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Supplemental Data

Article

A Prostatic Intraepithelial Neoplasia-Dependent $p27^{Kip1}$ Checkpoint Induces Senescence and Inhibits Cell Proliferation and Cancer Progression

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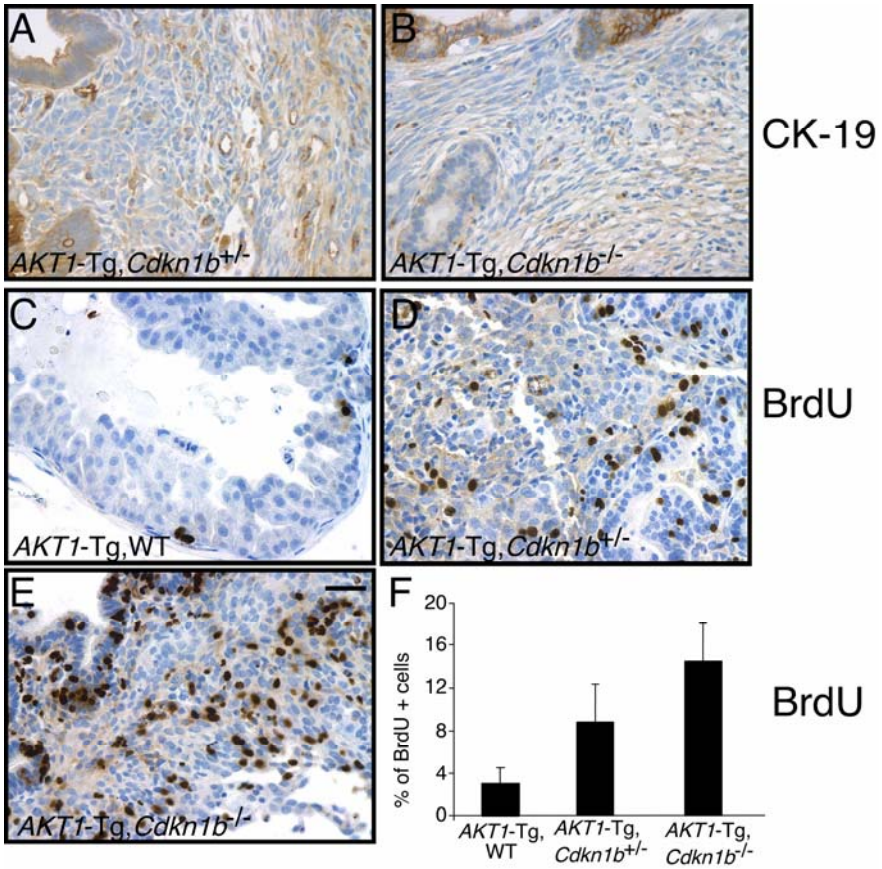

Figure S1. Prostate Cancers Arising in *AKT1*-Tg/*Cdkn1b*$^{+/-}$ and *AKT1*-Tg/*Cdkn1b*$^{-/-}$ Mice Are of Epithelial Origin and Are Highly Proliferative

(A and B) Cytokeratin (CK)-19 staining was performed in invasive prostate tumors developed either in *AKT1*-Tg/*Cdkn1b*$^{+/-}$ and *AKT1*-Tg/*Cdkn1b*$^{-/-}$ mice.

(C) Tissue sections of *AKT1*-Tg ventral prostate were stain with antibody directed against BrdU.

(D and E) Tissue sections of *AKT1*-Tg/*Cdkn1b*$^{+/-}$ and *AKT1*-Tg/*Cdkn1b*$^{-/-}$ ventral prostate were stain with antibody directed against BrdU. Scale bar = 50 μM (A-E).

(F) Numbers of BrdU positive and negative cells were counted in all lobes of VP and percentage of BrdU positive cells were calculated. Data are presented as mean $\pm$ SD.

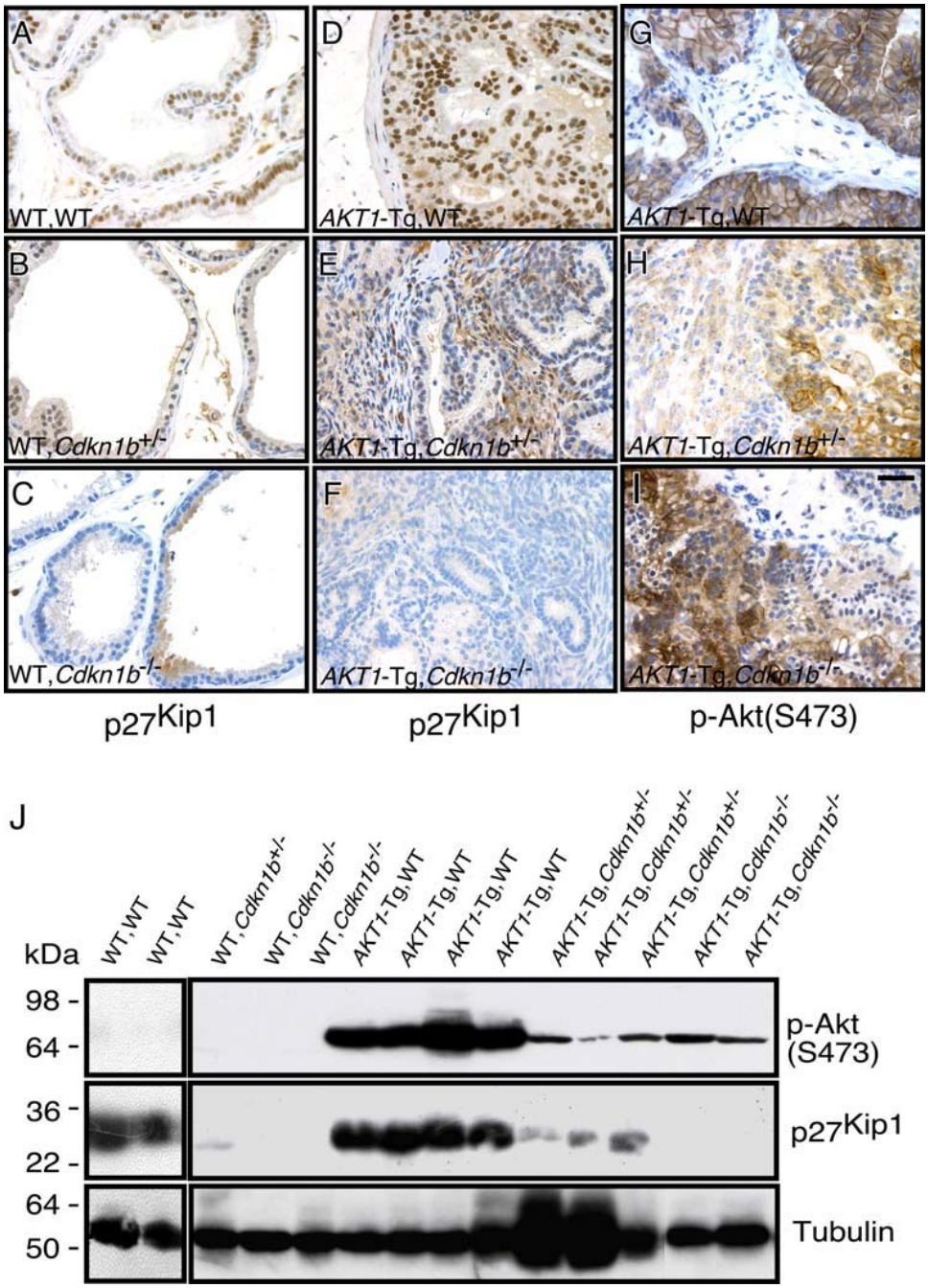

Figure S2. Inactivation of One Allele of *Cdkn1b* Is Sufficient for the Development of Invasive Prostate Cancer in *AKT1*-Transgenic Mice

(A–I) Tissue sections from VPs of wild type (WT/WT, A), *Cdkn1b* heterozygous (WT/*Cdkn1b*$^{+/-}$, B), *Cdkn1b* homozygous (WT/*Cdkn1b*$^{-/-}$, C), *AKT1*-Tg/WT (D and G), *AKT1*-Tg/*Cdkn1b*$^{+/-}$ (E and H) and *AKT1*-Tg/*Cdkn1b*$^{-/-}$ (F and I) were stained with antibodies directed against p27^{Kip1} (A–F) or phospho-Akt (S473) (G–I). Scale bar = 50 μM.

(J) Protein lysates from the above prostate tissues were immunoblotted with antibodies directed against phospho-Akt (S473) (upper panel), p27^{Kip1} (middle panel) and Tubulin (lower panel) as indicated.

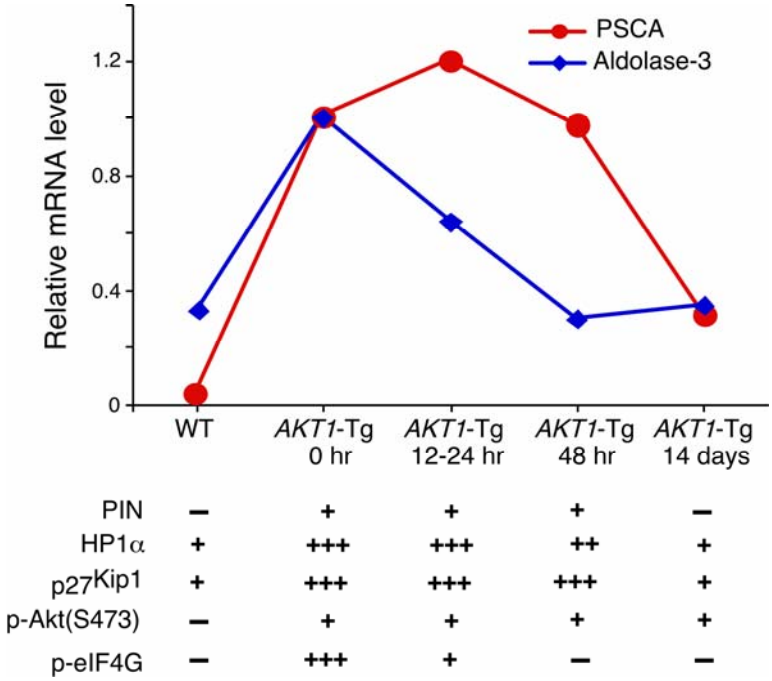

Figure S3. The Induction of p27^{Kip1} Is Temporally Correlated with the PIN Lesion and Related PIN-Dependent Biomarkers

Relative expression of Prostate Stem Cell Antigen (PSCA) mRNA or Aldolase-3 mRNA from wild type prostate treated with placebo (WT), *AKT1*-Tg prostate treated with placebo (*AKT1*-Tg/0hr), treated with RAD001 for 12hr (*AKT1*-Tg/12hr), 48hr (*AKT1*-Tg/48hr) and treated for 14 days (*AKT1*-Tg) as indicated (upper panel). Summarized data (IHC score) the correlating the induction of p27^{Kip1} with the PIN phenotype, up-regulation of senescence marker HP1α, activation of Akt and activation of mTOR (lower panel).

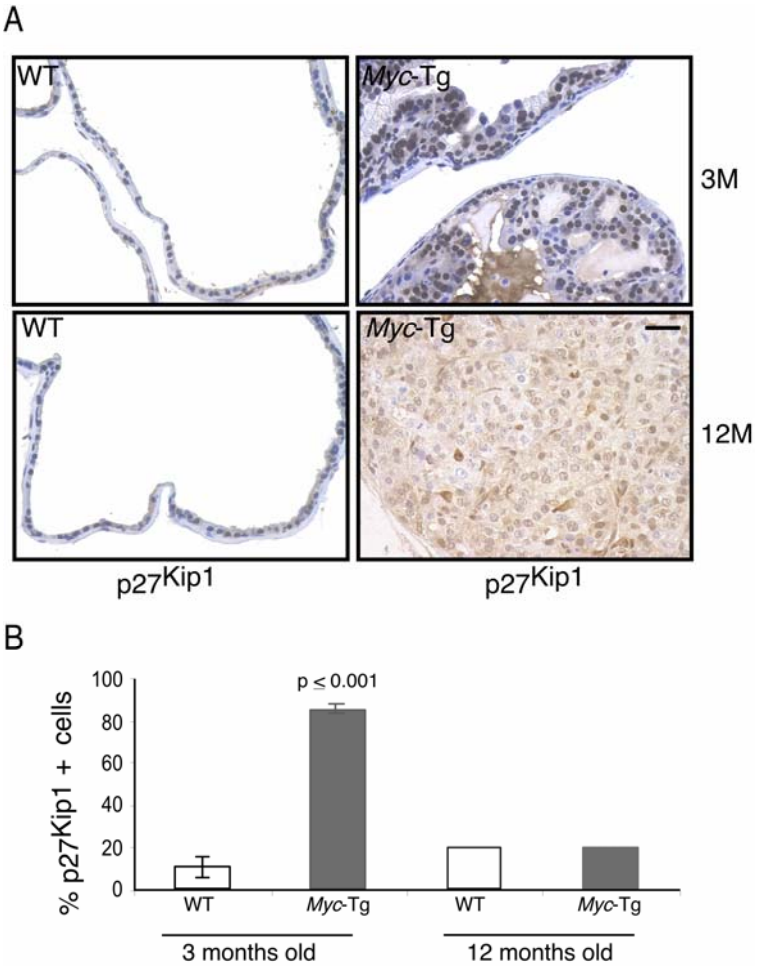

Figure S4. Induction of p27^{Kip1} in PIN Lesions from c-*Myc*-Tg Mice

(A) Tissue sections of the VPs of a 3 month-old (3M) wild-type (WT) mouse (upper panel)), of a c-*Myc*-Tg (*Myc*-Tg) mouse harboring PIN lesions, of the VP of a 12-month old (12M) wild type mouse (lower panel), and of an invasive tumor from a c-*Myc*-Tg mouse harvested at 12 months were stained with antibodies directed against p27^{Kip1}. Data are representative of the analysis of 4 wild-type and c-*Myc*-Tg mice. Scale bar = 50 μM.

(B) The ratio of p27^{Kip1} positively staining cells over total cells was determined in the VPs of wild type (WT) and *Myc*-Tg mice of the indicated ages. Data are presented as mean $\pm$ SEM.

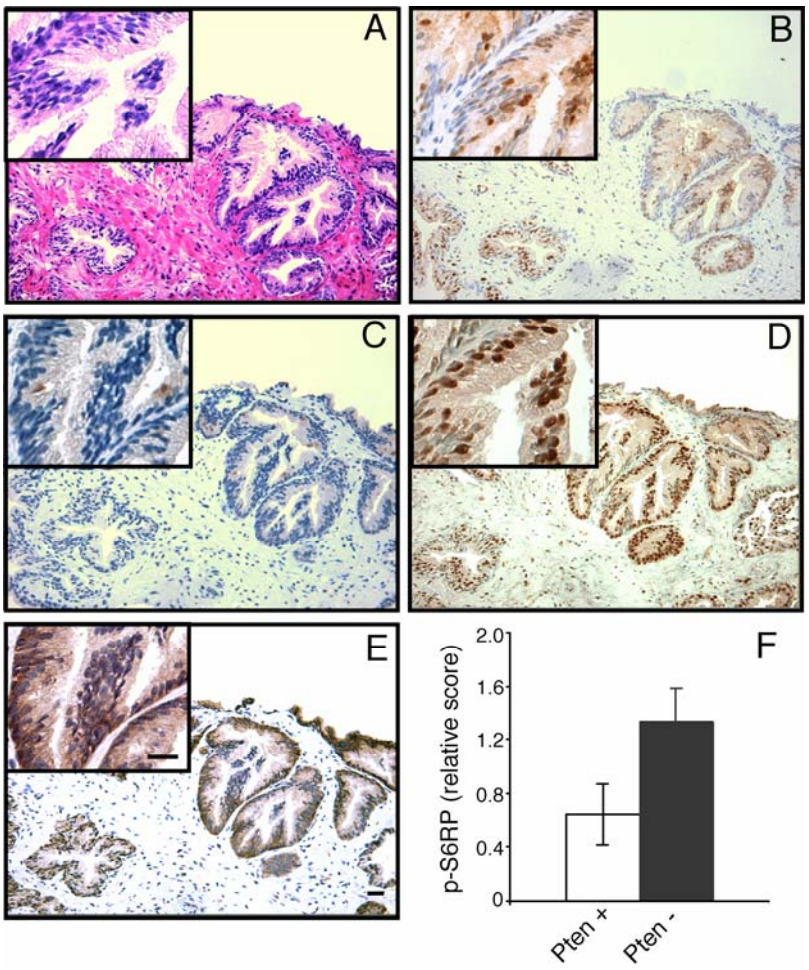

Figure S5. Correlation between PTEN Staining with $p27^{Kip1}$, HP1α, and Phospho-S6RP Staining in Human Prostate Intraepithelial Neoplasia

(A) Prostatic intraepithelial neoplasia tissue sections were stained with H&E.

(B) Prostatic intraepithelial neoplasia tissue sections were stained with antibody directed against $p27^{Kip1}$.

(C) Prostatic intraepithelial neoplasia tissue sections were stained with PTEN antibody.

(D) Tissue sections from human PIN were stained with antibody directed against HP1α.

(E) Human prostate intraepithelial tissue sections were stained with antibody against phospho-S6RP. Scale bar = 50 μM in (A)-(E); scale bar = 100 μM in inset in (A)-(E).

(F) The phospho-S6RP was graded by H-score. The relative score was plotted against PTEN status (presence or absence of cytoplasmic PTEN staining). Data are presented as mean ± SEM.