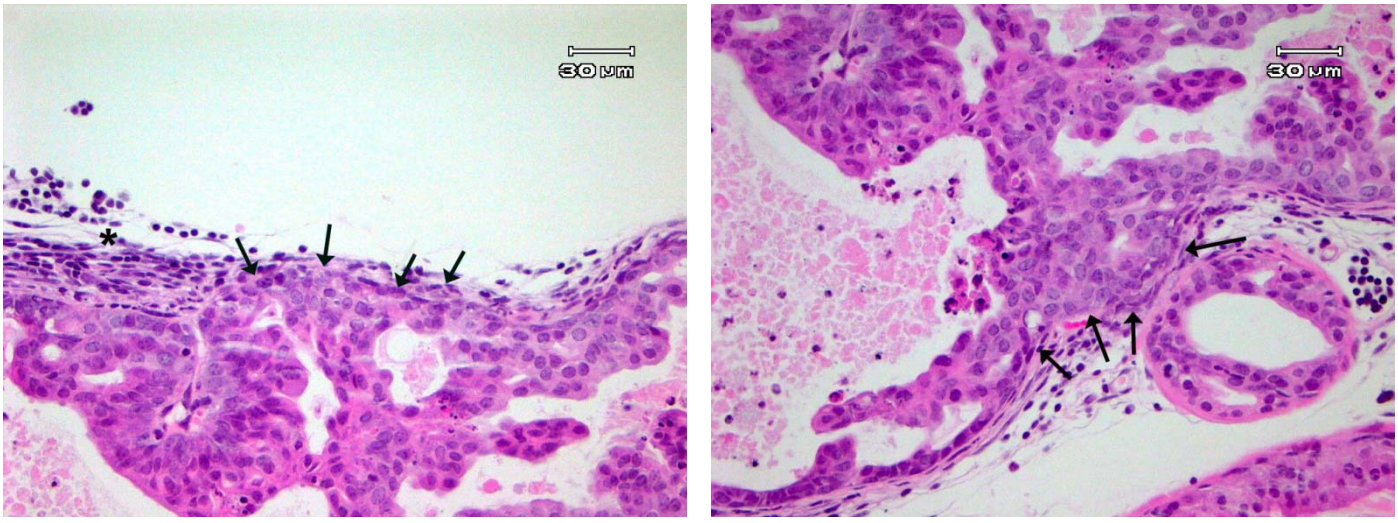
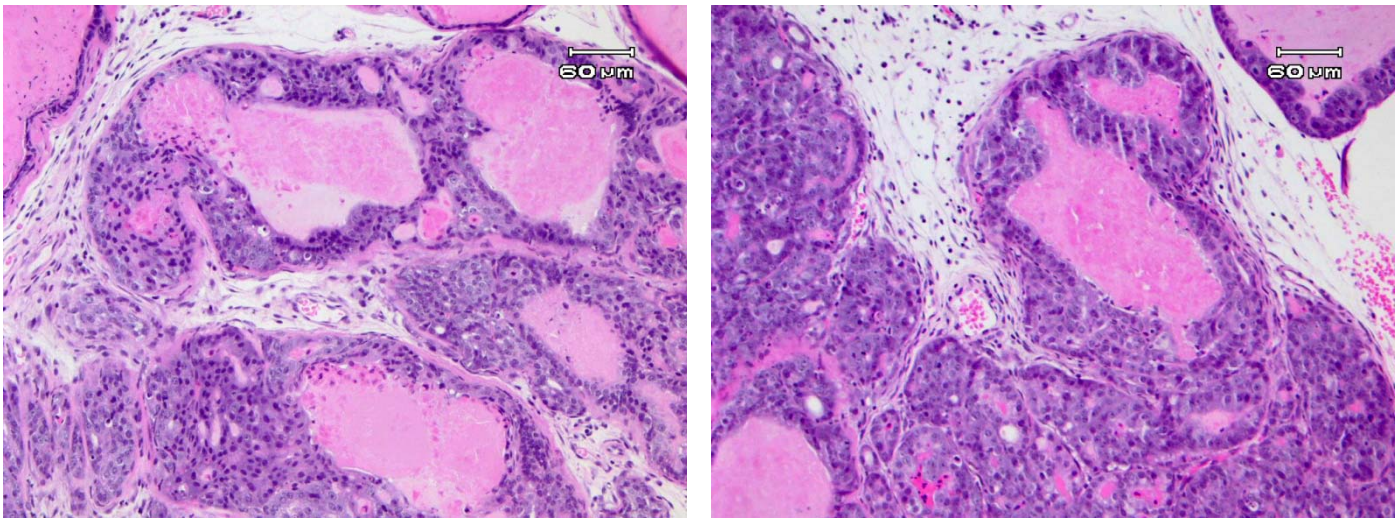


Supplementary Figure 1. Evidence of microinvasion in a *Pten+/-; TMPRSS2-ERG* mouse. H&E stain at 6 months of age (400X). Arrows point to areas of stromal disruption and inflammation in reaction to transformed epithelium.

ARR2PB-c-myc *ARR2PB-c-myc; TMPRSS2-ERG*



Supplementary Figure 2. No evidence of cooperativity of *TMPRSS2-ERG* and *c-Myc* in a mouse model of prostate cancer. Similar histological appearance of ventral prostate from *ARR2PB-c-Myc* and *ARR2PB-c-Myc ;TMPRSS2-ERG* mice at 6 months of age showing high grade PIN and invasive cancer.

Supplementary Methods

Constructs, Western blot, and qRT-PCR

The TMPRSS2-ERG fusion and full length ERG constructs were made by PCR cloning from an ERG variant 2 cDNA (IOH41014) from Invitrogen. For the fusion, the 5' primer included exon 1 of TMPRSS2 and basepairs 226-246 of ERG and the 3' primer included a Flag tag sequence and basepairs 1552 to 1572 exon of ERG. Both PCR products were cloned into PCR 2.1 (Invitrogen) and were sequence verified. They were subsequently cloned into pcDNA3.1 expression vector. The ATG to AAG mutation at codon 304 was made with the Quikchange mutagenesis Kit (Stratagene). ERG C-20 antibody was used for the Western blot (Santa Cruz). cDNA was generated using the Super Script III First-Strand Synthesis system for RT-PCR (Invitrogen). Quantitative RT-PCR was performed using the SYBR Green PCR light cycler protocol (Qiagen). Primers were designed for: ERG forward: TTA TCA GTT GTG AGT GAG GAC CA and reverse: AAG TCT GTC CAT AGT CGC TGG.

Mass spectrometry

Flag tagged versions of full length, truncated, or TMPRSS2-ERG fusion were transfected into 293FT cells. Protein was isolated using RIPA lysis buffer (50mM Tris pH 8.0, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate) and immunoprecipitated using anti-flag antibody. Gel-resolved proteins were digested with trypsin, batch fractionated on a Poros 50 R2 RP micro-tip, and resulting peptide pools analyzed by matrix-assisted laser-desorption/ionization reflectron time-of-flight (MALDI-reTOF) MS using a BRUKER UltraFlex TOF/TOF instrument (Bruker Daltonics; Bremen, Germany), as described (Erdjument-Bromage et al., 1998; Sebastiaan Winkler et al., 2002). Selected experimental masses (m/z) were taken to search the human segment of a non-redundant protein database ('NR'; ~134,198 entries; National Center for Biotechnology Information; Bethesda, MD), utilizing the MASCOT Peptide Mass Fingerprint (PMF) program, version 2.0.04 for Windows (Matrix Science Ltd.; London, UK), with a mass accuracy restriction better than 35 ppm and maximum one missed cleavage site allowed per peptide. To confirm PMF results with scores <40, mass spectrometric sequencing of selected peptides was done by MALDI-TOF/TOF (MS/MS) analysis on the same prepared samples, using the UltraFlex instrument in 'LIFT' mode. Fragment ion spectra were taken to search NR using the MASCOT MS/MS Ion Search program (Matrix Science). Any tentative confirmation (Mascot score $\geq$ 30) of a PMF result thus obtained was verified by comparing the computer-generated fragment ion series of the predicted tryptic peptide with the experimental MS/MS data.

Transgenic mice and genotyping

TMPRSS2-ERG was cut out of PCR2.1, blunt cloned into the pBS-ARR2PB-Poly-A-Intron backbone[1] and sequence verified. BssHII was used to release the entire ARR2PB-TMPRSS2-ERG-PAI transgene. Injections into FVB blastocysts were performed by the

Mouse Genetics core facility at Memorial Sloan-Kettering Cancer Center. A 1050bp BamHI fragment from the start of the fusion construct to an internal BamHI site was used as the Southern probe and genomic DNA was cut with BamHI. After stable transgene expression was obtained, animals were genotyped by PCR using primers 5'PB-CAATGTCTGTGTACAACTGCCAACTGGG and 3'ERG-GGCGTTGTAGCTGGGGGTGAG. PTEN +/-[2] and Tg-Akt[3] mice were kindly provided by P.P. Pandolfi and W.R. Sellers respectively. Smooth muscle actin staining was done by the Memorial Sloan-Kettering Molecular Cytology core using Sigma antibody A-2547. All animal work was done humanely and according to IACUC approved guidelines.

Analysis of human tumors

Copy number and exon expression levels were determined with Agilent 244K aCGH and Affymetrix human exon 1.0 ST arrays respectively. Primary and metastatic tumors were micro-dissected to ensure greater than 70 percent tumor content. As further assurance against contaminating normal cells, only those tumors with clear evidence of copy number alteration in their genomes were included in subsequent analyses (non-polymorphic regions of amplification or deletion spanning >0.1% of the autosomal genome). Samples having both aCGH data and exon expression data were used in this analysis, but the results were similar if the subset of samples having either data type were used. The complete aCGH and expression datasets from these tumors will be reported separately.

Tumors were assessed for copy number and expression changes in Myc, PTEN, and ERG. They were assessed for genomic gain in MYC and losses in PTEN and the 21q22.2-3 region spanning between ERG and TMPRSS2 by RAE (FDR < 0.1)[4].Tumors were also assessed for increased expression of Myc, decreased expression of PTEN and evidence of ERG fusion by Affymetrix exon array. Altered expression was defined as signal that exceeded two standard deviations from the mean expression in normal prostate samples (n = 29) assayed at the same time. Exon expression data was used to define ERG rearrangement-positive samples by examining the ratio of ERG exon expression before and after common ERG translocation breakpoints (exons 1 and 2 versus 5-8 respectively)[5]. This detects the change from low ERG expression in early unexpressed exons to high expression of ERG exons translocated downstream of a highly expressed promoter, such as that of TMPRSS2. In detail, the log signal from each probe for each sample was first normalized to the average log signal from the normal samples for that probe. In each sample, we calculated the difference between average normalized log signal for exons 5-8 and the same for exons 1-2. Samples were classified as ERG rearrangement-positive when this difference exceeded two standard deviations from the mean of the normal samples, while the balance classified as ERG fusion-negative. This ratio approach is in agreement with an alternative definition of ERG fusion using only the signal from ERG exons after common translocation breakpoints (i.e., defining ERG fusion positive cases as having a average normalized ERG exon 5 to 8 signal that is greater than two standard deviations above this mean signal for normal samples). TMPRSS2-ERG interstitial deletion was defined in individual tumors by RAE as bearing

a heterozygous or homozygous deletion of sequence spanning the 21q22.2-3 locus or having 5' and 3' segmentation breakpoints for deletions intragenic for ERG or TMPRSS2 respectively. The significance of co-occurrence of these events was determined by Fisher's exact test (right tail).

Agilent 244K CGH arrays for all tumors were processed, quantified, normalized, and segmented as previously described[6]. For exon-level expression, Affymetrix CEL files were processed using Aroma Affymetrix with the HuEx-1_0-st-v2,main,A20071112,EP CDF file with standard RMA background correction. Signal levels for each exon were produced using the RMA model and quantile normalization

The human prostate tissues analyzed in this study were from patients treated at Memorial Sloan-Kettering Cancer Center (MSKCC), all of whom provided informed consent. The study was approved by the MSKCC Institutional Review Board and the MSKCC Human Tissue Utilization Committee.

Accession Numbers and Data Availability

NCBI reference sequences for TMPRSS2-ERG construct cloning are:
TMPRSS2: NM_005656.2
ERG (v2): NM_004449.3

All mouse pathology slides have been scanned and the images are publicly available at:
http://imagearchive.compmed.ucdavis.edu/publications

aCGH and exon array data is available at:
http://cbio.mskcc.org/cancergenomics/king_2009/

References for Supplementary Methods

1. Ellwood-Yen, K. et al. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell* **4**, 223-38 (2003).
2. Di Cristofano, A., Pesce, B., Cordon-Cardo, C. & Pandolfi, P.P. Pten is essential for embryonic development and tumour suppression. *Nat Genet* **19**, 348-55 (1998).
3. Majumder, P.K. et al. Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. *Proc Natl Acad Sci U S A* **100**, 7841-6 (2003).
4. Taylor, B.S. et al. Functional copy-number alterations in cancer. *PLoS ONE* **3**, e3179 (2008).
5. Jhavar, S. et al. Detection of TMPRSS2-ERG translocations in human prostate cancer by expression profiling using GeneChip Human Exon 1.0 ST arrays. *J Mol Diagn* **10**, 50-7 (2008).
6. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455**, 1061-8 (2008).