Supporting Information

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SI Materials and Methods

Genotyping. Genomic DNA was genotyped by using a PCR-based procedure. Amplimers 5′-CGCCAGTCCAAAATCAAGAATC-3′, 5′-GCCATTCTGGTAGAGGAAGTTTCTC-3′, and 5′-CCAGCTCATTCCTCCACTCATG-3′ were used to detect *Jnk1*$^{+}$ (460-bp) and *Jnk1*$^{-}$ (390-bp) alleles. Amplimers 5′-GGAGCCCGATAGTATCGAGTTACC-3′, 5′-GTTAGACAATCCCAGAGGTTGTGTG-3′, and 5′-CCAGCTCATTCCTCCACTCATG-3′ were used to detect *Jnk2*$^{+}$ (400-bp) and *Jnk2*$^{-}$ (270-bp) alleles. Amplimers 5′-AGGATTTATGCCCTCTGCTTGTC-3′ and 5′-GAACCACTGTTCCAATTTCCATCC-3′ were used to detect *Jnk1*$^{+}$ (540-bp) and *Jnk1*$^{\mathrm{LoxP}}$ (330-bp) alleles. Amplimers 5′-CCTCAGGAAGAAAGGGCTTATTTC-3′ and 5′-GAACCACTGTTCCAATTTCCATCC-3′ were used to detect *Jnk1*$^{\mathrm{LoxP}}$ (1,000-bp) and *Jnk1*$^{\Delta}$ (410-bp) alleles. Amplimers 5′-GACATTGAGTTCCTTGCG-3′ and 5′-TCCTATGTAGTAGGAGTTTG-3′ were used to detect *Mkk4*$^{+}$ (390-bp) and *Mkk4*$^{\mathrm{LoxP}}$ (490-bp) alleles. Amplimers 5′-TCCTATGTAGTAGGAGTTTG-3′ and 5′-GGCAGCTTGTCAGATG-3′ were used to *Mkk4*$^{\mathrm{LoxP}}$ (950-bp) and *Mkk4*$^{\Delta}$ (450-bp) alleles. Amplimers 5′-TCATCCCTCACCCTAACACTA-3′ and 5′-CTCTGGTTTCAGGCTGTCTTGC-3′ were used to detect *Mkk7*$^{+}$ (365-bp) and *Mkk7*$^{\mathrm{LoxP}}$ (406-bp) alleles. Amplimers 5′-TCATCCCTCACCCTAACACTAC-3′ and 5′-ATCTTGCCCAGGATTCGCTC-3′ were used to *Mkk7*$^{\mathrm{LoxP}}$ (1.8-kb) and *Mkk7*$^{\Delta}$ (406-bp) alleles. Amplimers 5′-ACTCAAGGCAGGGATGAGC-3′ and 5′-AATCTAGGGCCTCTTGTGCC-3′ were used to detect *Pten*$^{+}$ (1.3-kb) and *Pten*$^{\mathrm{LoxP}}$ (1.0-kb) alleles. Amplimers 5′-ACTCAAGGCAGGGATGAGC-3′ and 5′-GCTTGATATCGAATTCCTGCAGC-3′ were used to detect the *Pten*$^{\Delta}$ (300-bp) allele. Amplimers 5′-TTACTGACCGTACACCAAATTTGCCTGC-3′ and 5′-CCTGGCAGCGATCGCTATTTTCCATGAGTG-3′ were used to detect *Cre recombinase* (450 bp).

Cell Culture. Prostate epithelial cells were prepared from tumor tissue of 12–16-wk-old mice using Stem Cell Technologies Protocol no. 05640. Briefly, prostates were washed in cold PBS and digested for 3 h at 37 °C in 1× collagenase/hyaluronidase (Stem Cell Technologies) in DMEM/F-12 supplemented with 5% FBS. Tissue pieces were collected by centrifugation at 350 × *g* for 5 min, resuspended in 5–6 mL of cold 0.25% trypsin-EDTA (Stem Cell Technologies), and incubated on ice for 1 h. After addition of 10 mL of cold Hanks balanced salt solution (4 °C) supplemented with 2% FBS, the tissue pieces were harvested by centrifugation at 350 × *g* for 5 min. The supernatant was carefully removed, and the tissue pieces were resuspended in 2 mL of prewarmed (37 °C) dispase solution (Stem Cell Technologies) supplemented with 200 μL of 1 mg/mL DNase I solution (Stem Cell Technologies). The tissue pieces were disrupted mechanically by titration with a 1-mL pipette, 10 mL of cold Hanks balanced salt solution (4 °C) supplemented with 2% FBS was added, and cells were filtered through a 40-mm strainer (BD Falcon) into a 50-mL conical tube and then collected by centrifugation at 350 × *g* (5 min). The cells were resuspended in cold Hanks balanced salt solution. Cells of endothelial and hematopoietic origin were removed by lineage (Lin) depletion using the Mouse Mammary Stem Cell Enrichment Kit (Stem Cell Technologies). Subsequently, enrichment for Sca-1–positive cells was performed by magnetic sorting (anti–Sca-1 Microbead Kit; Miltenyi Biotec). Finally, the cells were resuspended in 1:1 Matrigel (BD Biosciences)/prostate epithelial growth medium (PrEGM) (Lonza) (1). The cells were plated at a density of 3×10^4 cells per well in multiwell plates (12 well). Prostasphere cultures were maintained using methods described previously (1).

Immunofluorescence Analysis of Prostaspheres. Samples were embedded in 4% low-melting agarose (Sigma). Agarose plugs containing prostaspheres were fixed over night in 10% buffered neutral formalin (VWR), processed in a tissue processor, and embedded in paraffin. Sections (7 μm) were cut and stained using H&E (American Master Tech Scientific). Sections were also deparaffinized using standard protocols and used for immunofluorescence using antibodies to p63 (Santa Cruz; mouse), nucleostemin (Abcam; rabbit), CK5 (Covance; mouse), CK8 (Covance; rabbit), CD49F-phycoerythrin (BD Pharmingen; rat), and Bcl2 (Cell Signaling; rabbit). Secondary antibodies were purchased from Invitrogen and were raised in goats and coupled to Alexa Fluor 488 (goat–anti-mouse) or 633 (goat–anti-rabbit). The sections were examined using a Leica SP2 confocal microscope.

Analysis of Frozen Tumor Sections. Sections were stained to detect acidic β-galactosidase using X-Gal (Cell Signaling).

Transplantation Assays. Prostasphere cells (passage 1) were dissociated and 5×10^5 cells were injected under the renal capsule of immunodeficient NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjll}/*SzJ* mice (2). Transplantation assays were also performed using s.c. injection.

Statistical Analysis. Differences between groups were examined for statistical significance using the Student's test (Microsoft Excel software). Kaplan–Meier analysis was performed using JMP software, and statistically significant differences were identified with the log-rank test.

1. Xin L, Lukacs RU, Lawson DA, Cheng D, Witte ON (2007) Self-renewal and multilineage differentiation in vitro from murine prostate stem cells. *Stem Cells* 25:2760–2769.
2. Shultz LD, Ishikawa F, Greiner DL (2007) Humanized mice in translational biomedical research. *Nat Rev Immunol* 7:118–130.
3. Burger PE, et al. (2005) Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proc Natl Acad Sci USA* 102:7180–7185.

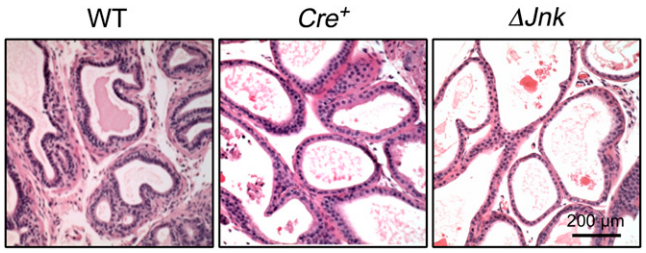

Fig. S1. Effect of JNK deficiency on prostate gland development. Representative sections of H&E-stained tissue sections prepared from the anterior prostate gland of WT, *PB-Cre4*$^{+}$, and *ΔJnk* mice at age 10 wk are presented.

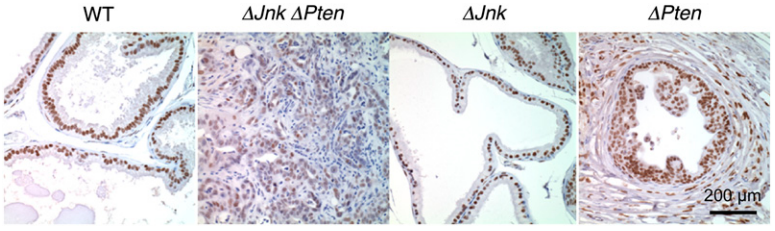

Fig. S2. Effect of *ΔJnk* and *ΔPten* on the androgen receptor. Representative tissue sections prepared from the anterior prostate gland of WT, *ΔJnk ΔPten*, *ΔJnk*, *and ΔPten* mice (age, 20 wk) were stained with an antibody to the androgen receptor.

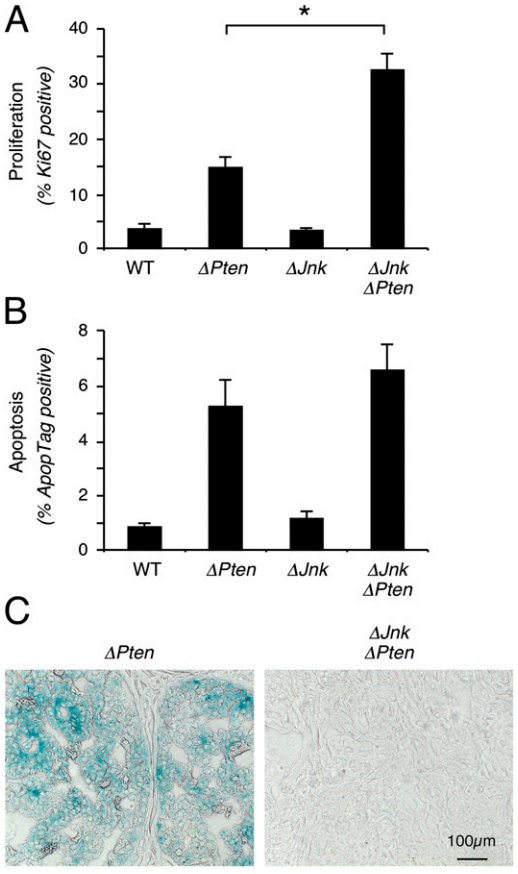

Fig. S3. Effect of *ΔJnk* and *ΔPten* on proliferation and apoptosis. (*A*) Representative tissue sections prepared from the anterior prostate gland of WT, *ΔPten*, *ΔJnk*, and *ΔJnk ΔPten* mice (age, 10 wk) were stained with an antibody to the proliferation marker Ki67 (mean ± SEM; $n = 3$ mice). Statistically significant differences between *ΔPten* and *ΔJnk ΔPten* mice are indicated (*$P < 0.05$). (*B*) Apoptotic cells were identified by staining with the ApopTag reagent. The percentage of Ki67 and ApopTag-positive cells was measured (mean ± SEM; $n = 3$ mice). No statistically significant differences between *ΔPten* and *ΔJnk ΔPten* mice were detected. (*C*) Frozen sections of *ΔPten* and *ΔJnk ΔPten* tumors were stained for the senescence marker β-galactosidase.

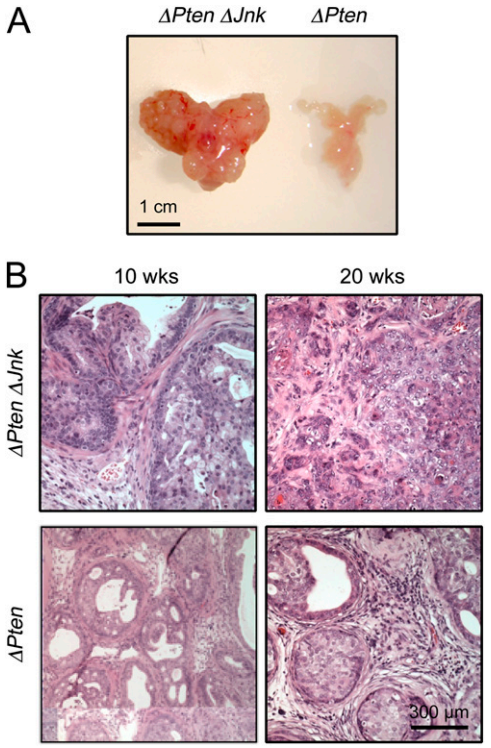

Fig. S4. Loss of JNK and PTEN cooperate to promote castration-resistant prostate cancer. (*A*) Mice were surgically castrated at age 6 wk. Representative prostate glands of *ΔPten* and *ΔJnk ΔPten* mice at 20 wk postcastration are illustrated. (*B*) Representative sections of H&E-stained tissue sections prepared from the anterior prostate gland of *ΔPten* mice and *ΔJnk ΔPten* mice at 10 and 20 wk postcastration are presented.

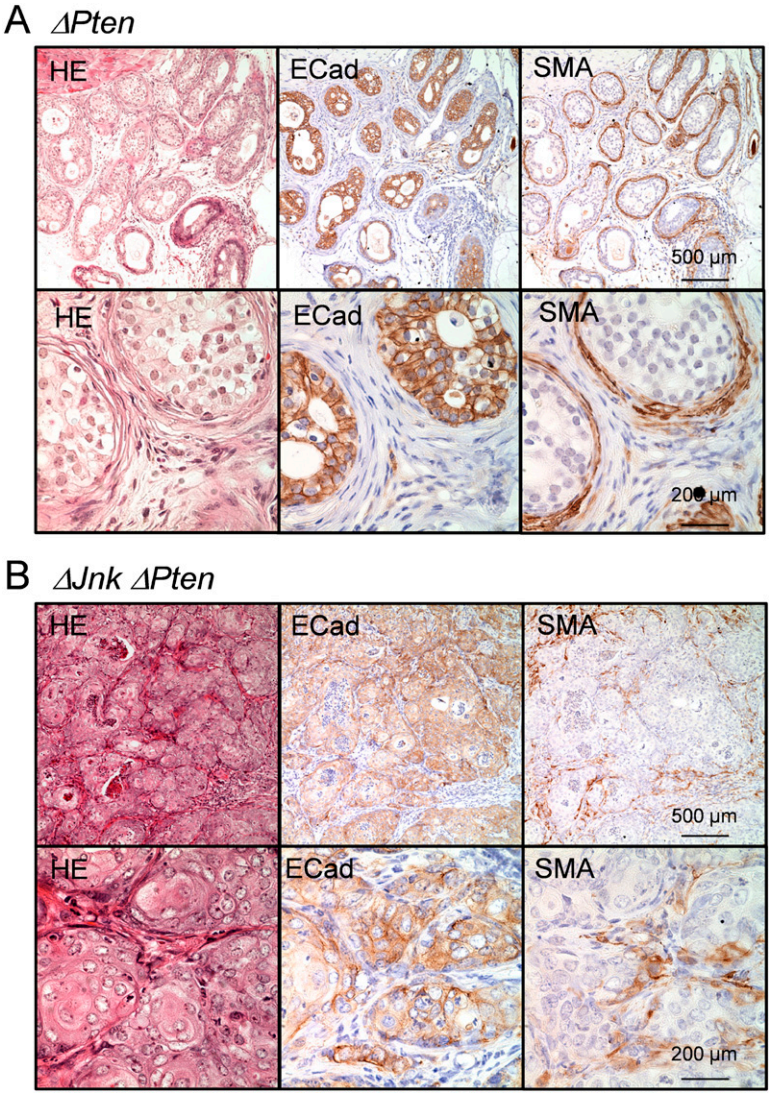

Fig. S5. Effect of androgen withdrawal on prostate tumor development in *ΔPten* and *ΔJnk ΔPten* mice. Mice were surgically castrated at age 6 wk. Representative tissue sections prepared from the anterior prostate gland at 20 wk postcastration were stained with H&E and with antibodies to E-cadherin (ECad) or smooth muscle actin (SMA). (*A*) *ΔPten* mice. (*B*) *ΔJnk ΔPten* mice.

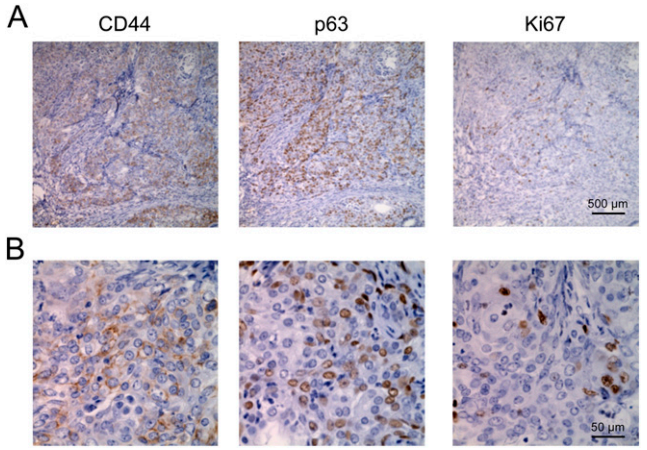

Fig. S6. Androgen-independent expansion of immature prostate cells in *ΔJnk ΔPten* mice. Representative tissue sections prepared from the anterior prostate gland of 20 wk-old *ΔJnk ΔPten* mice (castrated at age 6 wk) were stained with antibodies to CD44, p63, and Ki67. The images demonstrate the presence of large numbers of immature prostate cells (CD44 and p63) and also proliferation (Ki67). Low-magnification (*A*) and high-magnification (*B*) images are presented.

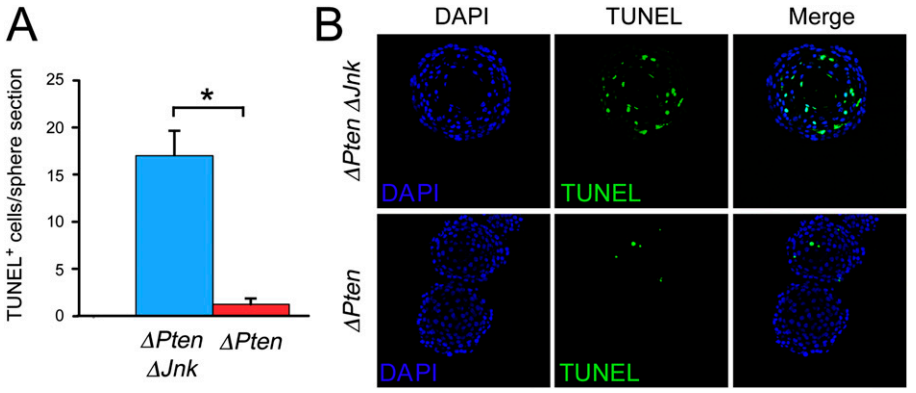

Fig. S7. Loss of JNK promotes increased luminal apoptosis in ΔPten prostaspheres. (*A* and *B*) TUNEL assays were performed on sections of prostaspheres. The number of TUNEL-positive cells per prostasphere in each section was measured (mean ± SD; $n = 9$). Significant differences are indicated (*$P < 0.05$).

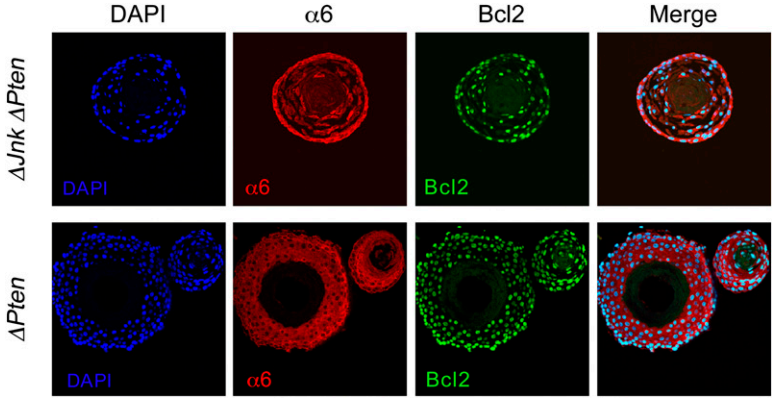

Fig. S8. Expression of integrin α6 and Bcl2 by cultured prostate cells of *ΔPten* and *ΔJnk ΔPten* mice. *Lin*$^-$ *Sca1*$^+$ cells isolated from primary prostate tumors of *ΔPten* and *ΔJnk ΔPten* mice were cultured in vitro. Sections of prostaspheres were stained for DNA (DAPI) and integrin α6 (CD49f) plus Bcl2. It is established that prostate stem cells are present within the *Sca1*$^+$ population that expresses integrin α6 and Bcl2 (3).

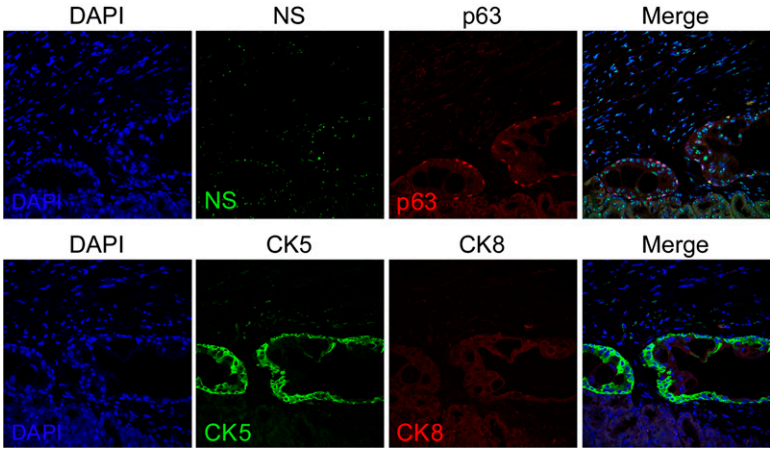

Fig. S9. Loss of JNK promotes tumor formation by immature *ΔPten* prostate cells. Cells dissociated from prostaspheres were injected under the renal capsule of immunodeficient mice. The mice were euthanized after 16 wk. Pathological analysis demonstrated growth of *ΔPten ΔJnk* prostate tumor cells but no growth of *ΔPten* cells. Representative sections of *ΔPten ΔJnk* tumors stained for DNA (DAPI), CK5, CK8, p63, and nucleostemin (NS) are presented.