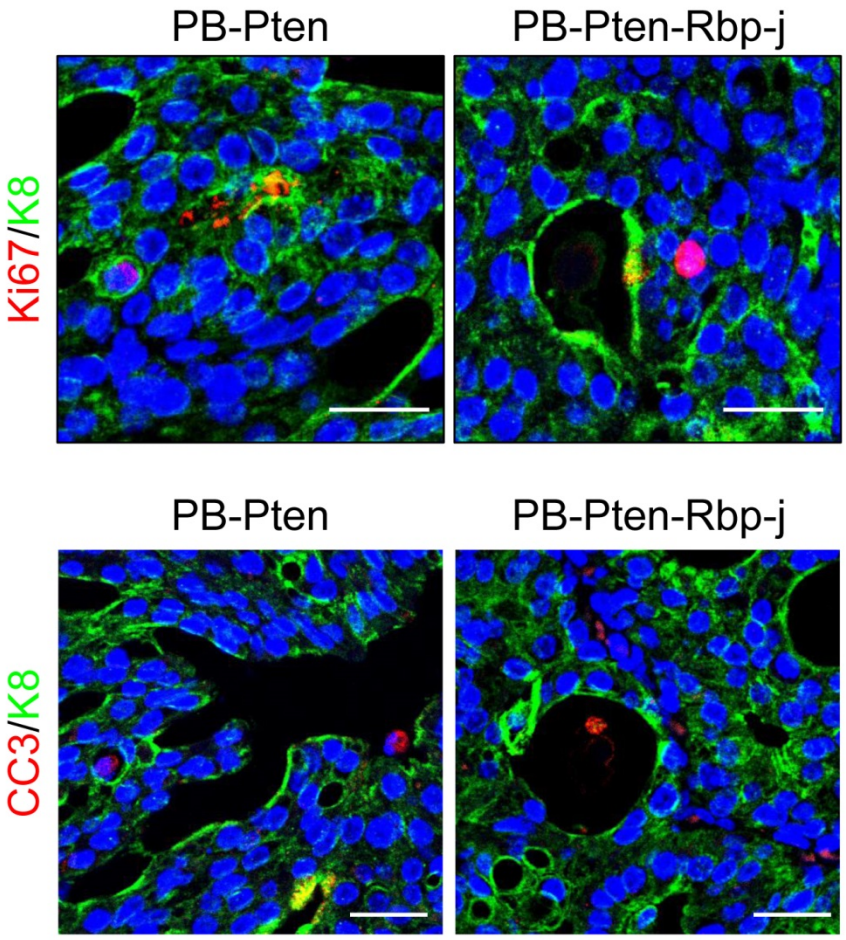
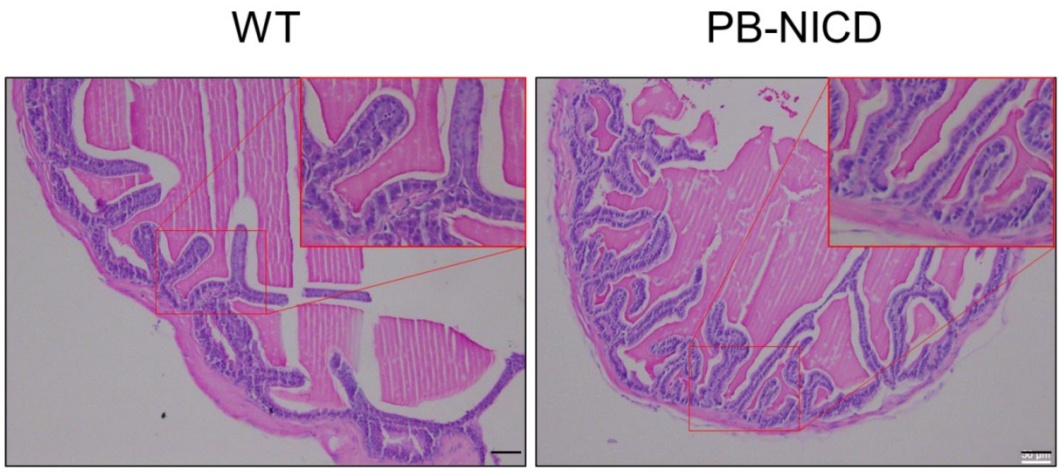
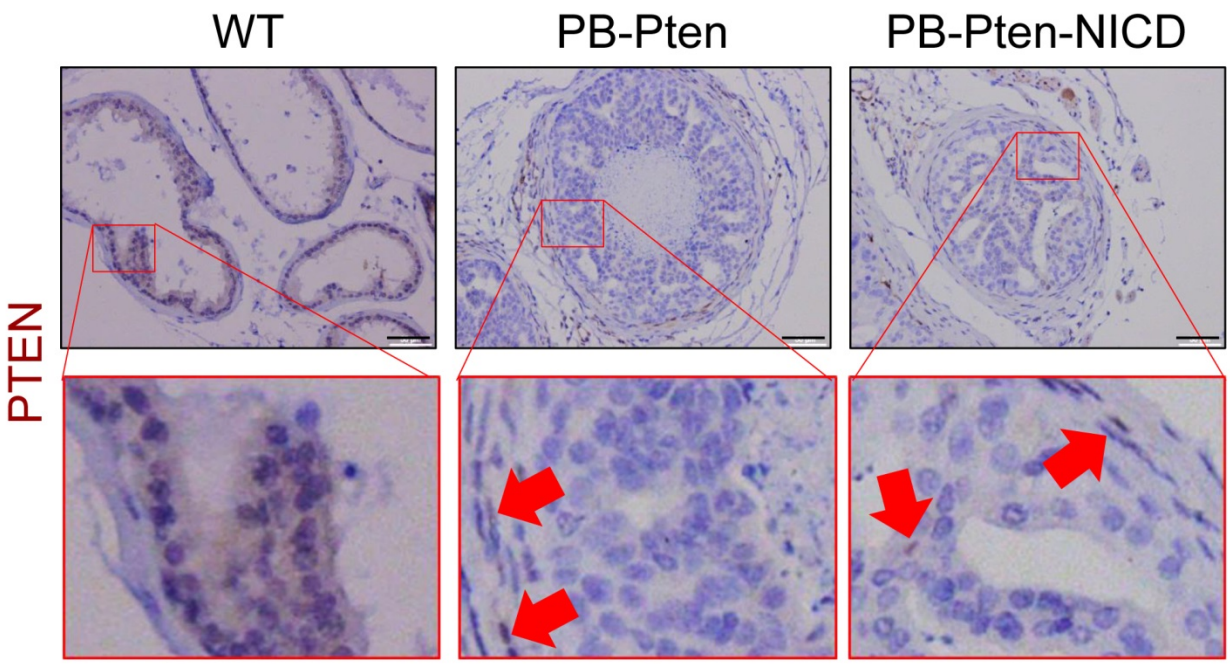


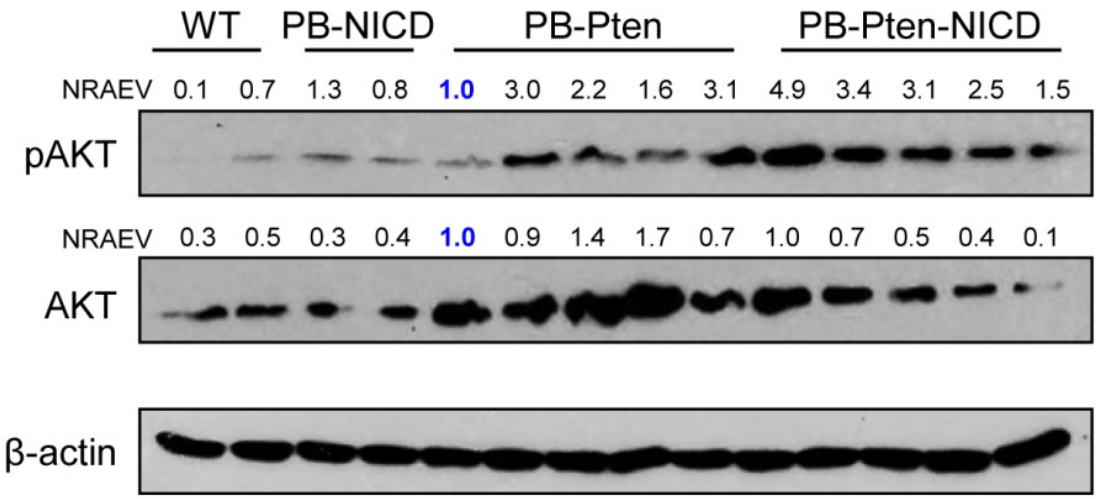
Supplementary Figure 1: Coimmunostaining of Ki67 and K8, and cleaved caspase 3 (CC3) and K8 of prostate tissues from 1-yr-old PB-Pten and PB-Pten-Rbp-J mice. Bars=50µm. These images are cropped versions of Figure 2D and 2E, and are zoomed and shown here to facilitate reading of the staining.



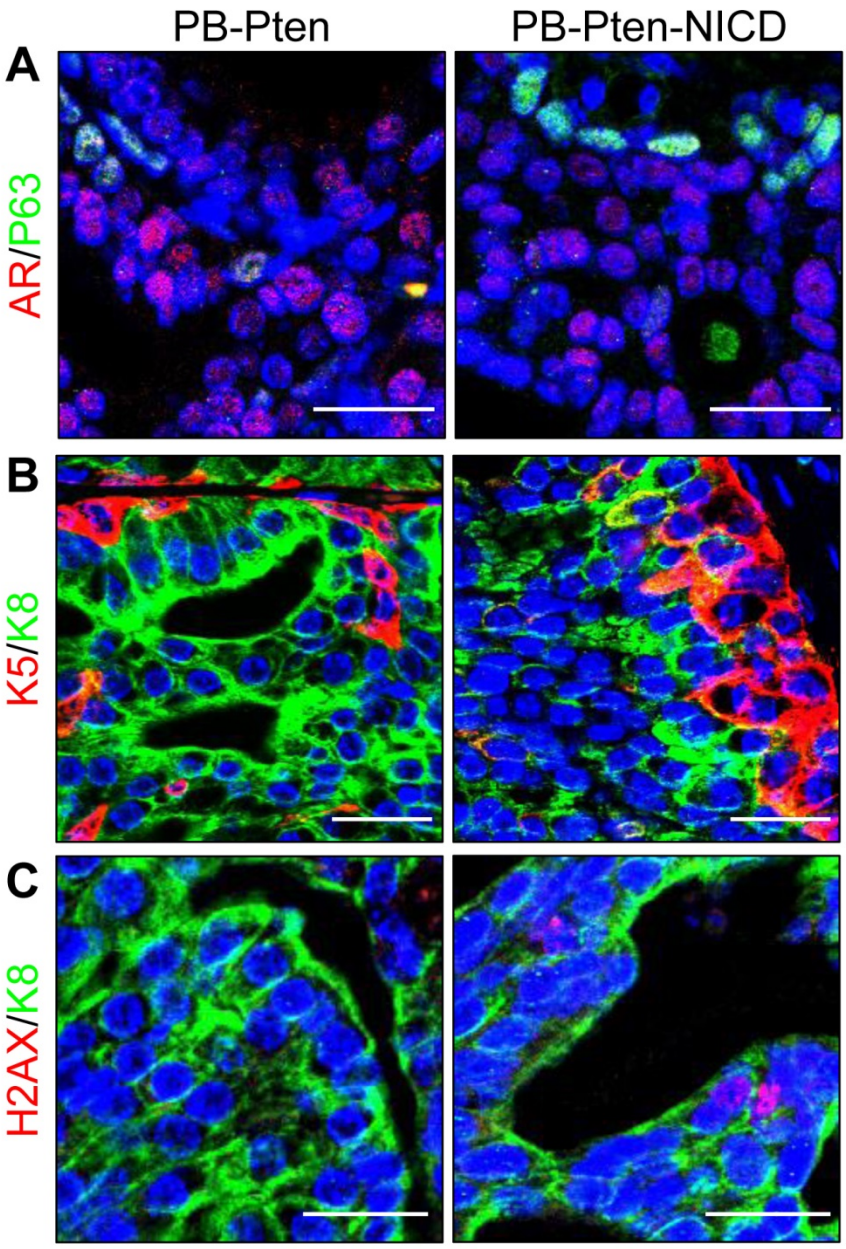
Supplementary Figure 2: H&E staining of seminal vesicles of 1.5-yr-old wild type and PB-NICD mice. Bars=50 μm.



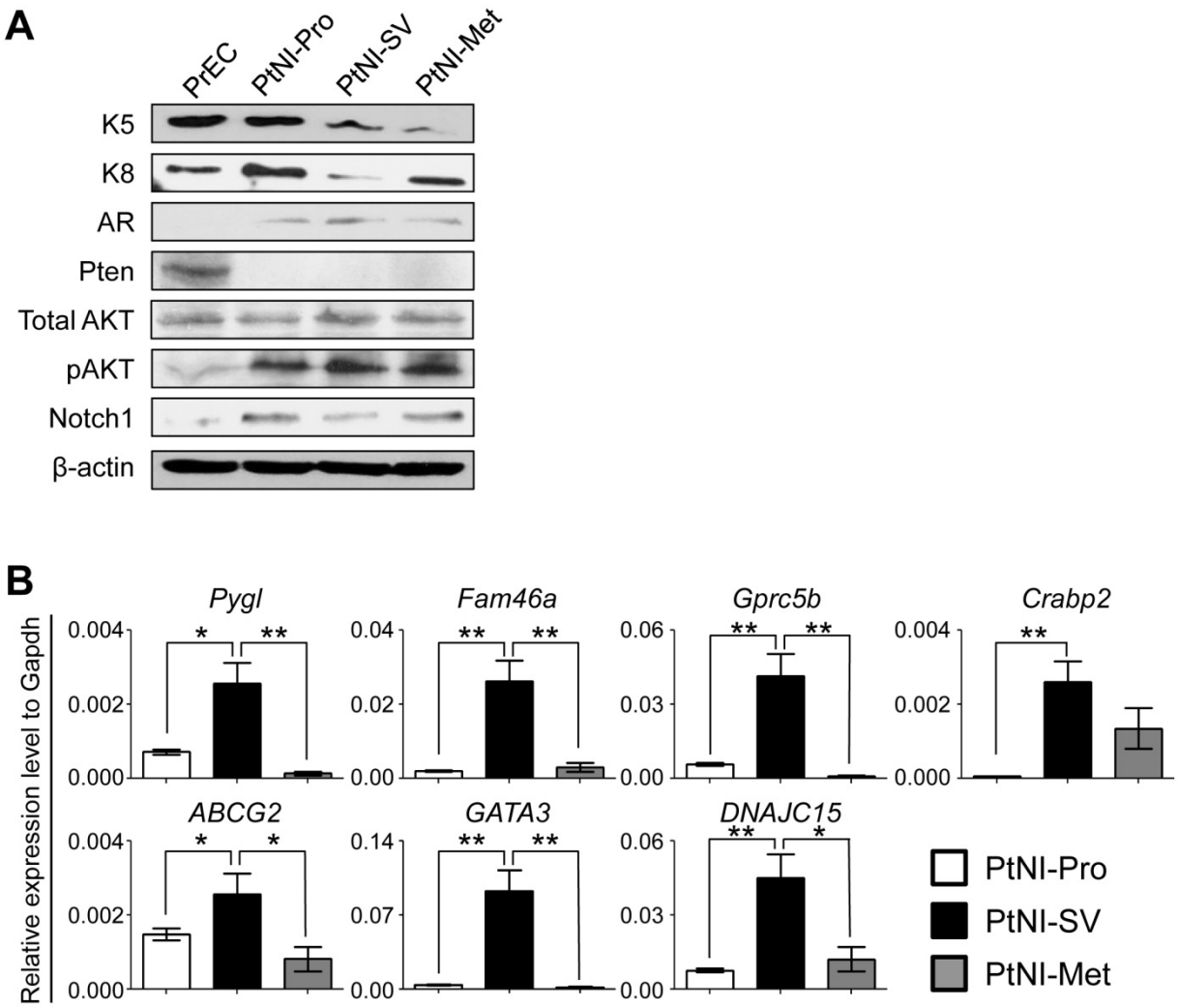
Supplementary Figure 3: Immunostaining of PTEN in prostate tissues of 16-wk-old PB-Pten and PB-Pten-NICD mice. Bars=50μm. Arrows point to PTEN staining in stromal cells, which serves as internal positive controls.



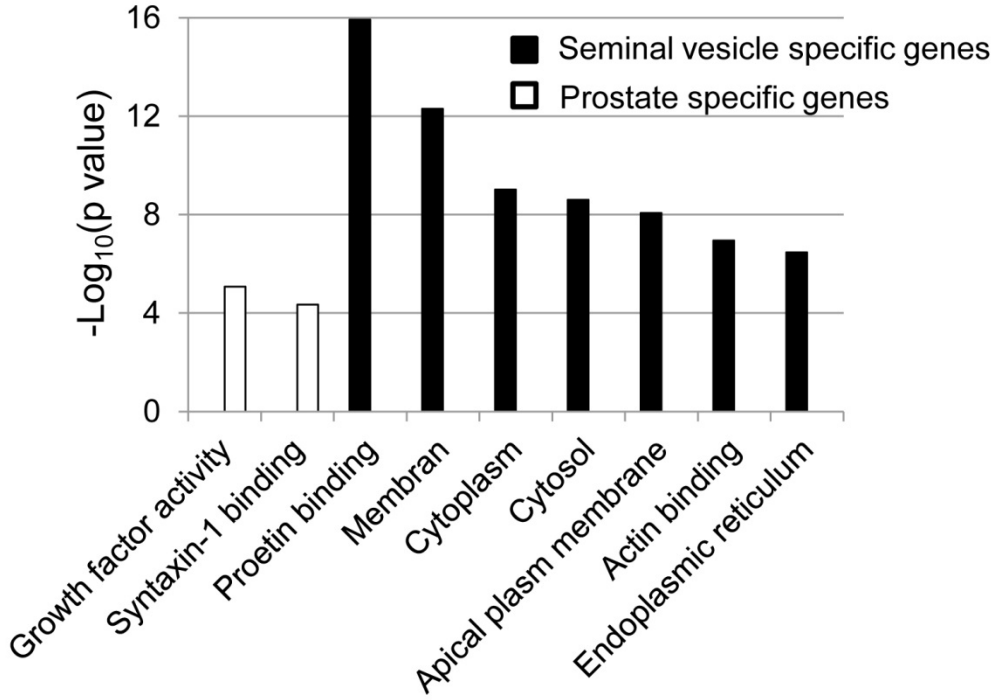
Supplementary Figure 4: Western blot analysis of expression of pAKT in prostate tissues of 16-wk-old wild type (WT), PB-NICD, PB-Pten, and PB-Pten-NICD mice. Each lane represents an independent specimen. NRAEV: normalized relative arbitrary expression value by β-actin. Results of AKT and pAKT from PB-Pten (lane 5-9) and PB-Pten-NICD (lane 10-14) were also shown in Figure 8C.



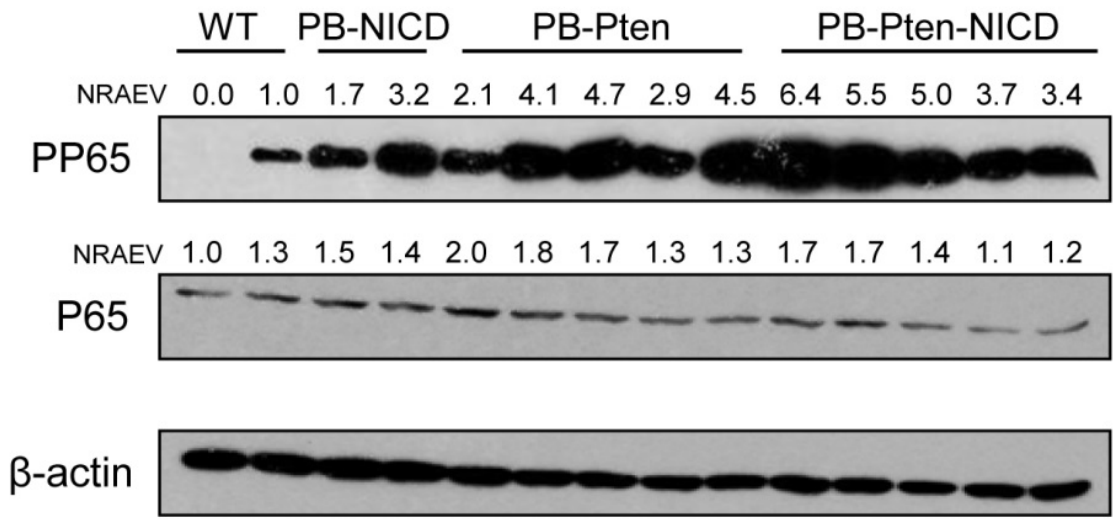
Supplementary Figure 5: Coimmunostaining of AR and P63 (A), K5 and K8 (B), and pH2A.X and K8 (C) in prostate tissues of 16-wk-old PB-Pten and PB-Pten-NICD mice. Bars=50μm.



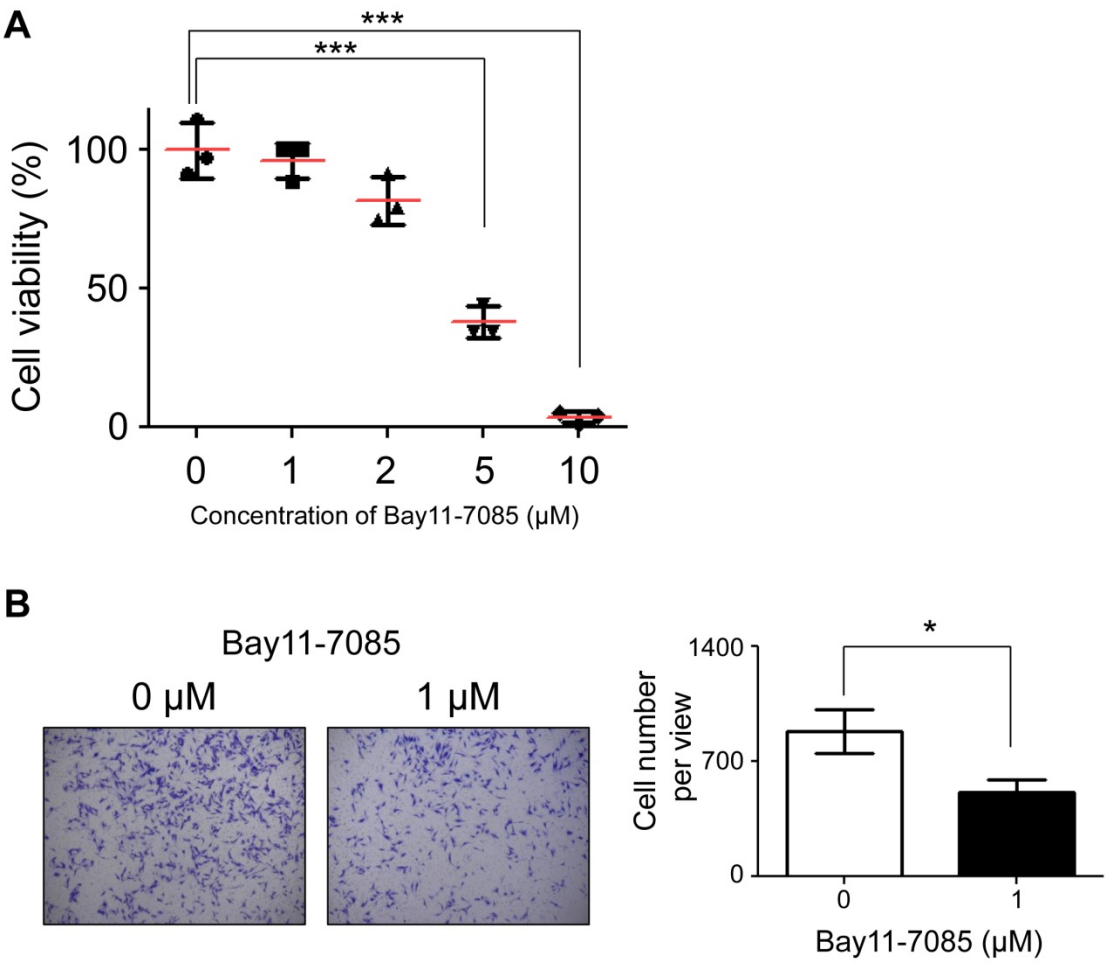
Supplementary Figure 6: Characterization of the cell lines established from primary prostate tumors (PtNI-Pro), primary seminal vesicle tumors (PtNI-SV) and lung metastasis (PtNI-Met) in PB-Pten-NICD mice. **(A)** Western blot analysis of expression of Keratin5 (K5), Keratin 8 (K8), androgen receptor (AR), Pten, AKT, pAKT, and Notch1. The primary human prostate basal epithelial cells (PrEC) were used as a control. **(B)** Quantitative RT-PCR analysis of 7 genes in the established cell lines from 3 different experiements. These are representative genes expressed at a much higher level in rodent seminal vesicles than in prostate. *:$p<0.05$, **:$p<0.01$, Student's t-test.



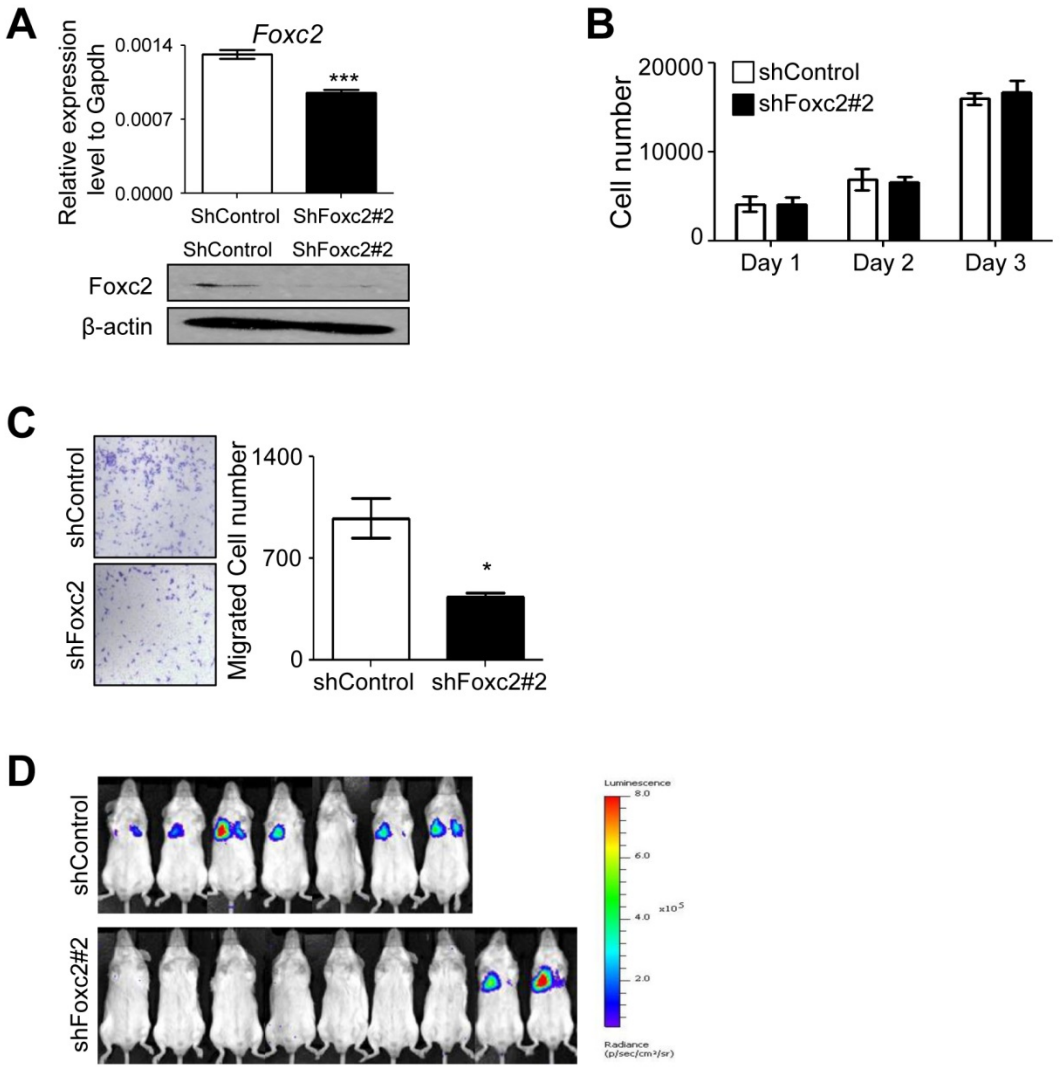
Supplementary Figure 7: Gene Ontology pathways that are differentially enriched (-log10 p-value) in primary prostate and seminal vesicle tumors in PB-Pten-NICD mice, respectively. P values were by one-sided Fisher's exact test.



Supplementary Figure 8: Western blot analysis of expression of pP65 in prostate tissues of 16-wk-old wild type (WT), PB-NICD, PB-Pten, and PB-Pten-NICD mice. Each lane represents an independent specimen. NRAEV: normalized relative arbitrary expression value by β-actin.



Supplementary Figure 9: NF-κB signaling is necessary for the survival and migratory capability of PB-Pten-NICD metastatic cell line. (A) Cell viability assay. $2x10^4$ cells per well were seeded in 24-well plates and cultured without or with Bay11-7085 at the specified concentration. Cells were counted 2 days later. Dot graphs show means ± SD of percentage of final cell numbers normalized by that of the no treatment group from 3 independent experiments. ***: $p < 0.001$, Student's t-test. **(B)** Transwell migration assay of PB-Pten-NICD cells treated without or with 1μM Bay11-7085. Bar graphs show means ± SD of migrated cells per well from 3 independent experiments. *: $p < 0.05$, Student's t-test.



Supplementary Figure 10: Suppressing FoxC2 by a second shRNA also reduces the capacities of PB-Pten-NICD cells for invasion in vitro and distal metastasis in vivo. (A) QRT-PCR and Western blot analyses show successful knockdown of *FoxC2* by shRNA#2. ***: p<0.001. **(B)** Growth curves of PB-Pten-NICD metastatic cell line infected with scrambled shRNA control and *FoxC2* shRNA#2 lentivirus. **(C)** Transwell migration assay. Bar graphs show means ± SD of migrated cells per well from 3 independent experiments. *: $p < 0.05$, Student's t-test. **(D)** NOD/SCID mice were inoculated with $2x10^6$ cells each in individual groups via tail vein. Mice were imaged 5 weeks later. Foxc2 shRNA reduced the capacity of PB-Pten-NICD cells to form distal metastases in the lung (p=0.019, Fisher's exact test).

Supplementary Table 1: Mouse genotyping primers

Primers	Sequences (5' to 3')
Cre forward	CCTGACAGTGACGGTCCAAAG
Cre reverse	CATGACTCTTCAACTCAAACT
RBP-J forward	GAAGGTCGGTTGACACCAGATAGC
RBP-J reverse 1	ATGTACATTTTGTACTCACAGAGATGGATG
RBP-J reverse 2	TAATGCACACAAGCATTGTCTGAGTTC
ICN forward	TAAGCCTGCCCAGAAGACTC
ICN reverse 1	GAAAGACCGCGAAGAGTTTG
ICN reverse 2	AAAGTCGCTCTGAGTTGTTAT
Pten forward	CAAGCACTCTGCGAACTGAG
Pten reverse	AAGTTTTTGAAGGCAAGA

Supplementary Table 2: Mouse qRT-PCR Primers

Primers	Sequences (5' – 3')
Notch1 forward	ACTGTGAGGACGAGGTGGAC
Notch1 reverse	ACAGGCACTCGTTGATCTCC
Hey1 forward	GGTACCCAGTGCCTTTGAGA
Hey1 reverse	GTGCGCGTCAAAATAACCTT
Jag1 forward	CCCAACTGTGAAATTGCTGA
Jag1 reverse	CAGCCTGGAGAACACTCACA
CcnD1 forward	AGTGCGTGCAGAAGGAGATT
CcnD1 reverse	AGCGGGAAGACCTCCTCTT
CcnD2 forward	GCTGTGCATTTACACCGACA
CcnD2 reverse	CCACTTCAGCTTACCCAACA
Nrarp forward	AGGGCCAGACAGCACTACAC
Nrarp reverse	CGGTTAGCTAGGCGGATGT
Puma forward	TGTGGAGGAGGAGGAGTGG
Puma reverse	CGATGCTGCTCTTCTTGTCTC
Noxa forward	GCTACAGCAAGTGCCCAAG
Noxa reverse	ACAGAAGCCACCACCTTAGC
Bax forward	GCTGATGGCAACTTCAACTG
Bax reverse	GATCAGCTCGGGCACTTTAG
Fn1 forward	TCTGGGACTGTACCTGCATC
Fn1 reverse	TGTAGGACTGACCCCCTTCA
Cdh2 forward	TGGATCGAGAGCTGATAGCC
Cdh2 reverse	CAATGTCAATGGGGTTCTCC
Foxc2 forward	CAGCTACTGGACGCTCGAC
Foxc2 reverse	GGGCACATCCTTCTTCTTGA
Snai1 forward	CTTGTGTCTGCACGACCTGT
Snai1 reverse	GAGCAGGAGAATGGCTTCTC
Snai2 forward	GATCTGTGGCAAGGCTTTCT
Snai2 reverse	CCTATTGCAGTGAGGGCAAG
Ocln forward	CCTGGAGGTACTGGTCTCTACG
Ocln reverse	AATCATGAACCCCAGGACAA
Cdh1 forward	AATGAAAAGGGCGAATTTCC
Cdh1 reverse	GCCGGTGATGCTGTAGAAAA
DSP forward	GAGCGACAAGAACACCAACA
DSP reverse	TTCCTTTTCCTTGACCTCCA

Supplementary Table 3: Antibodies for Western blot analyses

Antigen	Supplier	Species	Dilution
K5	Covance #PRB-160P	Rabbit	1:2000
K8	Covance #MMS-162P	Mouse	1:1000
AR	Santa Cruz, sc-816	Rabbit	1:200
Fibronectin 1	BD bioscience, 610077	Mouse	1:1000
Notch1	Cell Signaling, 3608	Rabbit	1:1000
Notch1	Thermal Fisher, MA1-81888	Mouse	1:500
Foxc2	Gift from Dr. Naoyuki Miura at Hamamatsu University, Japan	Rat	1:500
Vimentin	Cell Signaling, 5741	Rabbit	1:1000
β-actin	Sigma, A5441	Mouse	1:5000
AKT1	Cell Signaling, 9272	Rabbit	1:1000
phoAKT	Cell Signaling, 4060	Rabbit	1:1000
MAPK	Cell Signaling, 9102	Rabbit	1:1000
pMAPK	Cell Signaling, 4370	Rabbit	1:1000
P53	Protein tech,10442-1-AP	Rabbit	1:500
Pp53	Cell Signaling,9284	Rabbit	1:1000
α-Rat HRP	Santa Cruz, sc-2032	Goat	1:5000
α-Mouse HRP	Vector Lab. PI-2000	Goat	1:5000
α-Rabbit HRP	Vector Lab. PI-1000	Goat	1:5000

Supplementary Table 4: Antibodies for IHC analyses

Antigen	Supplier	Species	Dilution
K5	Covance #PRB-160P	Rabbit	1:2000
K8	Covance #MMS-162P	Mouse	1:1000
K14	Biogenex MU146-UC	Mouse	1:100
GFP	Clontech, JL8	Mouse	1:1000
P63	Santa Cruz, 4A4	Mouse	1:200
Ki67	Novocastra, NCL-Ki67-P	Rabbit	1:1000
AR	Santa Cruz, sc-816	Rabbit	1:200
pAKT	Cell Signaling, 4060	Rabbit	1:500
Cleaved caspase3	Cell Signaling, 9661S	Rabbit	1:1000
Vimentin	Cell Signaling, 5741	Rabbit	1:500
Smooth muscle actin	Sigma-Aldrich	Mouse	1:2000
Pho-γH2A.X	Cell signaling, 9718	Rabbit	1:400
α-Mouse	Invitrogen/Molecular Probes	Goat	1:2000
α-Rabbit	Invitrogen/Molecular Probes	Goat	1:2000
α-Rabbit HRP	Vector Lab, PI-1000	Goat	1:5000