Supplementary Information and Figure legends

Table S1. Primers for quantitative RT-PCR

Target	Sequence (5' -> 3')	Target	Sequence (5' -> 3')
DAB2IP	F:TGGACGATGTGCTCTATGCC	Snail	F:CCTCCCTGTCAGATGAGGAC
	R:GGATGGTGATGGTTTGGTAG		R:CCAGGCTGAGGTATTCCTTG
CD24	F:GCCAGTCTCTTCGTGGTCTC	Twist	F:GGAGTCCGCAGTCTTACGAG
	R:CCTGTTTTTCCTTGCCACAT		R:TCTGGAGGACCTGGTAGAGG
CD44	F:AGCAACCAAGAGGCAAGAAA	Slug	F:GGGGAGAAGCCTTTTTCTTG
	R:GTGTGGTTGAAATGGTGCTG		R:TCCTCATGTTTGTGCAGGAG
CD117	F:CAAGGCTTCTCCAATTCTGC	FOXC2	F:GCCTAAGGACCTGGTGAAGC
	R:TGCAGTGGTCCACAGAAGAG		R:TTGACGAAGCACTCGTTGAG
CD133	F:GCCACCGCTCTAGATACTGC	Sox-2	F:AGCTACAGCATGATGCAGGA
	R:TGTTGTGATGGGCTTGTCAT		R:GGTCATGGAGTTGTACTGCA
Sca-1	F:TGGCTCTTCTTCCGCTACAT	KLF4	F:TCTCAAGGCACACCTGCGAA
	R:GCCTCGAAGACCTCACAGTC		R:TAGTGCCTGGTCAGTTCATC
ZEB1	F:TTCAAACCCATAGTGGTTGCT	Oct4	F:AGCGAACCAGTATCGAGAAC
	R:TGGGAGACACCAAACCAACTG		R:TTACAGAACCACACTCGGAC
SIP1	F:TTCCTGGGCTACGACCATAC	c-myc	F:ACTCTGAGGAGGAACAAGAA
	R:TGTGCTCCATCAAGCAATTC		R:TGGAGACGTGGCACCTCCTT
CD44S	F:AGCAGCGGCTCCTCCAGTGA	CD44v3	F:GCACTTCAGGAGGTTACATC
	R:CCCACTGGGGTGGAATGTGTCT		R:CTGAGGTGTCTGTCTCTTTC
CD44v6	F:AGGAACAGTGGTTTGGCAAC	CD44v8	F:TCAGCCTACTGCAAATCCAA
	R:CGAATGGGAGTCTTCTCTGG		R:GAGGTCCTGTCCTGTCCAAA

Table S2. The list of primers for ChIP detection; CD44 promoter region

Target	Sequence (5’ -> 3’)	Target	Sequence (5’ -> 3’)
P1	F: TGAGCTCTCCCTCTTTCCAC	P4	F: ATGGTGGATGGTTGTGGTTT
	R: GAGGATGACCGAACCGTAAA		R: CATCCTCCTGTCCATCCACT
P2	F: CCCTATGACAGGCCATCAGT	P5	F: AAAAGGCTTCCCCTGAAGAA
	R: GGGAGTTGGTGAATCTTCCA		R: CAACCATCCACCATCCTCTT
P3	F: AGTGGATGGACAGGAGGATG	P6	F: CTTGCCACAGCCACTGATAA
	R: ACTGATGGCCTGTCATAGGG		R: TTCTTCAGGGGAAGCCTTTT

Figure S1. Loss of DAB2IP increased clonogenicity and stemness in normal prostate epithelial cells.

(A) Cells were seeded in 6-well plates at a density of 1,000 cells per well, and cultured for 10 days. At the end, cells were stained with crystal violet, photographed. The colony numbers were counted using Image J analysis software. (B) Cells were suspended in growth medium with 0.3% agar, then plated onto a 0.6% agar foundation in 6-well plates at a density of 5×10^4 cells per well. After 2 weeks, colonies were counted under the microscope. (C) RWPE-1 and PZ-HPV7 cells were grown in sphere forming condition for 2 weeks, and the numbers of prostaspheres were counted in 5 randomly selected fields (×100) (Left panel). Error bars represented mean ± SD. *, $p<0.01$. And the size of prostaspheres in diameter was measured, and the percentage of each sphere was calculated (Right panel). (D) Cells were stained with Hoechst 33342 for determining SP cells using flow cytometry. Three independent experiments were performed and representative result was shown.

Figure S2. Loss of DAB2IP increased in vitro invasion and motility in normal prostate epithelial cells.

RWPE-1 and PZ-HPV7 **c**ells were plated onto Matrigel-coated Transwell chambers (A) or plated upper Transwell chambers without Matrigel (B) for 48 h, and quantitative measurements of invade or migratory cells were determined. Data were presented as mean ± SD of each sample measured in triplicate. *, $p<0.01$. Con, control; KD, DAB2IP knock down.

Figure S3. DAB2IP inhibits CD44 expression in human prostate epithelial cell lines.

(A and B) Expression levels of several stem cell markers and EMT markers were analyzed by qRT-PCR. After normalizing with 18S rRNA in each sample, the relative mRNA levels were calculated using control (=1). (C) Left panel: wild type PZ-HPV7 cells (WT) and PZ-HPV7T (T)

cells were stained with PE conjugated CD44 and analyzed by flow cytometry. PE conjugated mouse IgG was used for gating. Right panel: PZ-HPV7 WT and T cells were grown in semisolid culture condition for 14 days and sphere forming ability was compared. (D) Relationship between CD44 and DAB2IP immunostaining. Number represented the percentage of total sample analyzed. (E) Expression levels of CD44 mRNA in wild-type and KO mice were determined by qRT-PCR.

Figure S4. Wnt pathway correlates with CRPC progression.

(A) For measuring the CD44 gene promoter activity, cells were transfected with CD44-luc for 48 h and subjected to dual luciferase assay. The activity of Renilla reporter was used for the normalization of transfection efficiency. Each experiment was performed twice in triplicates. (B-D) The correlation between CD44 and β-catenin gene (CTNNB1) expression from PCa specimens were shown. β-catenin expressions (y-axis) are plotted against the measurements of CD44 expression (x-axis), both in log scale. The Pearson's correlation coefficient and p-value in each study are listed for each dataset.

Figure S5. AR inhibitor doesn't affect the expression of CD44.

RWPE-1 and PZ-HPV7 cells were treated with increasing dose of AR inhibitor, MDV3100 for 48 hr, and then CD44 mRNA expression was measured by qPCR.

Figure S6. All CD44 variants respond to Wnt inhibitor.

(A) Expression levels of CD44 isoforms were analyzed by qRT-PCR. The primers used in the experiment were shown in Table S1. (B) RWPE-1 and PZ-HPV7 KD cells were treated with 100 nM paclitaxel, 2.5 μM IWP-2, or combination for 48 h and the level of CD44 isoforms were analyzed by qRT-PCR.

Figure S7. Wnt signal regulates CD44 expression and stem cell properties.

(A) Prostaspheres from RWPE-1 and PZ-HPV7 KD cells were treated with 5 μM IWP-2 for 2 weeks. The media containing IWP-2 were replenished every 3 days. (B) PZ-HPV7 KD cells treated with increasing dose of IWP-2 (left panel) or LGK974 (right panel) were labeled with PE conjugated CD44 antibody, and CD44 expression were analyzed by flow cytometry.

Figure S8. Wnt signal pathway mediates the expression of CD44.

(A) RWPE-1 and PZ-HPV7 Con cells were co-transfected with CA β-catenin mutant (S37A) and CD44-luc plasmid for 48 h then subjected to dual luciferase assay. (B and C) RWPE-1 Con and PZ-HPV7 Con cells transfected with CA β-catenin mutant plasmid (S37A) were plated onto the upper chamber of Transwell for cell migration (B) and cell invasion (C). (D) RWPE-1 KD cells were co-transfected with GSK3-β (WT or constitutive active CA), PP2A (WT or catalytic inactive LP) and CD44-luc. After 48 h, reporter assay was performed. (E) RWPE-1 Con cells transfected with CD44-luc plasmid were treated with different dose of Okadaic acid for 24 h, and then, reporter activity were measured by dual luciferase assay system. OA, Okadaic acid.

Figure S9. Wnt inhibitors diminish the chemo-resistance of KD cells.

(A) $CD44^+$ and $CD44^-$ cells sorted from PZ-HPV7T were seeded in a 96-well and treated with docetaxel or LGK975 for 48 h and subjected to MTT assay. (B) Con or KD cells from RWPE-1 or PZ-HPV7 were treated with paclitaxel or IWP-for 48 h and subjected to MTT assay. (C) RWPE-1 KD or PZ-HPV7 KD cells were treated with 100 nM paclitaxel, 2.5 μM IWP-2, or combination for 48 h and subjected to MTT assay, and drug synergistic effects were determined. NT, non-treated; PTX, paclitaxcel; P+I, combination treatment of paclitaxel and IWP-2. (D and E) PZ-HPV7T cells (D) or $22Rv_1$ cells (E) were treated with 1 nM Docetaxel, 100 nM LGK974, or combination for 48 h. And cell viability was determined by MTT assay and drug synergistic effects were determined based on combination index (CI). NT, Non-treatment; DCT, docetaxel;

LGK, LGK974; D+I, docetaxel and LGK974 combination treatment. (F) 22Rv1 cells treated with docetaxel, LGK974, or combination for 48 h and labeled with PE conjugated CD44 antibody then subjected to flow cytometrical analyses.

Figure S10. Overexpression of CD44 rescue the growth inhibition by LGK974.

(A) PZ-HPV7 KD and RWPE-1 KD cells were transfected with CD44S plasmid for 24 h and CD44S expressions were analyzed by qPCR. (B) Cells transfected with CD44S were plated into 96 well plate and treated with LGK974 (200 nM) for 48 h. Cell viability was measured by MTT assay and normalized with untreated group.

Figure S11. Synergistic effect of Wnt inhibitors combined with docetaxel on Du145 cells.

(A) Cells were treated with docetaxel and LGK974, or combination of paclitaxel and IWP-2 for 48 h and subjected to MTT assay, and drug synergistic effects were determined. (B) Cells were treated with docetaxel and LGK974, or combination of paclitaxel and IWP-2, and expression levels of CD44 mRNA were analyzed by qRT-PCR.

Figure S1.

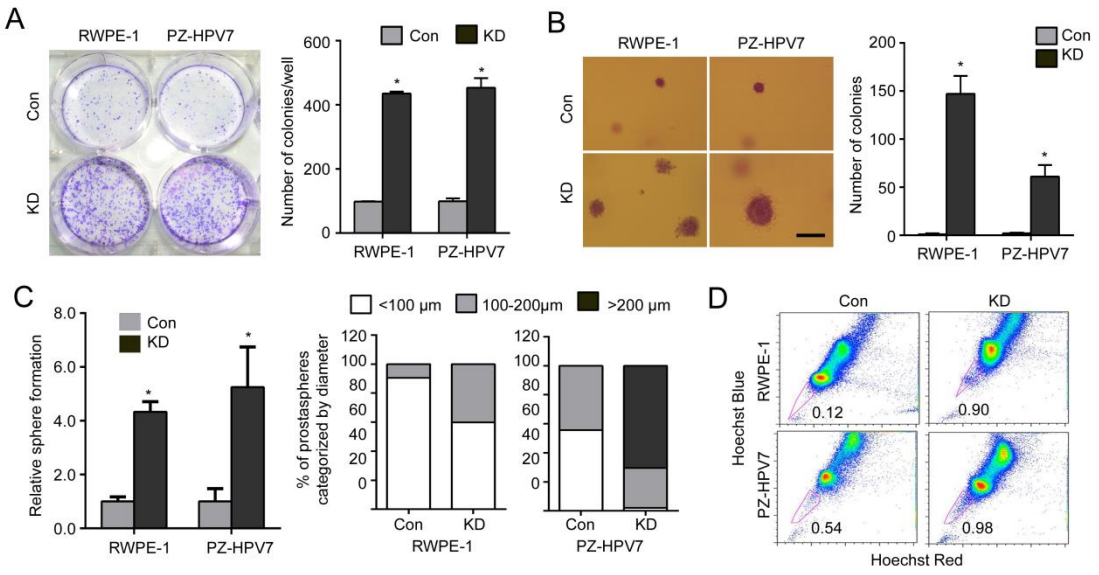

Figure S2.

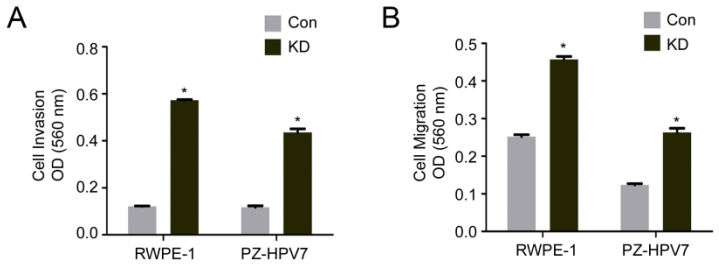

Figure S3.

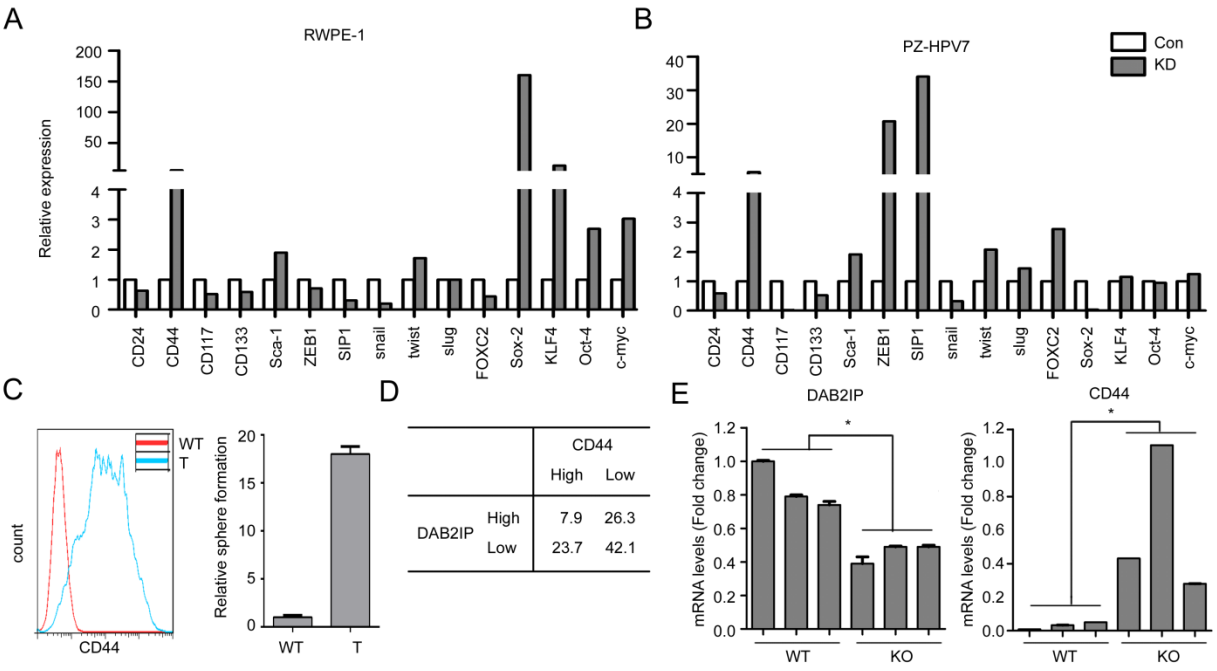

Figure S4.

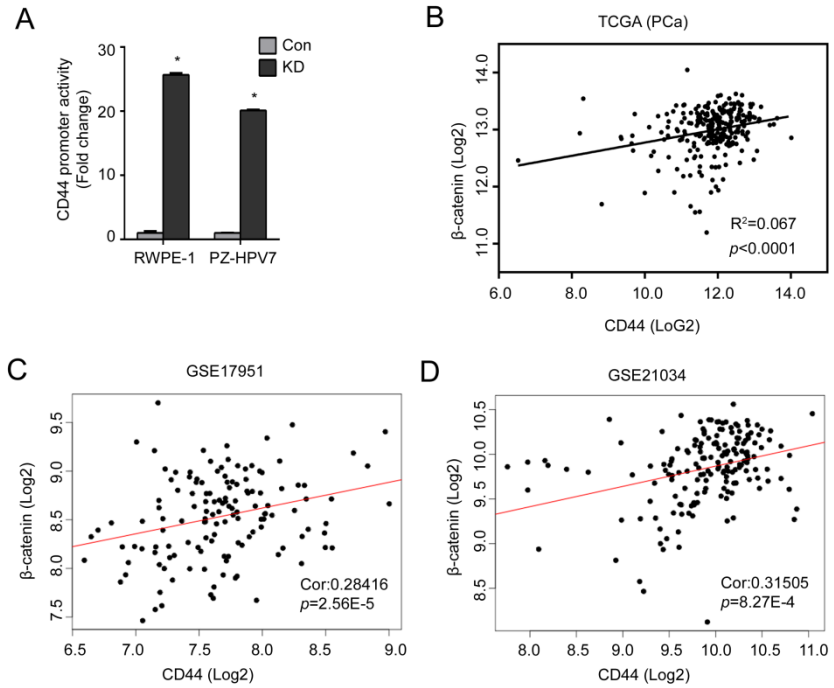

Figure S5.

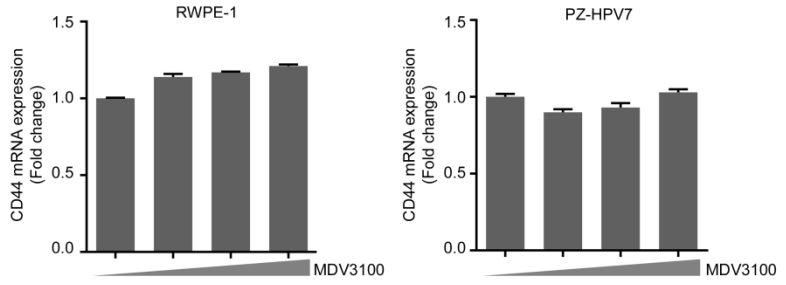

Figure S6.

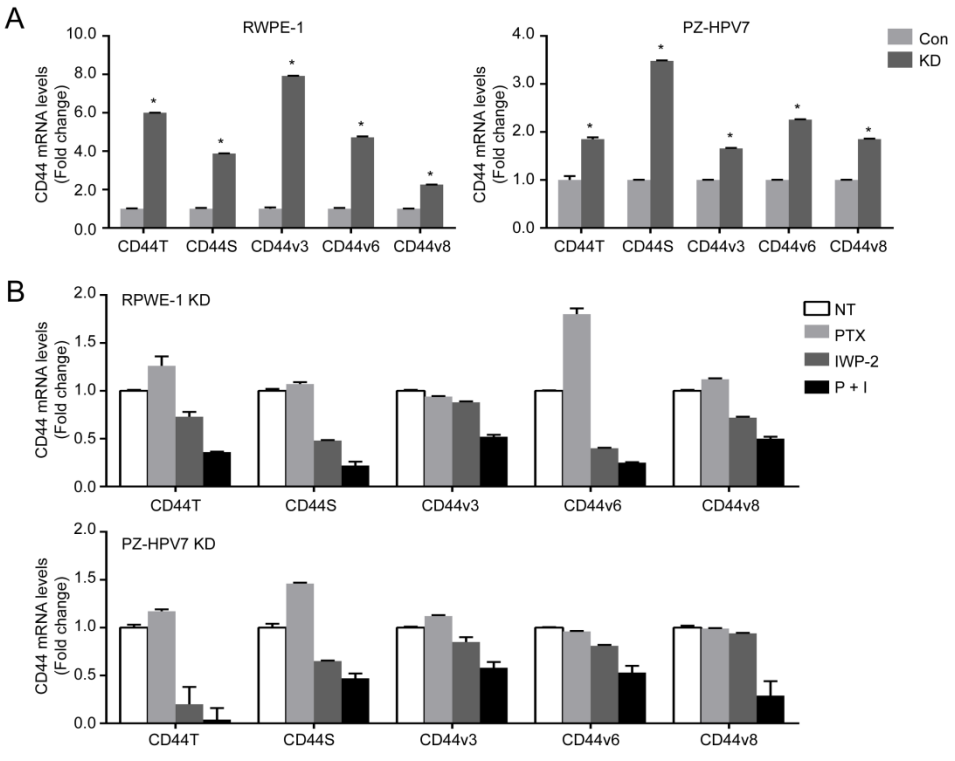

Figure S7.

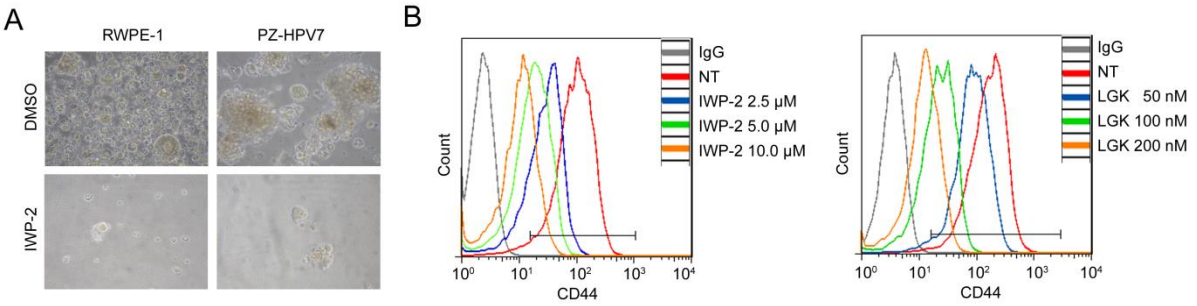

Figure S8.

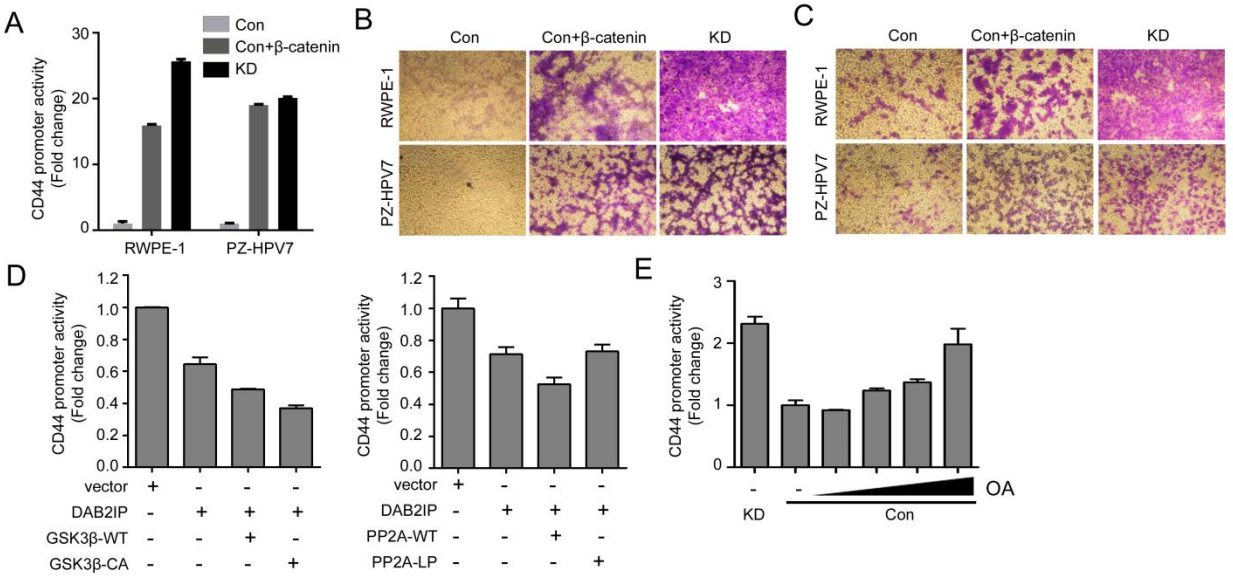

Figure S9.

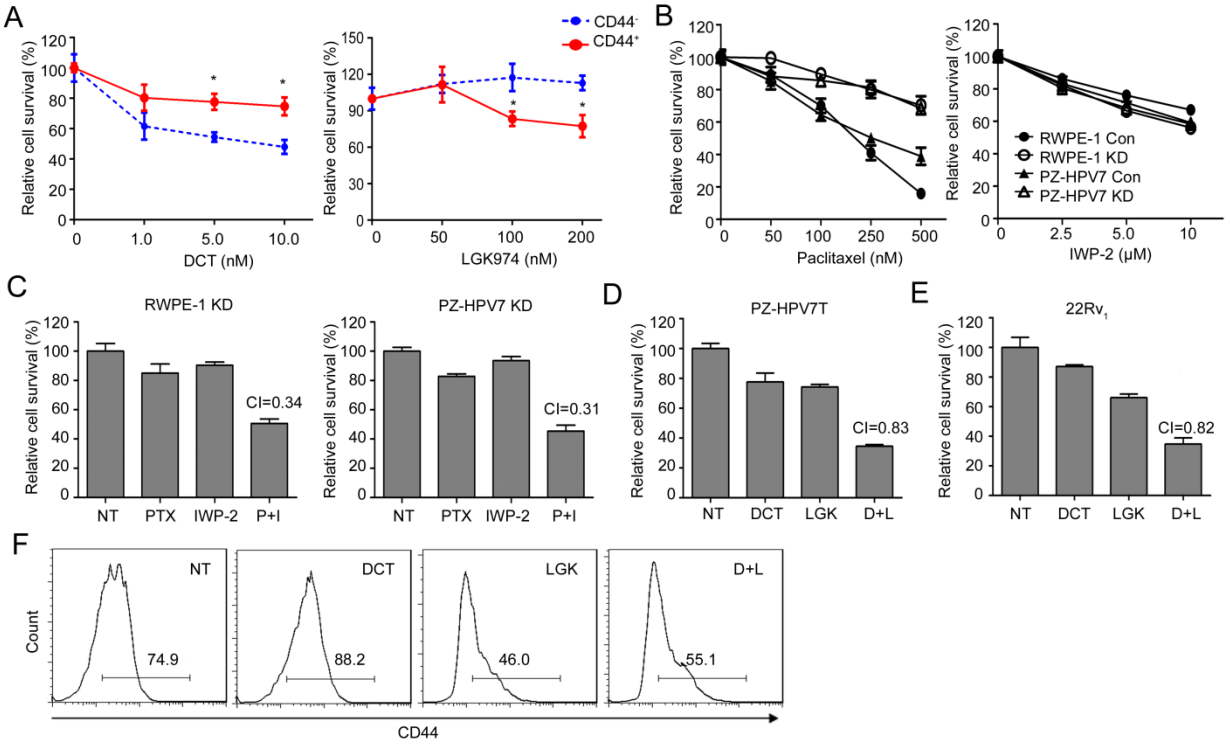

Figure S10.

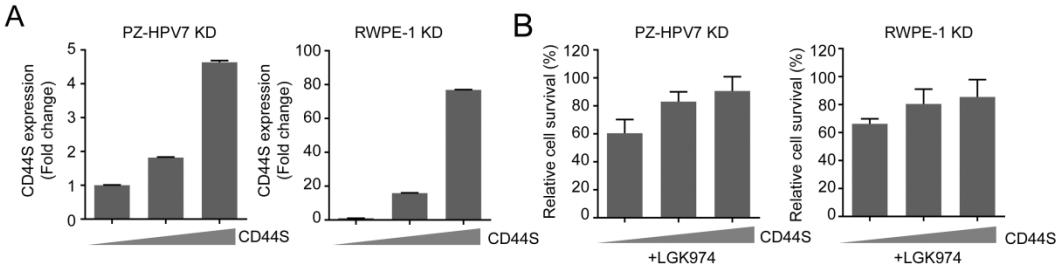

Figure S11.