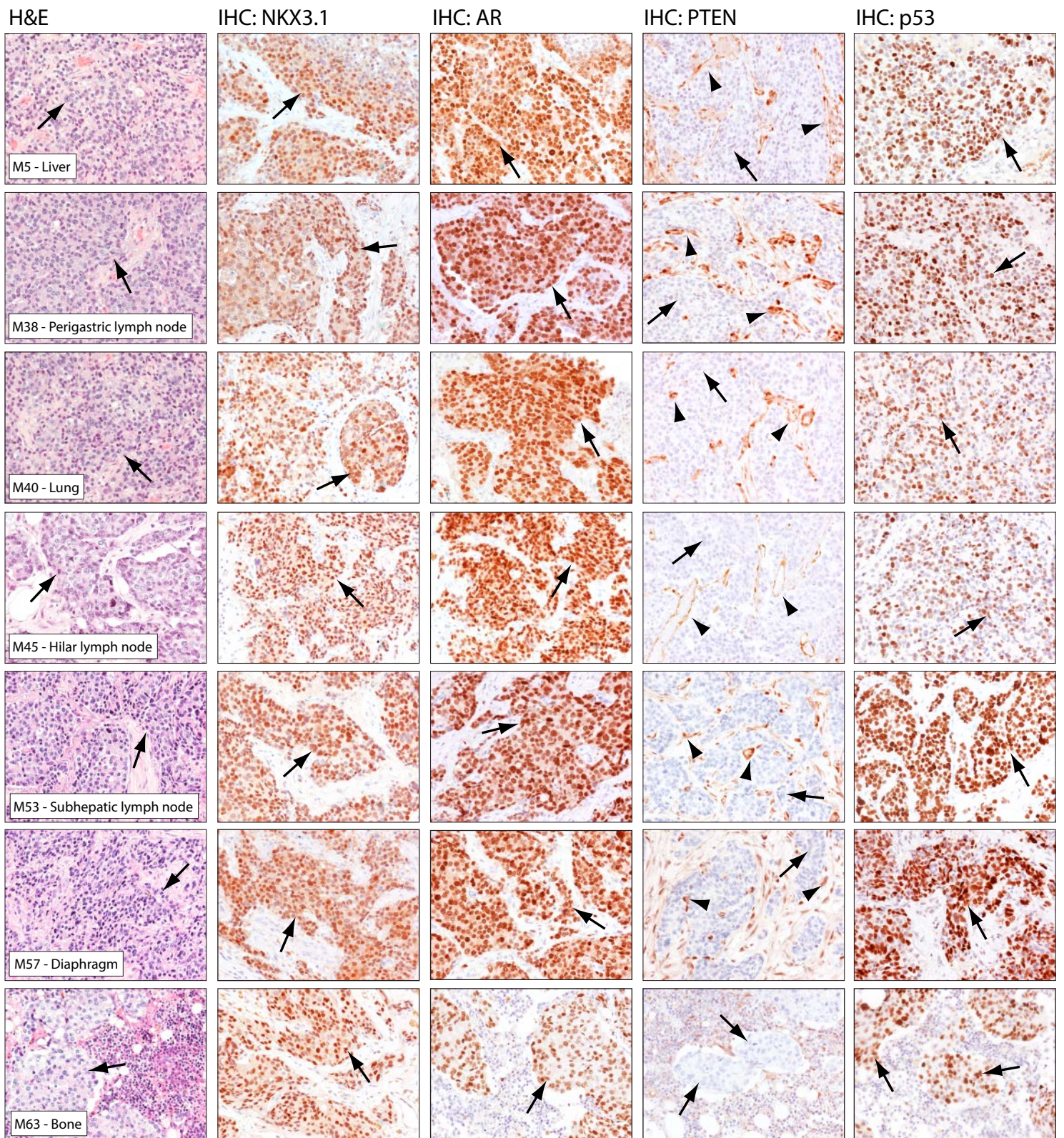
**Figure S3. Copy number analysis of chromosome X reveals high-level amplification of the *AR* locus.**

Panel A, Ploidy plots for chromosome X reveal a 10 Mb region harboring 19 genes with dramatically increased copy number (>28 fold) in all 3 metastases. The *AR* locus (highlighted in red) shows an estimated ploidy of 60x. Panel B, shows validation of *AR* amplification by FISH. Note that the *AR* locus (shown in red) was not amplified in the primary tumor (arrowheads), but showed increase copy number in distant metastases (arrows). Chromosome X-specific centromere probes are shown in green. Representative images are shown at 40x magnification.



**Figure S4. Validation of index mutations in *PTEN*, *SPOP* and *TP53* in distant metastases.**

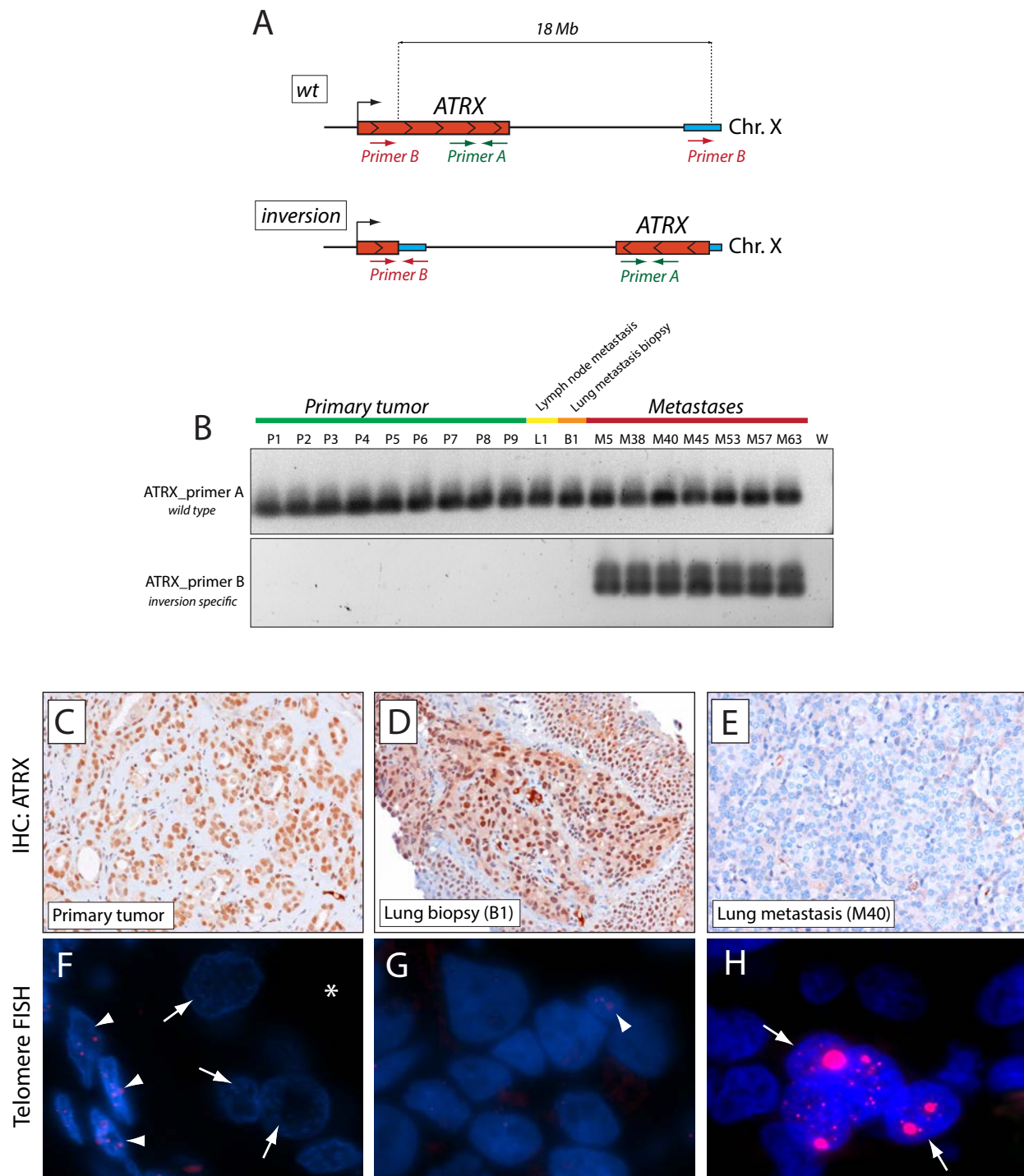
To confirm the presence of the index mutations in *PTEN*, *SPOP* and *TP53* in all distant metastases procured at autopsy, DNAs from all 7 lesions were amplified with primers targeting mutations in *PTEN* (*PTEN*:NM\_000314:exon8:c.955\_958del), *SPOP* (*SPOP*:NM\_001007227:exon6:c.C399G) and *TP53* (*TP53*:NM\_001126114:exon7:c.G743A). PCR products were subjected to direct Sanger sequencing and representative chromatograms are shown. Note that index mutations are only present in DNAs from distant metastases (indicated by arrows) and are absent in germline control DNA (GL). Red box indicates 4 bp region in exon 8 of *PTEN* deleted in metastases. Genomic coordinates are given for UCSC Genome build hg19.



**Figure S5. Histopathological characterization of distant metastases procured at autopsy.**

To confirm the prostatic origin of the metastatic lesions and functionally validate genomic lesions observed by whole genome sequencing, representative sections of 7 anatomically distinct metastases were immuno-labeled with antibodies specific to NKX3.1, AR, PTEN and p53.

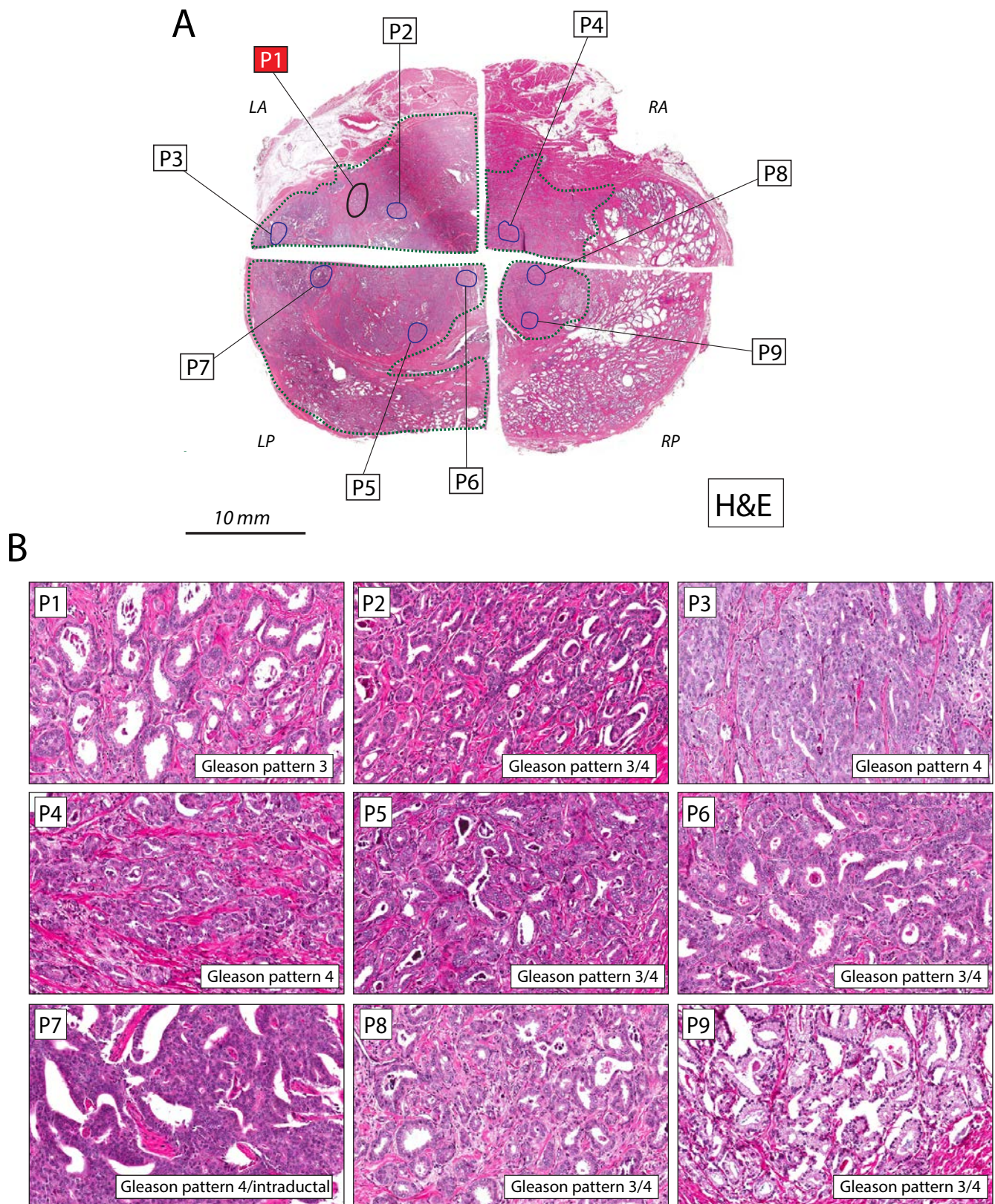
NKX3.1 is an established prostate lineage marker. All lesions showed high expression of NKX3.1 confirming their prostatic origin. The *AR* locus on chromosome X showed highly increased copy-number. Immunostaining revealed high *AR* expression levels in all metastases. *PTEN* harbors a 4 bp homozygous frame shift deletion in exon 8 resulting in structural changes in its c-terminus. Using an antibody that was raised against the c-terminus, we observed loss of *PTEN* staining in neoplastic cells (arrows), whereas normal stromal cells showed strong staining for *PTEN* (arrowheads) indicating that the mutant protein is not recognized by the antibody. Mutant p53 has been shown to accumulate in the nucleus of neoplastic cells. All metastatic lesions showed strong nuclear accumulation of p53 strongly suggesting the presence of a mutated p53. All images were taken at 20x magnification. Tumor cells are indicated with arrows. Arrowheads show non-neoplastic cells.



**Figure S6. Clonal evolution of an inversion affecting the *ATRX* gene and its association with ALT.**

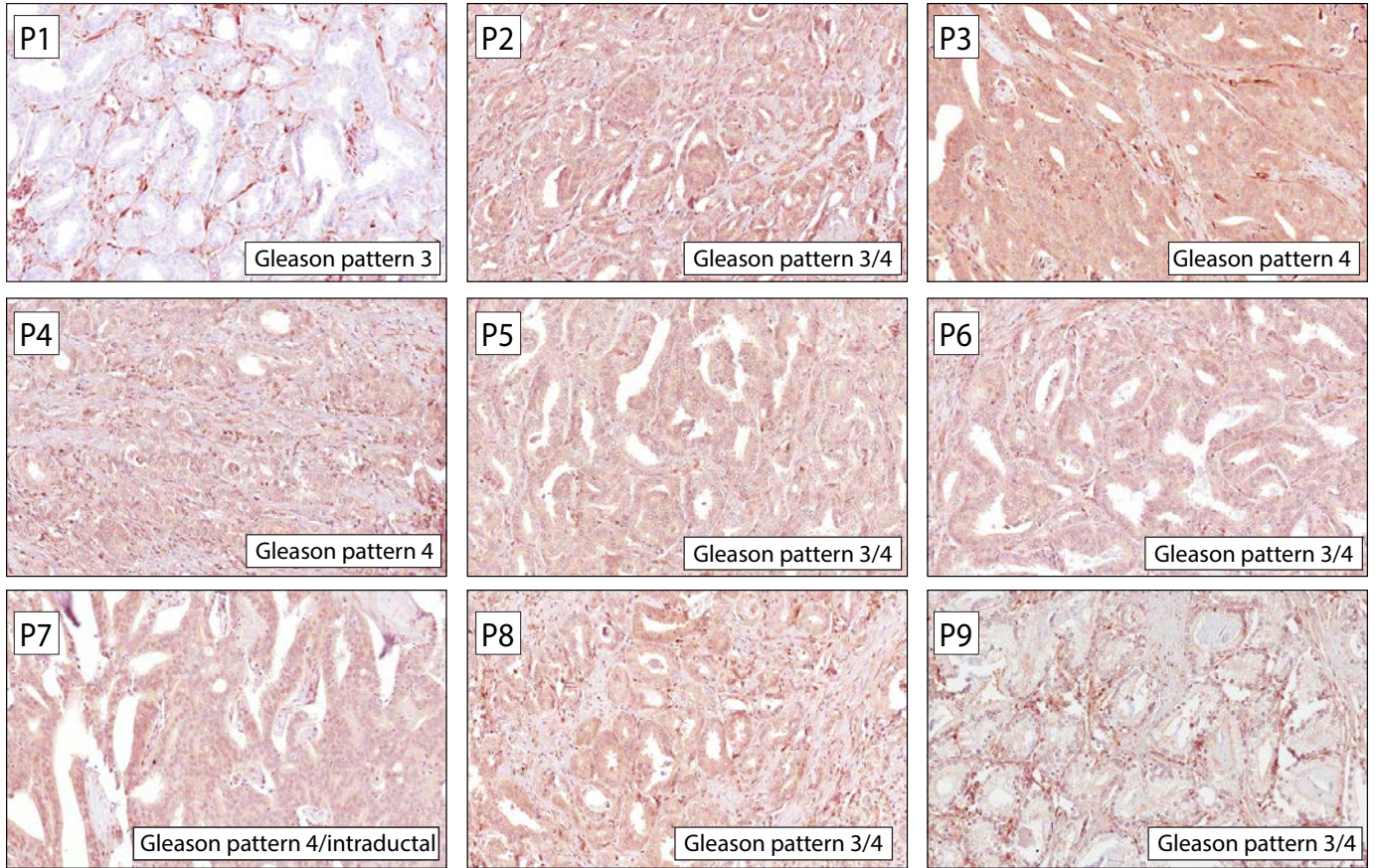
To validate a 18 Mb inversion on chromosome X encompassing the *ATRX* gene locus identified by whole genome sequencing in all 3 distant metastases, we designed PCR primers specific to *ATRX* in proximity to the breakpoint (*ATRX\_primer A*) and primers specific to the rearranged locus (*ATRX\_primer B*) (A). Using primers specific to the rearranged locus (*ATRX\_primer B*), we only observed amplification with DNAs derived from distant metastases (M5, M38, M40, M45, M53, M57, M63) but not with DNA from the primary tumor lesions (P1-P9), the lymph node (L1) metastasis or the biopsy of the lung metastasis (B1) indicating that the rearrangement in *ATRX* is only present in the distant metastases procured at autopsy. *ATRX\_primer A* located outside of the rearranged locus revealed robust amplification in all samples. Water controls are indicated with (W).

Since the observed inversion could result in an inactivation of the *ATRX* gene we investigated the expression of *ATRX* by immunohistochemistry. Whereas the primary tumor (C) and the lung biopsy (D) showed strong nuclear staining for *ATRX*, distant metastases were characterized by loss of *ATRX* expression (E). To assess telomere length in formalin fixed paraffin embedded tissue sections we used telomere specific fluorescence in situ hybridization (Telomere FISH, Panel F-H). Neoplastic cells (arrows) in the primary tumor and in the lung biopsy showed short telomeres. Adjacent normal stromal cells (arrowheads) showed detectable telomere signals. Telomeres in the distant metastases (arrows Panel H) however appeared ultra long characteristic of ALT. All IHC images are shown at 20x magnification. FISH images were taken at 100x magnification.



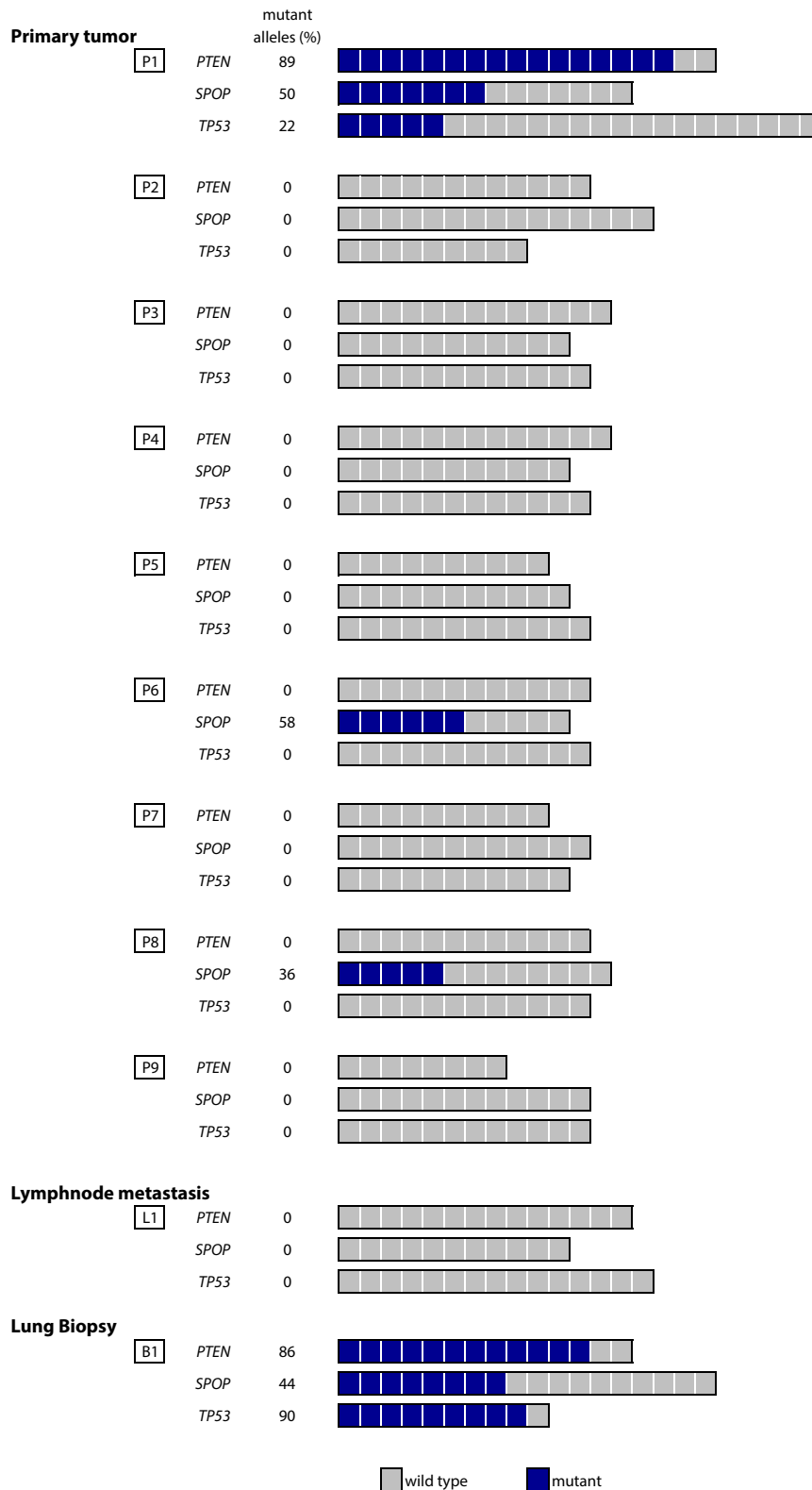
**Figure S7. Histopathological characterization of the primary prostatectomy specimen.**

Panel A shows a reconstructed cross-section of the radical prostatectomy specimen stained with hematoxylin and eosin (H&E). The green dotted lines highlight areas containing tumor glands. The black circle (P1) highlights a tumor focus with greatly reduced PTEN staining. Blue circles (P2 – P9) indicate additional tumor regions analyzed in the study. LA, RA, LP and RP denote left-anterior, right-anterior, left-posterior and right-posterior respectively. Panel B shows micrographs of individual regions (P1 – P9) at 20x magnification.

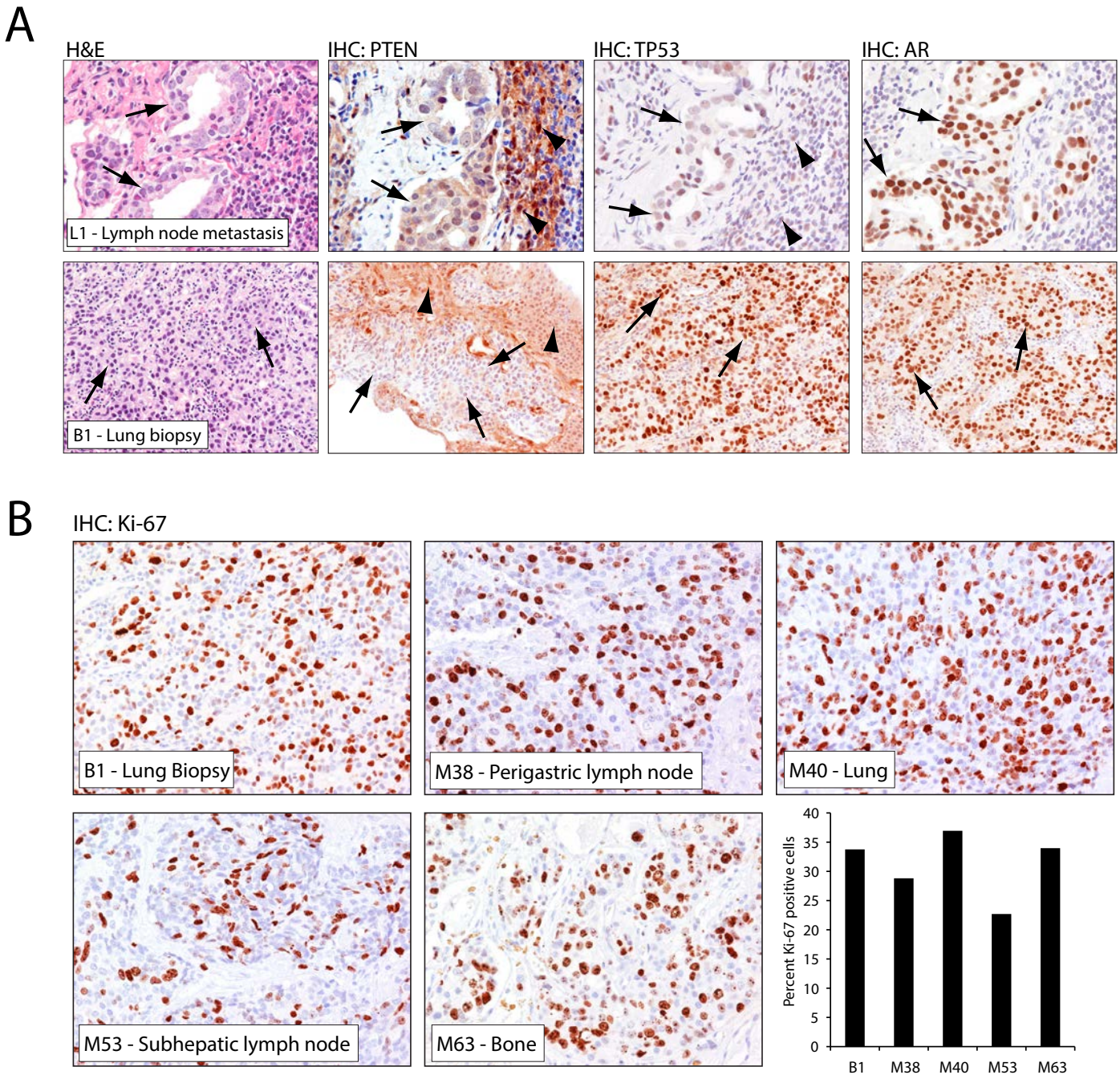


**Figure S8. PTEN staining in the primary prostatectomy specimen.**

PTEN protein expression was evaluated by immunohistochemistry in the primary radical prostatectomy specimen. Representative micrographs at 20x magnification of 9 distinct areas corresponding to regions highlighted in Figure S5 are shown. Note that the well differentiated P1 lesion is the only lesion with greatly reduced PTEN levels, whereas the intraductal carcinoma lesion (P7) shows robust PTEN expression.



**Figure S9. Mutation status of lesions in primary tumor, lymph node metastasis and lung biopsy tissue.** DNAs from indicated lesions were extracted and amplified with primers targeting index mutations in *PTEN*, *SPOP* and *TP53*. Amplified PCR products were subcloned and 8 to 23 individual clones were subjected to Sanger sequencing. Each sequenced clone is represented by a box. The presence of an index mutation is indicated in blue; a gray box indicates the absence of a mutation. The fraction of mutant alleles relative to the total number of analyzed clones is shown as percent of mutant alleles.



**Figure S10. Histopathological characterization of the lymph node metastasis resected at radical prostatectomy and the lung biopsy specimen.**

Formalin fixed paraffin embedded tissue sections from the lymph node metastasis resected at radical prostatectomy and a lung biopsy taken prior to autopsy were stained with hematoxylin and eosin or antibodies specific to PTEN, p53 and AR (Panel A). Hematoxylin and eosin staining confirms the presence of metastatic tumor cells (arrows). PTEN staining is present in metastatic cells in the lymph node but absent in the lung lesion. This is consistent with the observation that *PTEN* is not mutated in the lymph node metastasis resected at prostatectomy, but shows the index 4 bp deletion in the lung biopsy sample. p53 staining shows strong nuclear accumulation in the lung lesion, consistent with mutated *TP53*. The lymph node lesion shows low level nuclear staining in a subset of nuclei consistent with wild type *TP53*. Arrows indicate neoplastic cells, arrowheads show normal adjacent cells. Panel B shows staining for the cell proliferation marker Ki-67 in different metastases. Representative micrographs of the synchronous lymph node metastasis are shown at 40x magnification. All other images were taken at 20x.

**Table S1. Summary of sequencing coverage and rates of somatic alterations.**

Table S1 summarizes the sequencing coverage and the number of SNVs in the 4 samples analyzed by whole genome sequencing.

|                             | <b>Germline</b> | <b>M5</b>    | <b>M38</b>        | <b>M40</b>  |
|-----------------------------|-----------------|--------------|-------------------|-------------|
| <b>Organ site</b>           | <b>heart</b>    | <b>liver</b> | <b>lymph node</b> | <b>lung</b> |
| Average coverage            | 50              | 52           | 67                | 51          |
| min. 10x coverage           | 98.5%           | 98.5%        | 98.7%             | 99.4%       |
| min. 30x coverage           | 84.0%           | 82.2%        | 86.9%             | 91.8%       |
| High confidence SNVs        | *               | 13,642       | 14,343            | 13,457      |
| High confidence coding SNVs | *               | 113          | 125               | 112         |



**Table S3. Structural rearrangements identified in all 3 distant metastases.** Genomic coordinates (UCSC Genome Build hg19), strand orientation (+/-), Gene Symbol and RefSeq accession number are given for each genomic fusion sites. *Inter* indicates inter-chromosomal rearrangements. *Intra* indicates intra-chromosomal rearrangements. *Gene* indicates that the breakpoint resides in a coding region. *N* indicates breakpoints outside of coding genes.

| Position Left     | Gene Left           | Accession number | Position Right    | Gene Right      | Accession number | Junction Class   |
|-------------------|---------------------|------------------|-------------------|-----------------|------------------|------------------|
| chr1:92232063; +  | <i>TGFBFR3</i>      | NM_003243        | chr1:92233335; +  | <i>TGFBFR3</i>  | NM_003243        | Intra: Gene-Gene |
| chr1:187464828; + |                     |                  | chr1:187466727; - |                 |                  | Intra: N-N       |
| chr1:197361240; - | <i>CRB1</i>         | NM_201253        | chr4:168708274; - |                 |                  | Inter: Gene-N    |
| chr1:197527312; + | <i>DENND1B</i>      | NM_001142795     | chr1:197544974; + | <i>DENND1B</i>  | NM_001142795     | Intra: Gene-Gene |
| chr1:215272573; + | <i>KCNK2</i>        | NM_001017424     | chr1:215280133; + | <i>KCNK2</i>    | NM_001017424     | Intra: Gene-Gene |
| chr1:217227844; + | <i>ESRRG</i>        | NM_001134285     | chr1:237763237; - | <i>RYR2</i>     | NM_001035        | Intra: Gene-Gene |
| chr1:217252631; - | <i>ESRRG</i>        | NM_001134285     | chr1:242044266; + | <i>EXO1</i>     | NM_003686        | Intra: Gene-Gene |
| chr1:222719972; + | <i>HHIPL2</i>       | NM_024746        | chr1:240002951; - | <i>CHRM3</i>    | NM_000740        | Intra: Gene-Gene |
| chr1:238075731; - | <i>LOC100130331</i> | NR_027247        | chr1:240003267; + | <i>CHRM3</i>    | NM_000740        | Intra: Gene-Gene |
| chr1:240229251; + |                     |                  | chr12:17434582; + |                 |                  | Inter: N-N       |
| chr1:240239736; - |                     |                  | chr12:18160665; + |                 |                  | Inter: N-N       |
| chr1:244186578; + |                     |                  | chr1:244190576; + |                 |                  | Intra: N-N       |
| chr1:244416821; + |                     |                  | chr1:244418379; + |                 |                  | Intra: N-N       |
| chr2:53321119; +  |                     |                  | chr2:53330056; +  |                 |                  | Intra: N-N       |
| chr2:88847375; +  |                     |                  | chr7:122227934; - | <i>CADPS2</i>   | NM_001009571     | Inter: Gene-N    |
| chr2:88863004; -  | <i>EIF2AK3</i>      | NM_004836        | chr7:124378342; + |                 |                  | Inter: Gene-N    |
| chr2:88916508; -  | <i>EIF2AK3</i>      | NM_004836        | chr7:125448295; - |                 |                  | Inter: Gene-N    |
| chr2:88920314; -  | <i>EIF2AK3</i>      | NM_004836        | chr7:125564557; - |                 |                  | Inter: Gene-N    |
| chr2:89037880; +  | <i>RPIA</i>         | NM_144563        | chr7:124576015; - |                 |                  | Inter: Gene-N    |
| chr2:118606832; + |                     |                  | chr2:118608319; + |                 |                  | Intra: N-N       |
| chr2:123142316; + |                     |                  | chr2:126896611; + |                 |                  | Intra: N-N       |
| chr2:134578826; + |                     |                  | chr7:124578450; + |                 |                  | Inter: N-N       |
| chr2:134784407; - |                     |                  | chr7:125101132; + |                 |                  | Inter: N-N       |
| chr2:208474485; + | <i>FAM119A</i>      | NM_001127395     | chr2:208476787; + | <i>FAM119A</i>  | NM_001127395     | Intra: Gene-Gene |
| chr3:2438707; +   | <i>CNTN4</i>        | NM_175607        | chr3:2440189; +   | <i>CNTN4</i>    | NM_175607        | Intra: Gene-Gene |
| chr3:3180642; +   | <i>TRNT1</i>        | NM_182916        | chr3:3182832; +   | <i>TRNT1</i>    | NM_182916        | Intra: Gene-Gene |
| chr3:24489644; +  | <i>THRB</i>         | NM_000461        | chr3:24490841; -  | <i>THRB</i>     | NM_000461        | Intra: Gene-Gene |
| chr3:82185343; +  |                     |                  | chr3:82306944; +  |                 |                  | Intra: N-N       |
| chr3:82186894; +  |                     |                  | chr3:82226895; +  |                 |                  | Intra: N-N       |
| chr3:82189509; +  |                     |                  | chr3:82502576; -  |                 |                  | Intra: N-N       |
| chr3:82306815; +  |                     |                  | chr3:83002264; +  |                 |                  | Intra: N-N       |
| chr3:82547485; -  |                     |                  | chr3:83001909; +  |                 |                  | Intra: N-N       |
| chr3:83002262; -  |                     |                  | chr3:83083636; +  |                 |                  | Intra: N-N       |
| chr3:88177903; +  |                     |                  | chr4:12474466; +  |                 |                  | Inter: N-N       |
| chr3:88177938; -  |                     |                  | chr12:40013504; + | <i>ABCD2</i>    | NM_005164        | Inter: Gene-N    |
| chr3:105306007; + |                     |                  | chr3:105311042; + |                 |                  | Intra: N-N       |
| chr3:115306181; + |                     |                  | chr3:115322706; + |                 |                  | Intra: N-N       |
| chr3:164456857; + |                     |                  | chr3:164670242; - |                 |                  | Intra: N-N       |
| chr3:164475607; - |                     |                  | chr3:164703834; + | <i>SI</i>       | NM_001041        | Intra: Gene-N    |
| chr3:196056048; + | <i>TM4SF19</i>      | NM_138461        | chr3:196056976; + | <i>TM4SF19</i>  | NM_138461        | Intra: Gene-Gene |
| chr4:12962216; +  |                     |                  | chr12:40077594; - | <i>C12orf40</i> | NM_001031748     | Inter: Gene-N    |
| chr4:36015773; +  |                     |                  | chr4:36028224; +  |                 |                  | Intra: N-N       |
| chr4:60294500; +  |                     |                  | chr4:63904173; +  |                 |                  | Intra: N-N       |
| chr4:60802209; -  |                     |                  | chr4:61079154; +  |                 |                  | Intra: N-N       |
| chr4:60833598; +  |                     |                  | chr4:63675452; +  |                 |                  | Intra: N-N       |
| chr4:61330721; +  |                     |                  | chr4:61340124; +  |                 |                  | Intra: N-N       |
| chr4:61340367; +  |                     |                  | chr4:65703373; +  |                 |                  | Intra: N-N       |
| chr4:63763661; +  |                     |                  | chr4:65294413; +  |                 |                  | Intra: N-N       |
| chr4:91931557; +  | <i>FAM190A</i>      | NM_001145065     | chr4:91935837; +  | <i>FAM190A</i>  | NM_001145065     | Intra: Gene-Gene |
| chr4:93879352; -  | <i>GRID2</i>        | NM_001510        | chr4:106561473; - |                 |                  | Intra: Gene-N    |
| chr4:93894636; -  | <i>GRID2</i>        | NM_001510        | chr4:112144989; - |                 |                  | Intra: Gene-N    |
| chr4:93894855; +  | <i>GRID2</i>        | NM_001510        | chr4:112315045; + |                 |                  | Intra: Gene-N    |
| chr4:94195031; +  | <i>GRID2</i>        | NM_001510        | chr4:113985935; - | <i>ANK2</i>     | NM_001127493     | Intra: Gene-Gene |
| chr4:94197147; -  | <i>GRID2</i>        | NM_001510        | chr4:112314678; - |                 |                  | Intra: Gene-N    |
| chr4:104121165; - |                     |                  | chr4:106554702; + |                 |                  | Intra: N-N       |
| chr4:104382367; + |                     |                  | chr4:105956008; - |                 |                  | Intra: N-N       |
| chr4:104386918; - |                     |                  | chr4:112309714; + |                 |                  | Intra: N-N       |
| chr4:104485932; + |                     |                  | chr4:106166098; - | <i>TET2</i>     | NM_001127208     | Intra: Gene-N    |
| chr4:104496141; - |                     |                  | chr4:105967896; + |                 |                  | Intra: N-N       |
| chr4:106022494; - |                     |                  | chr4:106506022; + | <i>FLJ20184</i> | NM_017700        | Intra: Gene-N    |
| chr4:106552925; + |                     |                  | chr4:112601658; + |                 |                  | Intra: N-N       |
| chr4:107437804; + |                     |                  | chr4:107438587; + |                 |                  | Intra: N-N       |
| chr4:112111104; - |                     |                  | chr4:120018042; - |                 |                  | Intra: N-N       |
| chr4:112186117; - |                     |                  | chr4:115177784; - |                 |                  | Intra: N-N       |
| chr4:112293614; + |                     |                  | chr4:113267256; + | <i>ALPK1</i>    | NM_001102406     | Intra: Gene-N    |
| chr4:113107896; - | <i>C4orf32</i>      | NM_152400        | chr4:120068424; + | <i>MYOZ2</i>    | NM_016599        | Intra: Gene-Gene |
| chr4:113318397; - | <i>ALPK1</i>        | NM_001102406     | chr4:115261891; + |                 |                  | Intra: Gene-N    |
| chr4:114027003; - | <i>ANK2</i>         | NM_001127493     | chr4:119826006; - | <i>SYNPO2</i>   | NM_001128933     | Intra: Gene-Gene |
| chr4:114503579; + | <i>CAMK2D</i>       | NM_001221        | chr4:114836755; + | <i>ARSJ</i>     | NM_024590        | Intra: Gene-Gene |
| chr4:114573800; - | <i>CAMK2D</i>       | NM_001221        | chr4:118050408; - |                 |                  | Intra: Gene-N    |
| chr4:114803652; + |                     |                  | chr4:116568770; + |                 |                  | Intra: N-N       |
| chr4:115226266; - |                     |                  | chr4:115789361; + | <i>NDST4</i>    | NM_022569        | Intra: Gene-N    |
| chr4:115247970; + |                     |                  | chr4:119721202; - | <i>SEC24D</i>   | NM_014822        | Intra: Gene-N    |
| chr4:115565543; - | <i>UGT8</i>         | NM_001128174     | chr4:118080385; + |                 |                  | Intra: Gene-N    |
| chr4:115746627; + |                     |                  | chr4:119841869; + | <i>SYNPO2</i>   | NM_001128933     | Intra: Gene-N    |
| chr4:116564038; + |                     |                  | chr4:117738924; + |                 |                  | Intra: N-N       |
| chr4:116892281; + |                     |                  | chr4:116917089; + |                 |                  | Intra: N-N       |
| chr4:117704357; + |                     |                  | chr4:119732666; + | <i>SEC24D</i>   | NM_014822        | Intra: Gene-N    |
| chr4:120950454; + |                     |                  | chr4:120970800; + |                 |                  | Intra: N-N       |
| chr4:131326052; + |                     |                  | chr4:155775925; + |                 |                  | Intra: N-N       |
| chr4:133027749; + |                     |                  | chr4:133028777; + |                 |                  | Intra: N-N       |
| chr4:133933774; - |                     |                  | chr4:136168230; + |                 |                  | Intra: N-N       |
| chr4:152035816; - |                     |                  | chr4:152108907; - | <i>SH3D19</i>   | NM_001009555     | Intra: Gene-N    |
| chr4:154894897; + |                     |                  | chr4:155855653; + |                 |                  | Intra: N-N       |
| chr4:155704831; - | <i>RBM46</i>        | NM_144979        | chr4:155848929; - |                 |                  | Intra: Gene-N    |
| chr4:166480528; + |                     |                  | chr4:167621581; + |                 |                  | Intra: N-N       |
| chr5:8062804; -   |                     |                  | chr6:71262545; -  | <i>FAM135A</i>  | NM_001105531     | Inter: Gene-N    |
| chr5:19863922; +  | <i>CDH18</i>        | NM_001167667     | chr18:52939897; + | <i>TCF4</i>     | NM_001083962     | Inter: Gene-Gene |
| chr5:19867800; -  | <i>CDH18</i>        | NM_001167667     | chr18:50247314; + | <i>DCC</i>      | NM_005215        | Inter: Gene-Gene |
| chr5:23963889; +  |                     |                  | chr5:23969826; +  |                 |                  | Intra: N-N       |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        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| chr5:29069317; +<br>chr5:58658640; -<br>chr5:97392955; +<br>chr5:97400990; -<br>chr5:98237148; +<br>chr5:98523470; +<br>chr5:107181236; +<br>chr5:178012574; -<br>chr6:31193507; +<br>chr6:53924805; +<br>chr6:66913235; -<br>chr6:67601531; +<br>chr6:67630642; -<br>chr6:70515233; -<br>chr6:121717723; +<br>chr6:121718967; -<br>chr6:138277201; +<br>chr6:145487592; -<br>chr7:20787149; +<br>chr7:20788836; -<br>chr7:20795434; +<br>chr7:20800584; -<br>chr7:20800604; +<br>chr7:22823974; -<br>chr7:29459292; +<br>chr7:67635773; +<br>chr7:100327776; +<br>chr7:109436689; +<br>chr7:121162519; +<br>chr7:123580873; +<br>chr7:141808583; +<br>chr7:152539927; -<br>chr7:152630469; +<br>chr7:154392102; +<br>chr7:157951378; +<br>chr7:158387912; -<br>chr8:3017720; -<br>chr8:3244162; +<br>chr8:6010039; +<br>chr8:8198691; -<br>chr8:9376469; +<br>chr8:9785084; -<br>chr8:10359875; +<br>chr8:10497068; -<br>chr8:11631255; +<br>chr8:12928658; +<br>chr8:13353359; -<br>chr8:13536485; +<br>chr8:13857225; +<br>chr8:19252905; -<br>chr8:21179042; +<br>chr8:21649242; +<br>chr8:23186263; +<br>chr8:28247268; -<br>chr8:28510838; +<br>chr8:29921431; +<br>chr8:29921431; +<br>chr8:29927576; +<br>chr8:40757450; +<br>chr8:48816732; +<br>chr8:51512998; +<br>chr8:51973274; +<br>chr8:52936102; +<br>chr9:135759089; +<br>chr10:29775023; -<br>chr10:70786361; +<br>chr10:133652126; -<br>chr11:68731454; -<br>chr11:103921989; +<br>chr11:105263354; -<br>chr11:105354310; +<br>chr11:106113577; +<br>chr11:106165651; +<br>chr11:125149714; +<br>chr12:1028930; +<br>chr12:17427282; +<br>chr12:40013983; +<br>chr12:81210005; +<br>chr12:133049542; +<br>chr13:26516270; -<br>chr13:32562194; +<br>chr13:37745091; +<br>chr13:38039321; +<br>chr13:62895883; +<br>chr13:76675572; -<br>chr13:76763038; +<br>chr13:76833695; -<br>chr13:76853715; -<br>chr13:77604068; +<br>chr13:77937003; +<br>chr13:77982724; -<br>chr13:77983292; -<br>chr13:85397673; - | <i>PDE4D</i><br><br><i>CHD1</i><br><br><i>COL23A1</i><br><br><i>C6orf142</i><br><br><br><br><br><br><br><i>ABCB5</i><br><i>ABCB5</i><br><i>ABCB5</i><br><br><br><br><br><i>CHN2</i><br><br><br><br><i>SPAM1</i><br><br><i>ACTR3B</i><br><br><i>DPP6</i><br><i>PTPRN2</i><br><br><i>CSMD1</i><br><i>CSMD1</i><br><br><i>SGK223</i><br><br><br><br><br><br><br><i>RP1L1</i><br><i>NEIL2</i><br><br><i>DLC1</i><br><br><br><i>SH2D4A</i><br><br><br><br><i>LOXL2</i><br><br><br><br><br><i>TMEM66</i><br><i>TMEM66</i><br><i>TMEM66</i><br><br><i>PRKDC</i><br><i>SNTG1</i><br><br><br><i>C9orf9</i><br><i>SVIL</i><br><br><br><br><br><br><br><br><br><br><i>PKNOX2</i><br><i>RAD52</i><br><br><br><i>LIN7A</i><br><br><br><i>ATP8A2</i> | NM_001104631<br><br>NM_001270<br><br>NM_173465<br><br>NM_138569<br><br><br><br><br>NM_001163941<br>NM_001163941<br>NM_001163941<br><br><br><br><br>NM_004067<br><br><br><br>NM_001174044<br><br>NM_001040135<br><br>NM_001039350<br>NM_002847<br><br>NM_033225<br>NM_033225<br><br>NM_001080826<br><br><br><br><br><br><br>NM_178857<br>NM_001135746<br><br>NM_024767<br><br><br><br>NM_022071<br><br><br><br>NM_002318<br><br><br><br><br>NM_016127<br>NM_016127<br>NM_016127<br><br>NM_001081640<br>NM_018967<br><br><br>NM_018956<br>NM_003174<br><br><br><br><br><br><br><br><br><br>NM_025208<br><br><br><br>NM_022062<br>NM_134424<br><br><br>NM_004664<br><br><br>NM_016529 | chr5:29069625; -<br>chr5:58663790; -<br>chr5:102250475; -<br>chr5:125996199; +<br>chr5:99776806; +<br>chr18:50851985; -<br>chr5:109750410; +<br>chr5:178012742; -<br>chr6:31194660; +<br>chr6:53934830; +<br>chr6:71285774; +<br>chr6:67773605; +<br>chr6:70640607; -<br>chr6:128284244; +<br>chr6:122983363; -<br>chr6:122998893; +<br>chr6:140268501; -<br>chr6:145488062; -<br>chr7:20795416; -<br>chr12:12928895; +<br>chr7:30762231; -<br>chr12:8810960; -<br>chr7:20800906; -<br>chr7:30762261; +<br>chr7:29461676; +<br>chr7:98083201; -<br>chr7:100340919; +<br>chr7:109453901; +<br>chr7:121162621; -<br>chr7:125449898; +<br>chr7:154178565; -<br>chr7:154186118; +<br>chr7:153559400; +<br>chr7:154399951; +<br>chr7:157951922; +<br>chr7:158387981; -<br>chr8:8938090; -<br>chr8:6636967; -<br>chr8:6604104; +<br>chr8:15556863; +<br>chr8:19735831; -<br>chr8:17281326; +<br>chr8:22834604; +<br>chr8:13788311; +<br>chr8:17455487; -<br>chr8:12931011; +<br>chr8:13750498; -<br>chr8:31624540; +<br>chr8:28442174; -<br>chr8:25870831; +<br>chr8:30720105; +<br>chr8:29362830; +<br>chr8:23957124; +<br>chr8:29926865; -<br>chr8:28511558; -<br>chr8:29924292; +<br>chr8:29927156; +<br>chr8:29940363; +<br>chr8:42073066; +<br>chr8:48817297; +<br>chr8:52697372; +<br>chr8:52015671; +<br>chr8:53017899; +<br>chr9:135760133; +<br>chr10:29845877; -<br>chr10:70787344; -<br>chr10:133652343; -<br>chr11:68731614; -<br>chr11:118027231; -<br>chr11:106114806; +<br>chr11:117654980; +<br>chr11:106231711; +<br>chr11:106390941; -<br>chr11:125150310; +<br>chr12:1029739; +<br>chr12:17746295; +<br>chr12:40077869; +<br>chr12:82180509; +<br>chr12:133050800; +<br>chr13:26641707; -<br>chr13:32597220; +<br>chr13:37766199; +<br>chr13:49013451; +<br>chr13:63133935; +<br>chr13:89765279; -<br>chr13:89842428; +<br>chr13:89854515; -<br>chr13:92237631; -<br>chr13:89449260; +<br>chr13:90367709; +<br>chr13:89632285; -<br>chr13:89872954; -<br>chr13:89631312; + | <i>PDE4D</i><br><i>PAM</i><br><br><br><i>DCC</i><br><br><i>COL23A1</i><br><br><i>C6orf142</i><br><i>C6orf57</i><br><br><i>COL19A1</i><br><br><i>PKIB</i><br><i>PKIB</i><br><br><br><i>ABCB5</i><br>< |
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Table S3 continued.

|                    |                 |              |                    |                |              |                  |
|--------------------|-----------------|--------------|--------------------|----------------|--------------|------------------|
| chr13:85478461; +  |                 |              | chr13:85480008; +  |                |              | Intra: N-N       |
| chr13:86652840; +  |                 |              | chr13:89632401; +  |                |              | Intra: N-N       |
| chr13:86657635; -  |                 |              | chr13:89455783; +  |                |              | Intra: N-N       |
| chr13:88584892; +  |                 |              | chr13:88587449; +  |                |              | Intra: N-N       |
| chr13:88929918; +  |                 |              | chr13:89582145; +  |                |              | Intra: N-N       |
| chr13:88941159; -  |                 |              | chr13:89872967; +  |                |              | Intra: N-N       |
| chr13:89443915; -  |                 |              | chr13:91361718; -  |                |              | Intra: N-N       |
| chr13:89562487; +  |                 |              | chr13:91440093; +  |                |              | Intra: N-N       |
| chr13:89631137; +  |                 |              | chr13:90960399; +  |                |              | Intra: N-N       |
| chr13:89633675; +  |                 |              | chr13:90962250; +  |                |              | Intra: N-N       |
| chr13:89637766; -  |                 |              | chr13:91378322; +  |                |              | Intra: N-N       |
| chr13:90447661; -  |                 |              | chr13:92654277; +  | <i>GPC5</i>    | NM_004466    | Intra: Gene-N    |
| chr13:90858282; -  |                 |              | chr13:92600417; -  | <i>GPC5</i>    | NM_004466    | Intra: Gene-N    |
| chr13:113499584; + | <i>ATP11A</i>   | NM_015205    | chr13:113500181; + | <i>ATP11A</i>  | NM_015205    | Intra: Gene-Gene |
| chr14:49191110; +  |                 |              | chr14:49202994; +  |                |              | Intra: N-N       |
| chr14:66258292; -  |                 |              | chr14:66258410; -  |                |              | Intra: N-N       |
| chr14:68822870; -  | <i>RAD51L1</i>  | NM_002877    | chr14:68823419; -  | <i>RAD51L1</i> | NM_002877    | Intra: Gene-Gene |
| chr14:100993015; - | <i>WDR25</i>    | NM_001161476 | chr14:100993316; - | <i>WDR25</i>   | NM_001161476 | Intra: Gene-Gene |
| chr14:105289321; + | <i>MGC23270</i> | NR_024396    | chr14:105295690; + |                |              | Intra: Gene-N    |
| chr15:52388396; +  |                 |              | chr15:52389649; +  |                |              | Intra: N-N       |
| chr16:48966187; -  |                 |              | chr16:48966587; -  |                |              | Intra: N-N       |
| chr16:76667358; +  |                 |              | chr16:76668111; +  |                |              | Intra: N-N       |
| chr17:41379299; -  |                 |              | chr17:41401061; +  |                |              | Intra: N-N       |
| chr17:41401198; +  |                 |              | chr17:41467151; -  |                |              | Intra: N-N       |
| chr17:43097684; -  |                 |              | chr17:52635197; +  |                |              | Intra: N-N       |
| chr17:44287865; -  |                 |              | chr17:45175823; -  |                |              | Intra: N-N       |
| chr17:51508565; -  |                 |              | chr17:51956655; -  |                |              | Intra: N-N       |
| chr17:55687811; +  | <i>MSI2</i>     | NM_138962    | chr17:55689916; +  | <i>MSI2</i>    | NM_138962    | Intra: Gene-Gene |
| chr18:109601; +    |                 |              | chrY:13485983; -   |                |              | Inter: N-N       |
| chr18:50235832; +  | <i>DCC</i>      | NM_005215    | chr18:53540155; -  |                |              | Intra: Gene-N    |
| chr18:52375844; -  |                 |              | chr18:53252110; +  | <i>TCF4</i>    | NM_001083962 | Intra: Gene-N    |
| chr19:23935919; -  | <i>ZNF681</i>   | NM_138286    | chr19:23936383; -  | <i>ZNF681</i>  | NM_138286    | Intra: Gene-Gene |
| chr20:42325141; -  | <i>MYBL2</i>    | NM_002466    | chr20:42325645; -  | <i>MYBL2</i>   | NM_002466    | Intra: Gene-Gene |
| chr21:9834344; +   |                 |              | chr21:9835502; +   |                |              | Intra: N-N       |
| chr21:16588380; +  |                 |              | chr21:16591454; +  |                |              | Intra: N-N       |
| chr21:36072766; +  | <i>CLIC6</i>    | NM_053277    | chr21:36073629; +  | <i>CLIC6</i>   | NM_053277    | Intra: Gene-Gene |
| chr21:43226732; +  | <i>PRDM15</i>   | NM_001040424 | chr21:43227386; +  | <i>PRDM15</i>  | NM_001040424 | Intra: Gene-Gene |
| chrX:63645317; +   |                 |              | chrX:63646554; +   |                |              | Intra: N-N       |
| chrX:67768511; +   |                 |              | chrX:67769690; -   |                |              | Intra: N-N       |
| chrX:76943176; +   | <i>ATRX</i>     | NM_000489    | chrX:95432589; -   |                |              | Intra: Gene-N    |
| chrX:76943178; -   | <i>ATRX</i>     | NM_000489    | chrX:95432590; +   |                |              | Intra: Gene-N    |
| chrX:86879212; +   | <i>KLHL4</i>    | NM_019117    | chrX:86891551; +   | <i>KLHL4</i>   | NM_019117    | Intra: Gene-Gene |





**Table S4 continued.**

|                          |       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------|-------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| chr5:102300000-125999999 | 0.01  | PAM, GIN1, PPIP5K2, C5orf30, NUDT12, EFNA5, FBXL17, FER, PJA2, MAN2A1, AC011366.3, TMEM232, SLC25A46, TSLP, WDR36, CAMK4, STARD4, C5orf13, EPB41L4A, APC, SRP19, REEP5, ZRSR1, DCP2, MCC, TSSK1B, YTHDC2, KCNN2, TRIM36, PGGT1B, CCDC112, FEM1C, TICAM2, TMED7-TICAM2, TMED7, CDO1, ATG12, AP3S1, AC010282.1, COMMD10, SEMA6A, DTWD2, DMXL1, TNFAIP8, HSD17B4, FAM170A, PRR16, FTMT, SRFBP1, LOX, ZNF474, SNCAIP, SNX2, SNX24, PPIC, PRDM6, CEP120, CSNK1G3, ZNF608, RP11-43D2.2, GRAMD3, ALDH7A1, PHAX, C5orf48 |
| chr6:68800000-71299999   | -0.01 | BAI3, LMBRD1, COL19A1, COL9A1, FAM135A, C6orf57                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| chr7:20800000-22899999   | 0.04  | SP8, SP4, DNAH11, CDCA7L, RAPGEF5, STEAP1B, IL6, TOMM7                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| chr18:50900000-52399999  | -0.06 | DCC, MBD2, POLI, STARD6, C18orf54, C18orf26                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| chr18:53500000-66399999  | -0.1  | AC006305.1, TXNL1, WDR7, AC100775.1, ST8SIA3, ONECUT2                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                          | -0.01 | FECH, NARS, ATP8B1, AC090324.1, NEDD4L, ALPK2, MALT1, ZNF532                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                          | -0.08 | SEC11C, GRP, RAX, CPLX4, LMAN1, CCBE1, PMAIP1, MC4R, CDH20, RNF152, PIGN, KIAA1468, TNFRSF11A, ZCCHC2, PHLPP1, AC015989.1, BCL2, KDSR, VPS4B, SERPINB5, SERPINB12, SERPINB13, SERPINB4, SERPINB11, SERPINB3, SERPINB7, SERPINB2, SERPINB10, HMSD, SERPINB8, CDH7, CDH19, DSEL, TMX3                                                                                                                                                                                                                              |

**Table S5. Additional reagents and primers used in the study.**

**List of antibodies used for immunohistochemistry**

| <b>Antibody</b> | <b>Company</b>                    | <b>Catalog number</b> | <b>Pretreatment</b>  | <b>Dilution</b> | <b>Incubation</b> | <b>Secondary reagent</b> |
|-----------------|-----------------------------------|-----------------------|----------------------|-----------------|-------------------|--------------------------|
| <b>ATRX</b>     | Sigma-Aldrich                     | HPA001906             | Citrate steam 30 min | 1:400           | RT - 60 min       | PV+                      |
| <b>AR</b>       | Santa Cruz                        | sc-816                | HTTR steam 50 min    | 1:1000          | RT - 45 min       | PV+                      |
| <b>PSA</b>      | Dako                              | M0750                 | Citrate steam 25 min | 1:50            | RT - 45 min       | PV+                      |
| <b>PTEN</b>     | Cell Signaling                    | 9188                  | EDTA steam 45 min    | 1:100           | 4°C - over night  | PV+                      |
| <b>P53</b>      | Dako                              | M7001                 | HTTR steam 50 min    | 1:2000          | 4°C - over night  | PV+                      |
| <b>NKX3.1</b>   | C. Bieberich, UMBC, Baltimore, MD |                       | Citrate steam 25 min | 1:500           | RT - 45 min       | PV+                      |
| <b>Ki-67</b>    | Zymed                             | 18-0192               | HTTR steam 50 min    | 1:4000          | 4°C - over night  | PV+                      |

**Abbreviations**

|         |                                                                                          |
|---------|------------------------------------------------------------------------------------------|
| HTTR    | Target retrieval Solution (Dako, cat#S1700)                                              |
| Citrate | 10 mM Sodium Citrate (ph 6.0)                                                            |
| EDTA    | EDTA buffer (Invitrogen, #00-5500)                                                       |
| PV+     | PowerVision anti-mouse and anti-rabbit secondary antibodies (Leica, cat# PV6114, PV6119) |

**List of primers used in the study**

| <b>Name</b>          | <b>Sequence</b>              | <b>Annealing temperature</b> |
|----------------------|------------------------------|------------------------------|
| <b>PTEN_Ex8</b>      | F CAGGACCAGAGGAAACCTCA       | 55                           |
|                      | R AAGTCAACAACCCCAACAAA       |                              |
| <b>SPOP_Ex4</b>      | F GACTCACCTCGCAGAAGAGG       | 58                           |
|                      | R GGCTTTGATCTGGTTTTTGC       |                              |
| <b>TP53_Ex7</b>      | F GGCTCCTGACCTGGAGTCTT       | 58                           |
|                      | R GCACTGGCCTCATCTTGG         |                              |
| <b>ATRX_primer A</b> | F ACATGTCACAAATCCAGCAA       | 58                           |
|                      | R GATTCAGACATGCTTAAATGAACTG  |                              |
| <b>ATRX_primer B</b> | F CTGTCACAATTTAAATGAACTCTC   | 55                           |
|                      | R AAAATGACAGAGTTAGAAAATCACCA |                              |

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