RUNX2 overexpression and PTEN haploinsufficiency cooperate to promote CXCR7 expression and cellular trafficking, AKT hyperactivation and prostate tumorigenesis

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Supplementary Table 1. Primers for genotyping, RT-qPCR and ChIP-qPCR.

For genotyping			
Gene		**Forward**	**Reverse**
Cre		GATCCTGGCAATTTCGGCTAT	TTGCCTGCATTACCGGTCGAT
Pten		ACTCAAGGCAGGGATGAGC	GCCCCGATGCAATAAATATG
Runx2		CACTGCATTCTAGTTGTGGTTTGTCCAAAC	CGATGATCTCCACCATGGTGCGGTTGTCGT
For RT-qPCR (Human)			
Gene		**Forward**	**Reverse**
GAPDH		ACCCACTCCTCCACCTTTGAC	TGTTGCTGTAGCCAAATTCGTT
CXCR7		GGCTATGACACGCACTGCTACA	TGGTTGTGCTGCACGAGACT
RUNX2		CTAGGCGCATTTCAGGTGCT	TGGCAGGTAGGTGTGGTAGT
For RT-qPCR (Mouse)			
Gene		**Forward**	**Reverse**
Gapdh		AGGTTGTCTCCTGCGACTTCA	GGGTGGTCCAGGGTTTCTTACT
Pten		AATTCCCAGTCAGAGGCGCTATGT	GATTGCAAGTTCCGCCACTGAACA
Pten-phosphotase domain		ATGACAGCCATCATCAAAGAGATC	ATATCTTCACCTTTAGCTGGCAG
Runx2		GCCTTCAAGGTTGTAGCCCT	GGACCGTCCACTGTCACTTT
Pten Exons	**PCR size (bp)**		
Exons 1	998	TTTGAGAGTTGAGCCGCTGT	GCACGATCTAGAAATGCGCC
Exons 2	499	ATCACAGCTGTCAGGGATGA	CATCCAGTGACGCATCCA
Exons 3	474	TGCTAATATCGTTTTGTCAAGACG	CGCTTCGAGACCCAACAA
Exons 4	441	TGACTGTAAAAACACTTAGCGCA	GCTGTCCCACACCGTCAATA
Exons 5-1	650 bp (floxed), 500 bp (WT), 300 bp (deletion)	TCCCAGAGTTCATACCAGGA	GCAATGGCCAGTACTAGTGAAC
Exons 5-2		TCCCAGAGTTCATAC	AATCTGTGCATGAAGG

		CAGGA	GAAC
Exons 6	318	GCCACTTAAAGGAGAAACTTTGGG	GTTTTCCGACACACAGACAGC
Exons 7	424	AGAAGTCCTTACATGGGTTGGT	GCTTTAAGCAAAAGGTCTGTGGT
Exons 8	368	CCACAAGGTGTTTGCCTTC	CTCCCACCCCCAAATG
Exons 9	500	GTGCCCTTCAGAATTCATTTTG	ACAAGTGTCAAAACCCTGTGG
For ChIP-qPCR (Human)			
Gene Locus		**Forward**	**Reverse**
CXCR7-Promoter (P)		GGAGCTCTTGGTGTTAATGGGA	TGCAAAATCTGGTGAAGCCAC
CXCR7-Negative Control (NC)		GTTTGCTGCCCATCAGCTTT	TCAACGCTTCAACGACTTGC
CXCR7-Enhancer 1 (E1)		AATTCCGGCTTCACCCTCAG	ATGCACCTCTCGTTTCTGGG
CXCR7-Enhancer 2 (E2)		CAGGGCTAATTGCTGACCCA	TCACCATTTCACTCCCAGCC

Supplementary Figure Legends

Figure S1

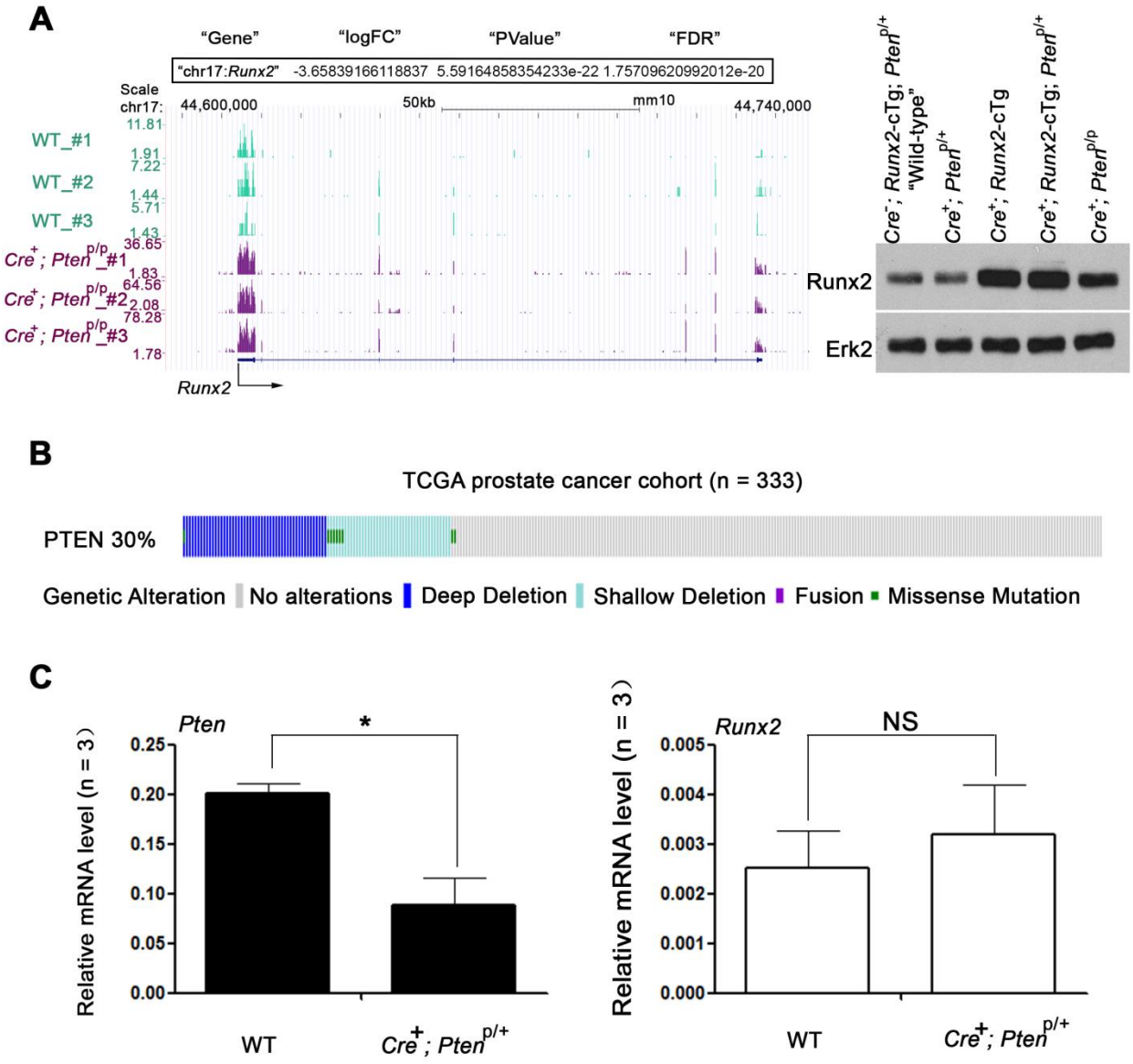

Figure S1. *Runx2* expression in *Pten* homozygous and heterozygous prostate tissues in mice and patient PCa specimens with or without *PTEN* gene deletion. (**A**), RNA-seq track views

from UCSC genome browser for *Runx2* gene in the prostate tissues of wild-type (WT) and prostate-specific *Pten* homozygous knockout mice at 4 months of age (left panel). Western blot showing Runx2 levels in murine prostate tissues from mice with the indicated genotypes. Erk2 was used as loading control (right panel). (**B**), Meta-analysis of TCGA datasets showing deep, shallow or no deletion of PTEN in primary PCa in this cohort. (**C**), RT-qPCR analysis of effectiveness of *Pten* deletion and relative *Runx2* mRNA level in the prostate of wild-type (WT) and prostate-specific *Pten* heterozygous deletion (Cre^{+};$Pten^{p/+}$) mice at age of 4 months. Data are means±S.D. (n = 3 mice/group). * $P < 0.05$.

Figure S2

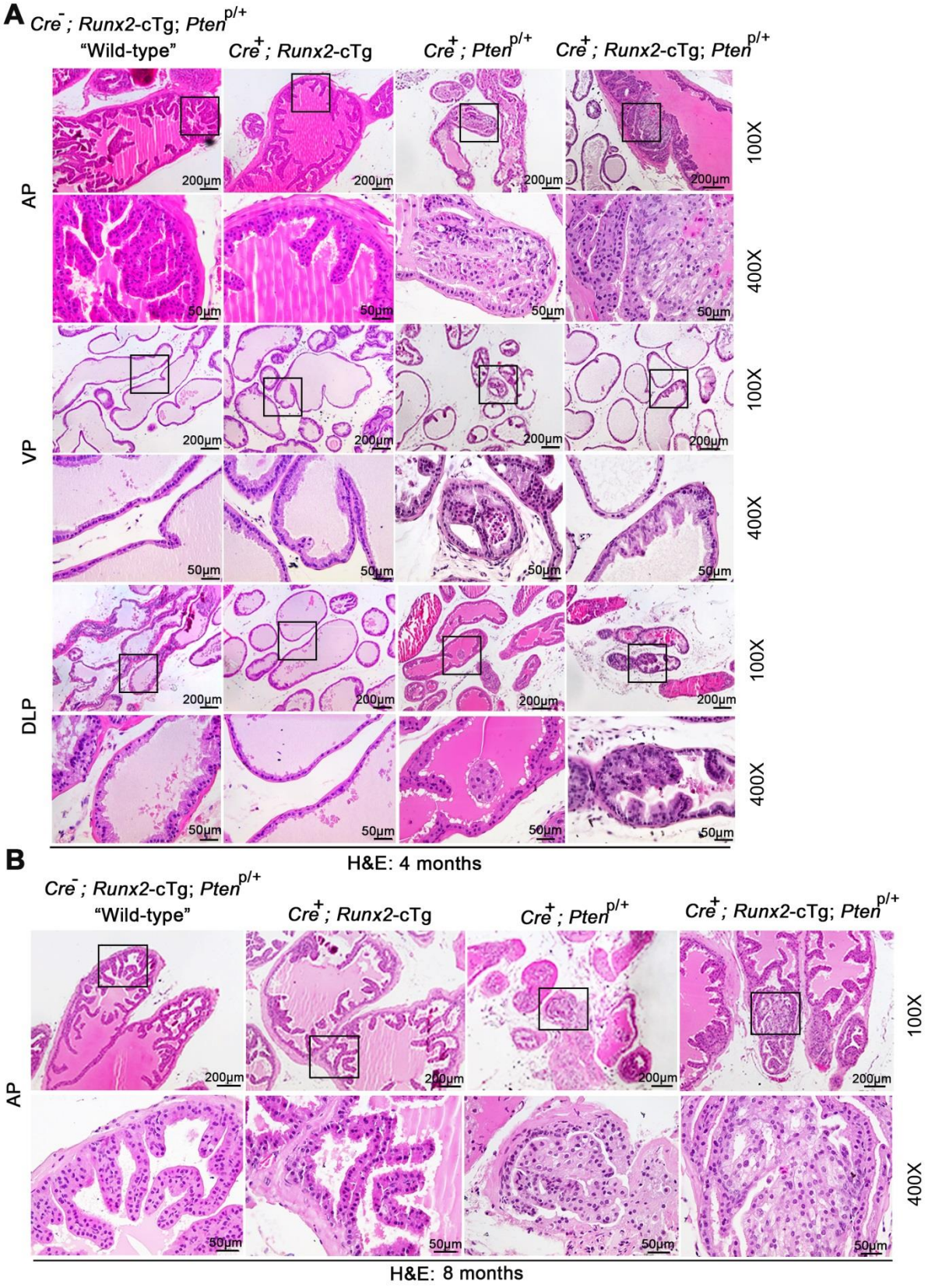
A
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg
Cre+; Pten p/+
Cre+; Runx2-cTg; Pten p/+
AP
VP
DLP
100X
400X
200μm
50μm
H&E: 4 months
B
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg
Cre+; Pten p/+
Cre+; Runx2-cTg; Pten p/+
AP
100X
400X
200μm
50μm
H&E: 8 months

Figure S2. Histology of different lobes of the prostate in mice with indicated genotypes at 4 or 8 months of age. (**A**), H&E staining of murine prostate tissue collected from the indicated mice at 4 months of age. AP, anterior prostate. VP, ventral prostate. DLP, dorsolateral prostate (n = 10 mice/genotype). (**B**), H&E staining of murine prostate tissue collected from indicated mice at 8 months of age (n = 10 mice/genotype). AP, anterior prostate. Upper row: 100X, scale bar, 200 μm. Lower row: 400X, scale bar, 50 μm.

Figure S3

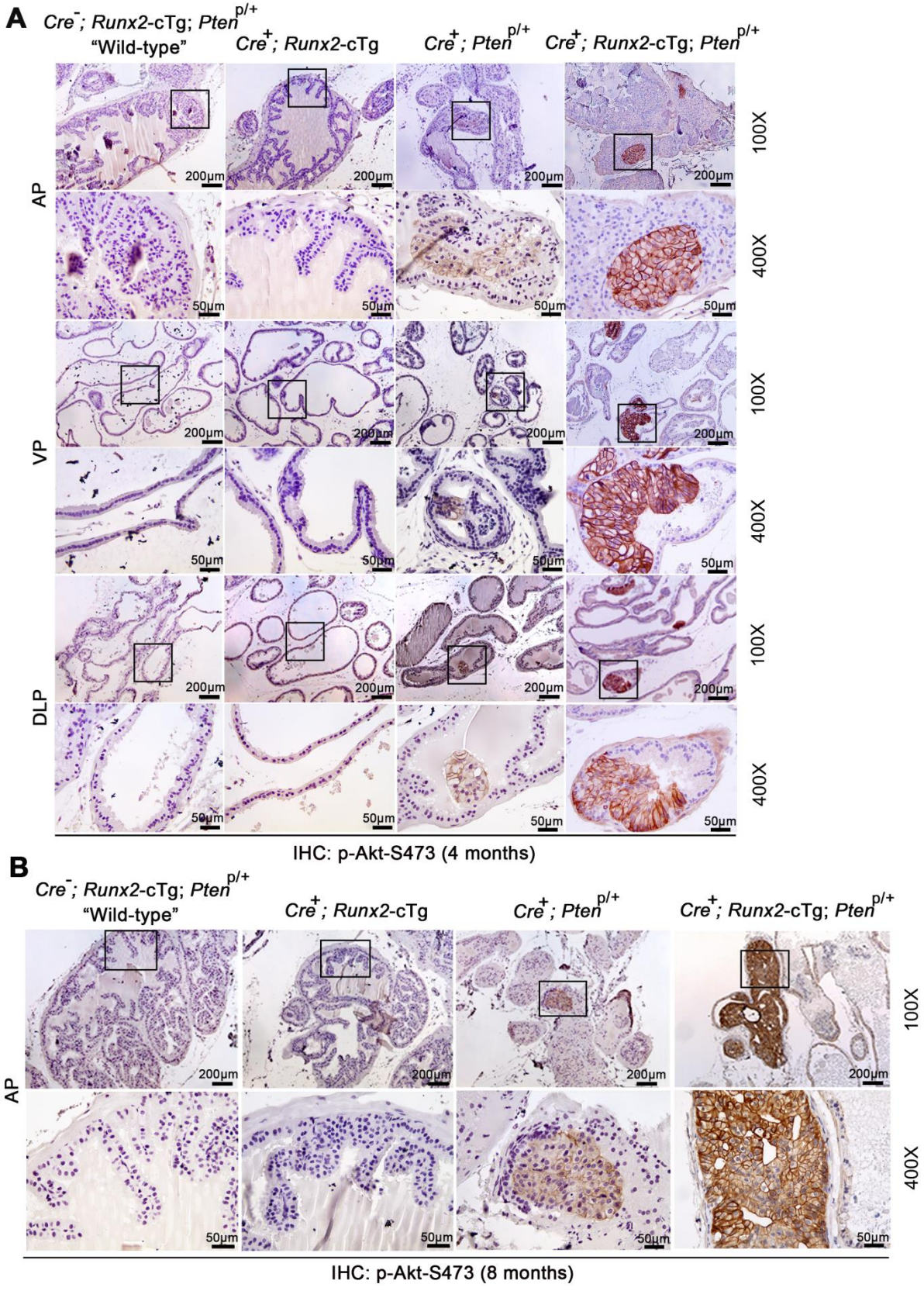
A
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg
Cre+; Pten p/+
Cre+; Runx2-cTg; Pten p/+
AP
VP
DLP
100X
400X
200μm
50μm
IHC: p-Akt-S473 (4 months)
B
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg
Cre+; Pten p/+
Cre+; Runx2-cTg; Pten p/+
AP
100X
400X
200μm
50μm
IHC: p-Akt-S473 (8 months)

Figure S3. IHC staining for phosphorylated Akt in different lobes of the prostate in mice with indicated genotypes at 4 and 8 months of age. (**A**), IHC for S473 phosphorylated Akt (p-Akt-473) in murine prostate tissues collected from indicated mice at 4 months of age (n = 10 mice/genotype). AP, anterior prostate. VP, ventral prostate. DLP, dorsolateral prostate. (**B**), IHC for S473 phosphorylated Akt (p-Akt-473) in murine prostate tissues collected from the indicated mice at 8 months of age (n = 10 mice/genotype). AP, anterior prostate. Upper row: 100X, scale bar, 200 μm. Lower row: 400X, scale bar, 50 μm.

Figure S4

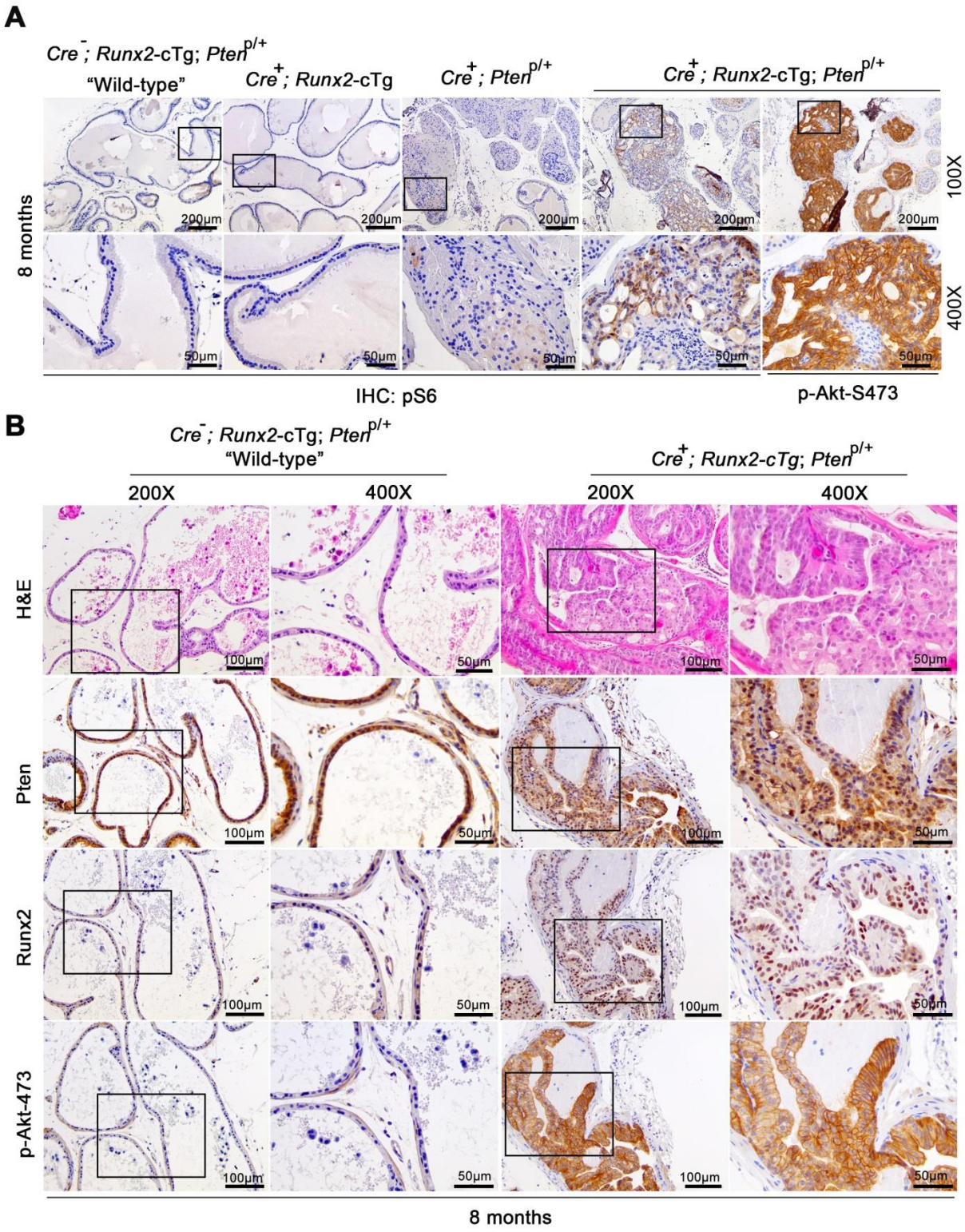
A
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg
Cre+; Pten p/+
Cre+; Runx2-cTg; Pten p/+
8 months
100X
400X
200μm
50μm
IHC: pS6
p-Akt-S473
B
Cre-; Runx2-cTg; Pten p/+
"Wild-type"
Cre+; Runx2-cTg; Pten p/+
200X
400X
H&E
Pten
Runx2
p-Akt-473
100μm
50μm
8 months

Figure S4. Expression of Pten protein and phosphorylation of S6 ribosomal protein in the prostate of *Runx2-Pten* double mutant mice. (**A**), IHC of S235/236 phosphorylated S6 (pS6) in the prostate tissues of mice at 8 months of age (n = 3 mice/genotype). p-Akt-473 IHC was included as an indicator of AKT hyperactivated tissues. Upper row: 100X, Scale bar, 200 μm. Lower row: 400X, Scale bar, 50 μm. (**B**), H&E (top) and IHC for Pten, Runx2, and S473 phosphorylated Akt (p-Akt-473) in murine prostate tissue collected at 8 months of age. Left column: 200X, Scale bar, 100 μm. Right column: 400X, Scale bar, 50 μm.

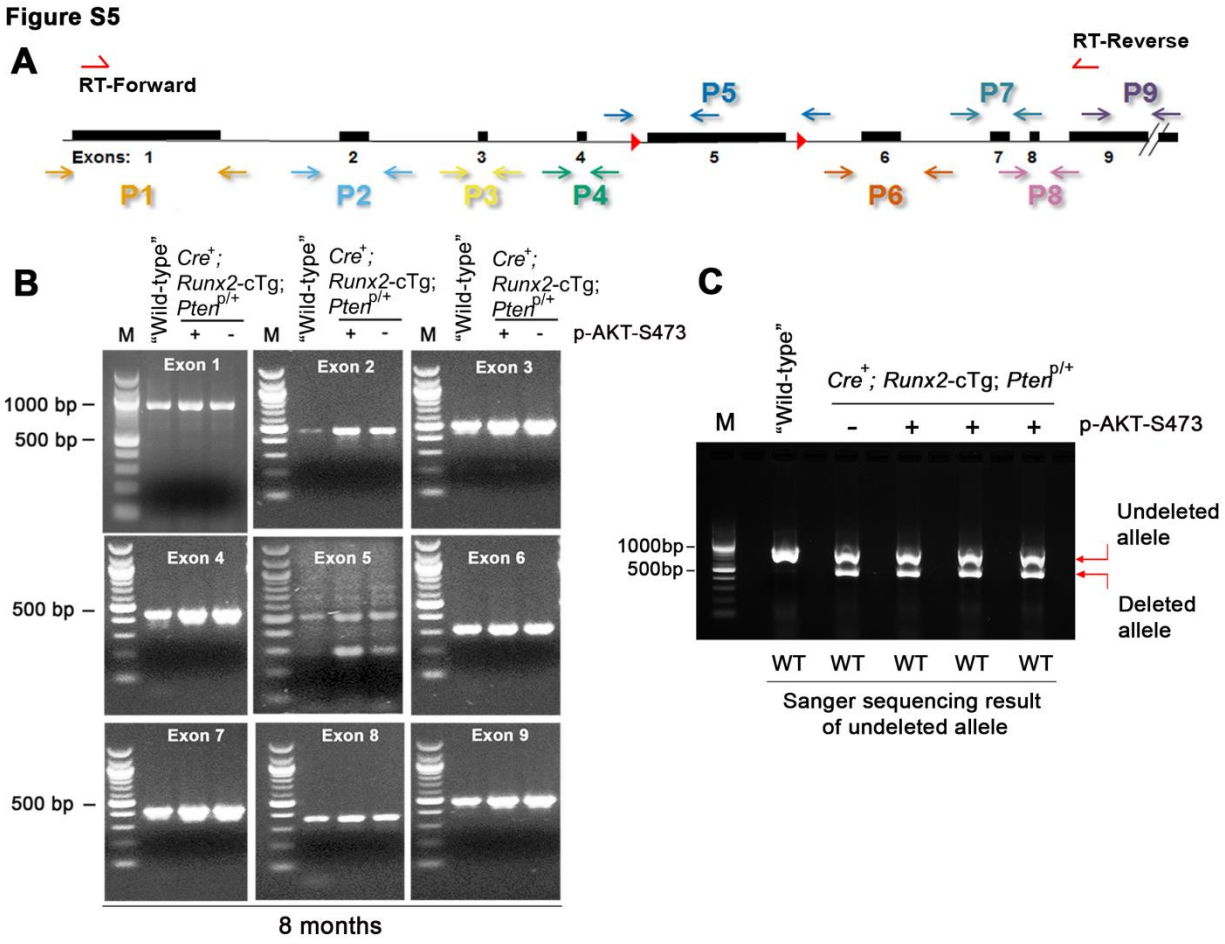

Figure S5. Assessment of undeleted allele of the *Pten* gene in the prostate of *Runx2-Pten* double mutant mice. (**A**), Schematic of the murine *Pten* gene indicating the position of primer pairs used for PCR amplification of different exons from prostate tissue in mice with indicated genotypes. Black bars and numbers indicate exons. Colored arrows and P# indicate primer pairs. Red triangles indicate LoxP sites. (**B**), Agarose gels showing PCR results for different exons from prostate tissue in mice with indicated genotypes. (**C**), Upper panel, an agarose gel showing the products of PCR amplification of undeleted and deleted alleles of the *Pten* gene in the prostate of mice with indicated genotypes using the RT-PCR primers shown in (**A**). Lower panel, Sanger sequencing result of undeleted allele in mouse prostate tissues.

Figure S6

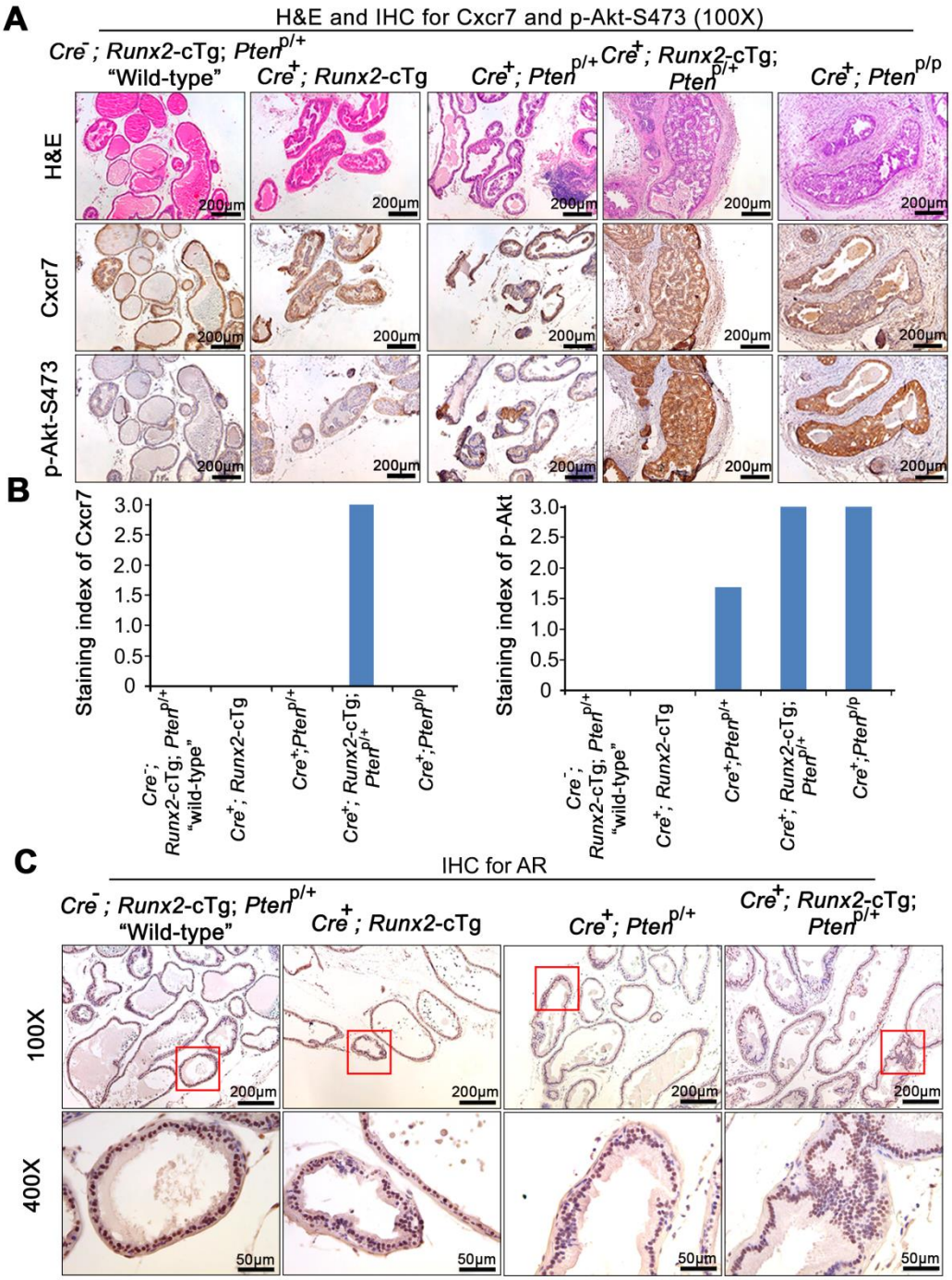

Figure S6. Cxcr7, Akt phosphorylation and AR protein expression in mouse prostate tissues. (**A-B**), H&E and IHC staining of Cxcr7 and phosphorylated Akt (p-Akt-473) in murine prostate tissues collected from mice with the indicated genotypes at 8 months of age (**A**). Scale

bar, 200 μm. Three individuals (including one GU pathologist) determined the IHC staining scores by evaluating both the intensity of the staining and the percentage of staining-positive cells. Membrane staining of p-Akt and Cxcr7 was considered as positive (**B**). (**C**), IHC for androgen receptor (AR) in murine prostate tissues collected from indicated mice at 8 months of age (n = 5 mice/genotype). Upper row: 100X, scale bar, 200 μm. Lower row: 400X, scale bar, 50 μm.

Figure S7

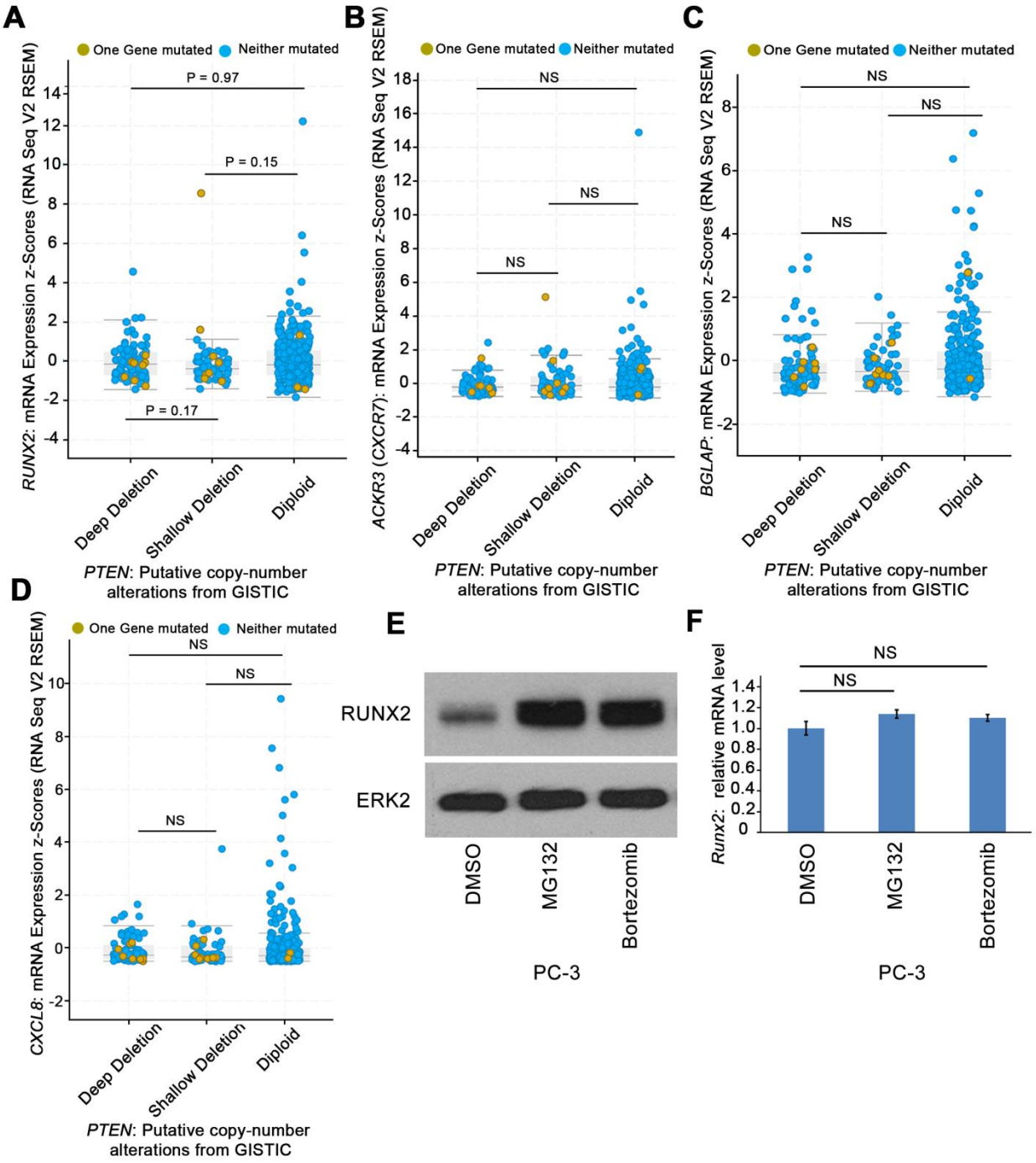

Figure S7. Runx2 mRNA and protein expression in prostate cancer patient samples and cells in culture. (**A-D**), Meta-analysis of *RUNX2* (**A**), *ACKR3* (*CXCR7*) (**B**), *BGLAP* (**C**), *CXCL8* (**D**) mRNA expression in primary prostate cancer patient specimens with deep, shallow

or no deletion of *PTEN* in the TCGA cohort. The *P* values were shown in the images. NS means no significance. (**E-F**), PC-3 cells were treated with DMSO, MG132 (20 μM) or bortezomib (200 nM) for 8 h and cells were harvested for western blot analysis of RUNX2 protein level (**E**) and RT-qPCR measurement of *RUNX2* mRNA expression (**F**). Data represent mean values ± SD (n = 3). NS means no significance.