Supplementary information

Tgfbr2 **inactivation facilitates cellular plasticity and development of** ***Pten*****-null prostate cancer**

Inventory of Supplementary information

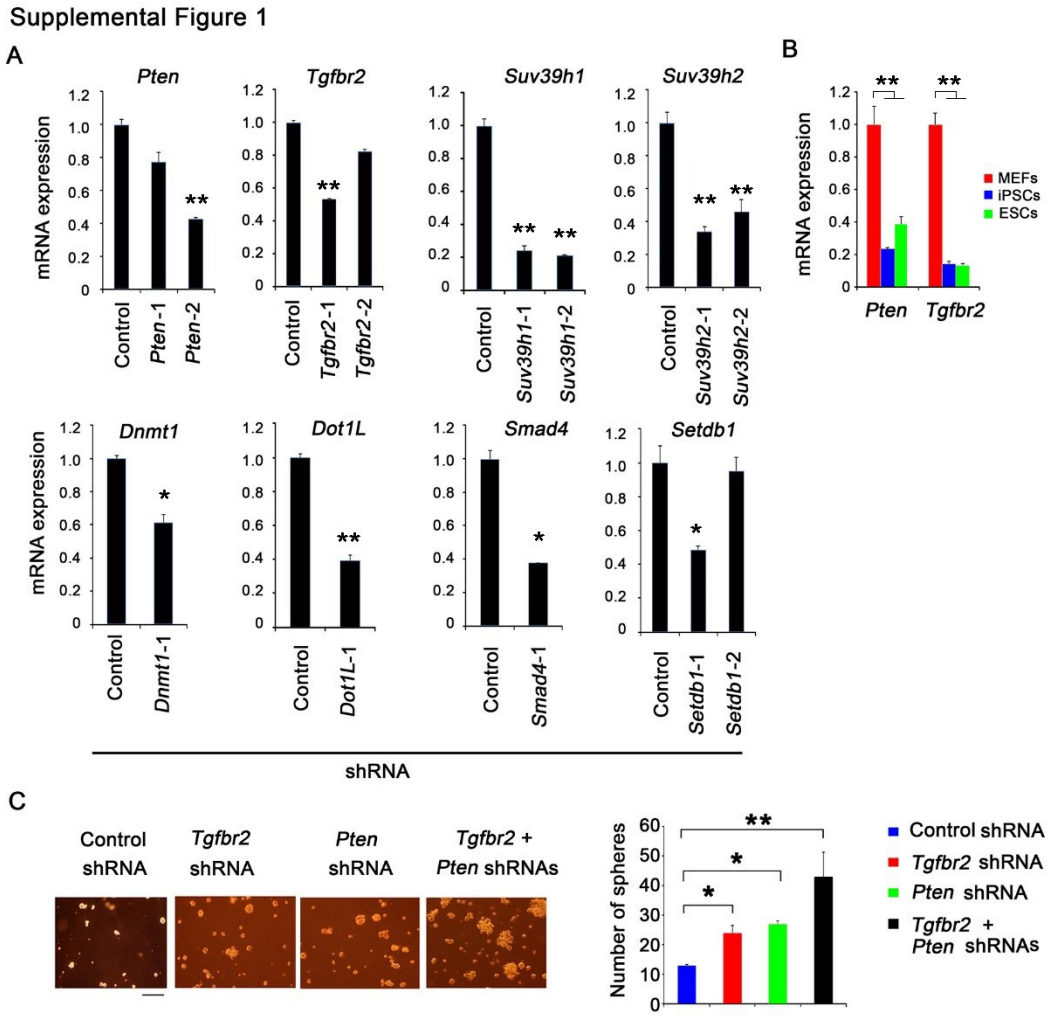


Figure S1. Validation of shRNAs Knockdown Efficiency by Real-time PCR. Related to Figure 1.

(A) MEFs were transduced with the lentivirus-mediated shRNA for 48 h, and total RNA was extracted for RT-real-time PCR analysis. For Suv39h2 shRNA KD experiment, we add 2 μg/ml puromycin at day 2 for positive selection (3 days).

(B) Real-time PCR analysis of *Pten* and *Tgfbr2* expression in MEFs, iPSCs, and ESCs. All data are normalized by internal control *18S* RNA.

(C) TRAMP-C3 cells were transduced with indicated shRNA for 48 hours. Growth of the cells in 3D Matrigel culture (left). Representative phase-contrast images of each group depict progression of organoid growth 7 days with scale (0.1 mm) as shown (right). Sphere (>0.1 mm) numbers of each group.

Data are represented as mean ± SD. Student's t test, *p<0.05, **p<0.01.

Supplemental Figure 2

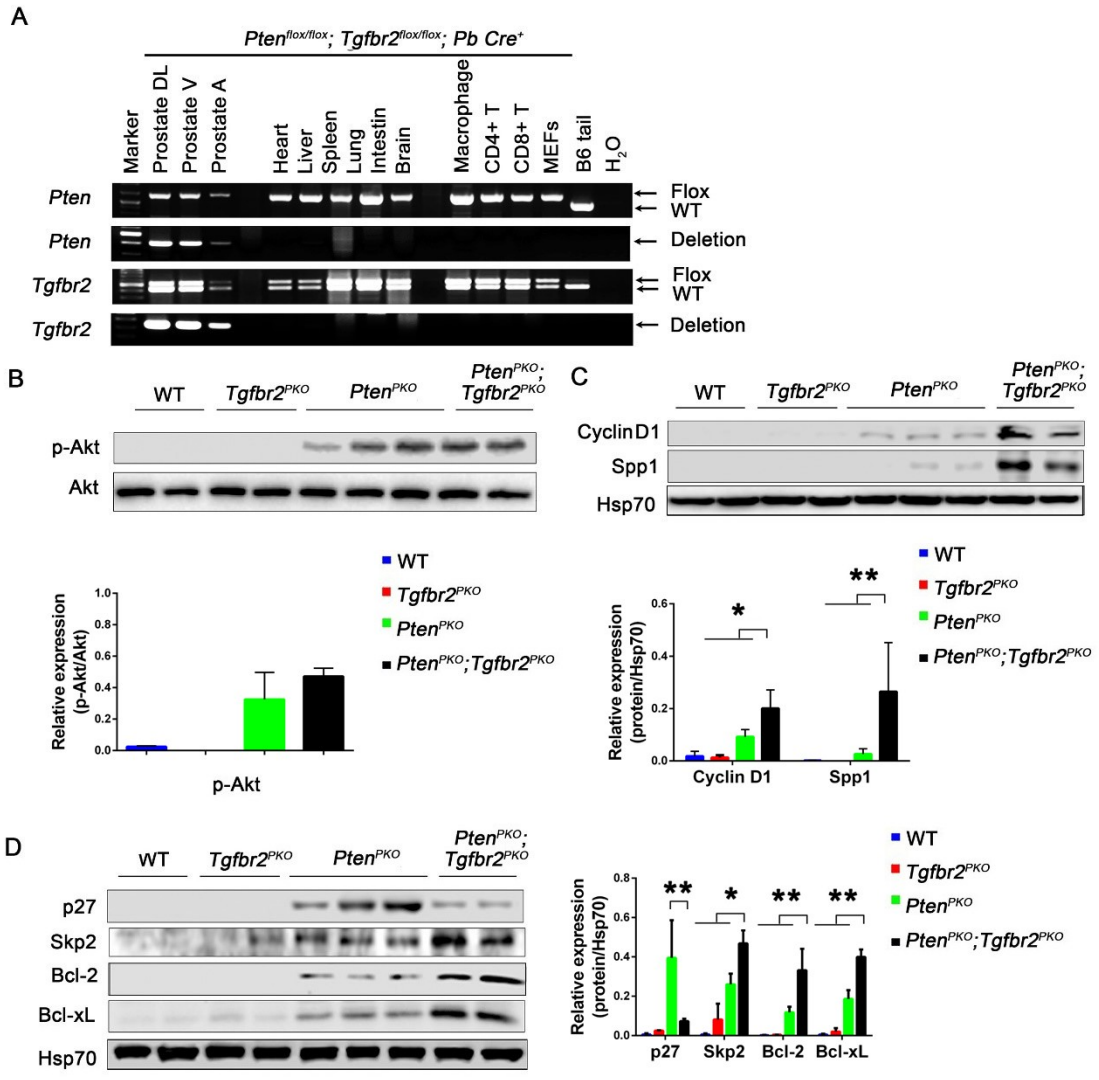


Figure S2. Validation of *Pten* and *Tgfbr2* Conditional Knockout Mice. Related to Figure 1.

(A) Genotyping analysis of *Pten* and *Tgfbr2* conditional knockout mouse strains. DL, dorsolateral; V, ventral; A, anterior.

(B) Western blot analysis of expression of phosphorylated Akt and Akt in prostates from mice with indicated genotypes.

(C) Western blot analysis of Cyclin D1 and Spp1 expression in the prostates from mice with the indicated genotypes.

(D) Western blot analysis of Bcl-xL, Bcl-2, Skp2, and p27 expression in prostate tissue from mice with the indicated genotypes.

Data are represented as mean ± SD. Student's t test, *p<0.05, **p<0.01.

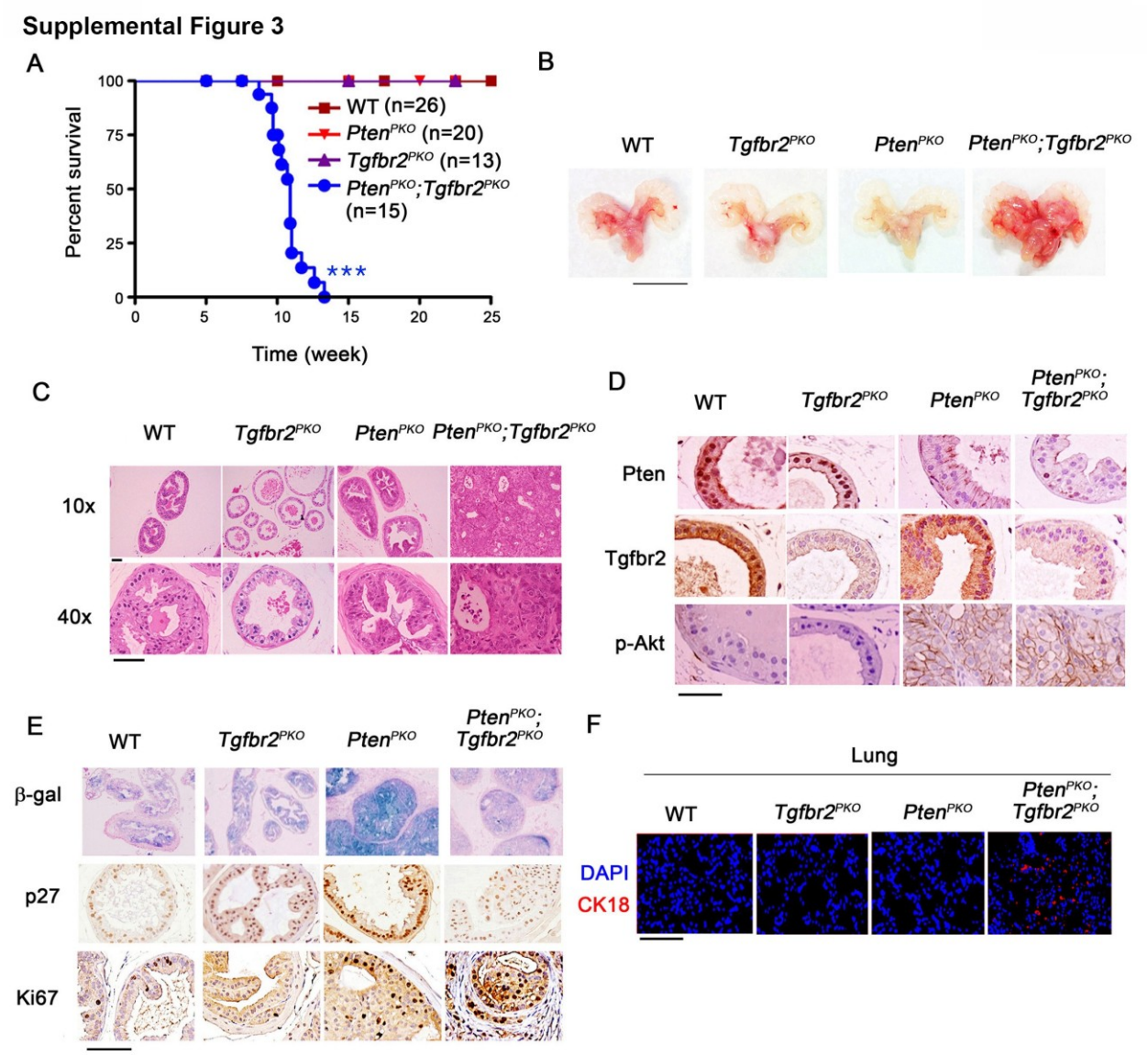


Figure S3. Prostate Tumor Progression in Mice with Deletion of *Pten* and *Tgfbr2*. Related to Figure 1.

(A) Kaplan–Meier plots for mice with prostate-specific homozygous deletion of *Pten* ($Pten^{PKO}$), *Tgfbr2* ($Tgfbr2^{PKO}$), or both genes ($Pten^{PKO}$; $Tgfbr2^{PKO}$). ***P <0.001 vs. corresponding control.

(B) Gross anatomy of representative prostates from 10-week-old mice. Scale bar = 10 mm.

(C) Hematoxylin and eosin (H&E)-stained sections of prostates from 10-week-old mice of the indicated genotypes. Increased number of cells in prostates from $Pten^{PKO}$; $Tgfbr2^{PKO}$, suggesting enhanced tumor formation. Scale bar = 100 μm.

(D) Immunohistochemical staining of Pten, Tgfbr2, and p-Akt (Ser 473) of prostates from mice with the indicated genotypes. Scale bar = 10 μm.

(E) Expression of β-galactosidase (β-Gal), p27, and Ki67 in prostates from mice with the indicated genotypes. Scale bar = 100 μm.

(F) Immunofluorescent staining of CK18 in lung tissues from mice with the indicated genotypes. Scale bar = 100 μm.

Supplemental Figure 4

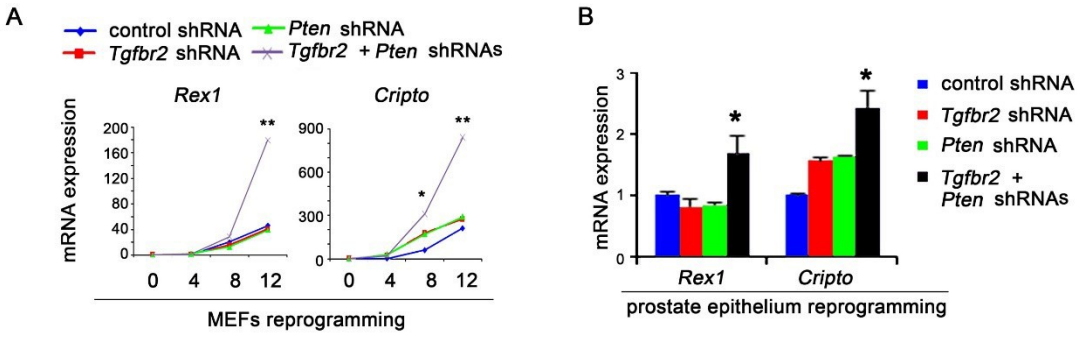


Figure S4. Induction of pluripotency factors in reprogramming cells upon KD of *Pten* and *Tgfbr2*. Related to Figure 2.

(A) *Tet-O-OSKM* cells were transduced with indicated shRNAs, with subsequent OSKM-mediated reprogramming for 12 days. RNA samples were collected at the indicated time points. Expression of *Rex1* and *Cripto* was analyzed by quantitative real-time PCR.

(B) TRAMP-C3 cells were transduced with indicated shRNAs, with subsequent OSKM-mediated reprogramming for 9 days. *Rex1* and *Cripto* expression was analyzed by quantitative real-time PCR.

Data are represented as mean ± SD. Student's t test, *p<0.05, **p<0.01.

Supplemental Figure 5

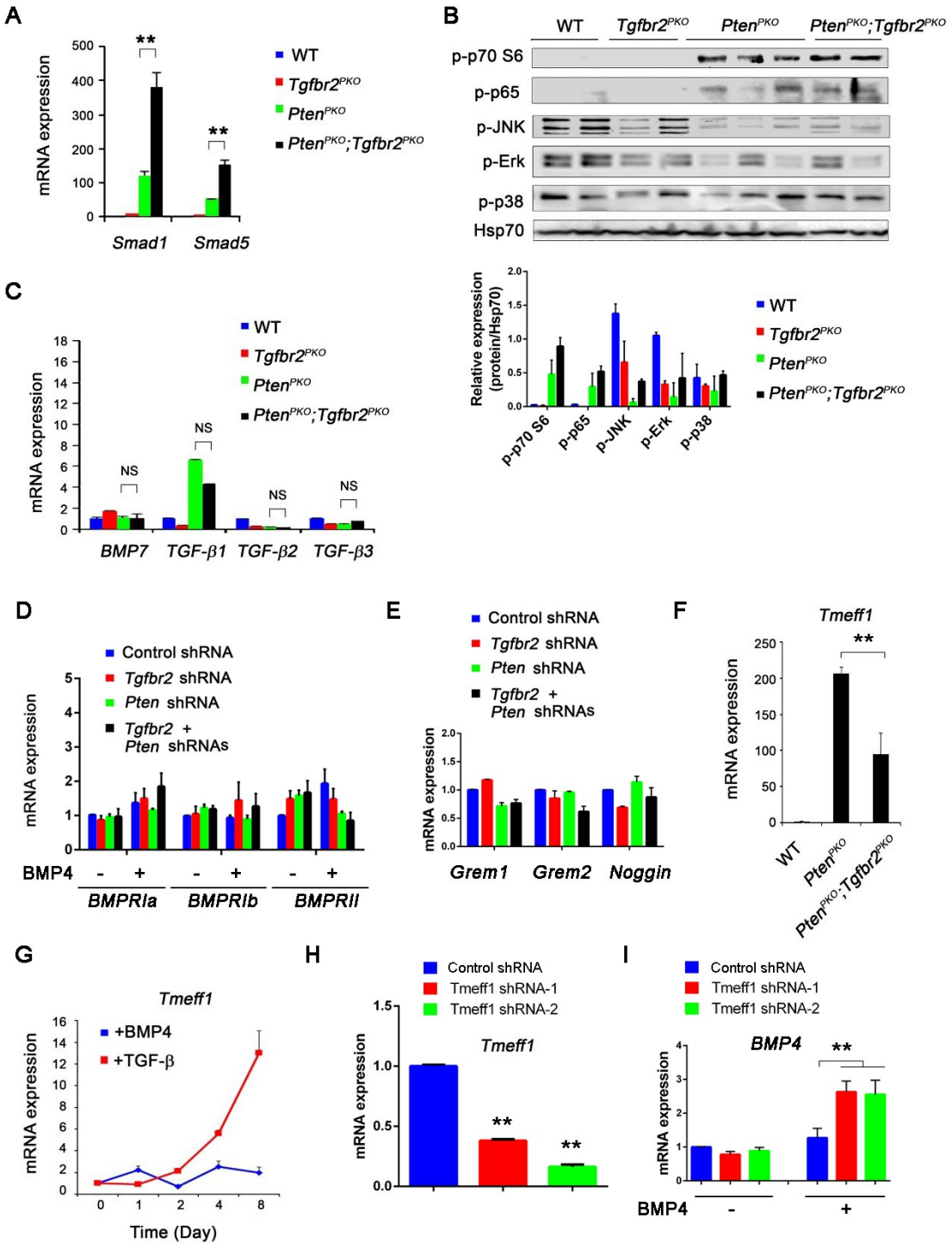


Figure S5. Interplay of TGF-β and BMP Signaling in Prostate Cancer Cells. Related to Figure 3.

(A) Expression of *Smad1* and *Smad5* was analyzed by real-time PCR in prostates from mice with indicated genotypes.

(B) Western blot analysis of expression of phosphorylated p70 S6, phosphorylated p65, phosphorylated JNK, phosphorylated Erk, and phosphorylated p38 in prostates from mice with indicated genotypes.

(C) Expression of *BMP7*, *TGF-β1*, *TGF-β2*, and *TGF-β3* was analyzed by real-time PCR in prostates from mice with indicated genotypes. NS, non-significant.

(D) TRAMP-C3 cells were transduced with the indicated shRNA for 48 hours, and subsequently treated with 50 ng/ml BMP4 for 24 hours. Real-time PCR analysis of expression of BMP receptors (*BMPRIa*, *BMPRIb,* and *BMPRII*) was performed.

(E) TRAMP-C3 cells were transduced with the indicated shRNA for 48 hours. Real-time PCR analysis of expression of BMP antagonists (*Gremlin1*, *Gremlin2*, and *Noggin*) was performed.

(F) Expression of *Tmeff1* was analyzed by real-time PCR in prostates from mice with indicated genotypes.

(G) TRAMP-C3 were treated with 50 ng/ml BMP4 or 10 ng/ml TGF-β for 8 days, expression of *Tmeff1* was analyzed by real-time PCR at indicated time points.

(H) TRAMP -C3 were transduced with the lentivirus-mediated *Tmeff1* shRNA for 48 h, and total RNA was extracted for RT-real-time PCR analysis of *Tmeff1*.

(I) TRAMP-C3 cells were transduced with the *Tmeff1* shRNA for 48 hours, and subsequently treated with 50 ng/ml BMP4 for 24 hours. Real-time PCR analysis of expression of *BMP4* was performed.

Data are represented as mean ± SD. Student's t test, *$p<0.05$, **$p<0.01$.

Supplemental Figure 6

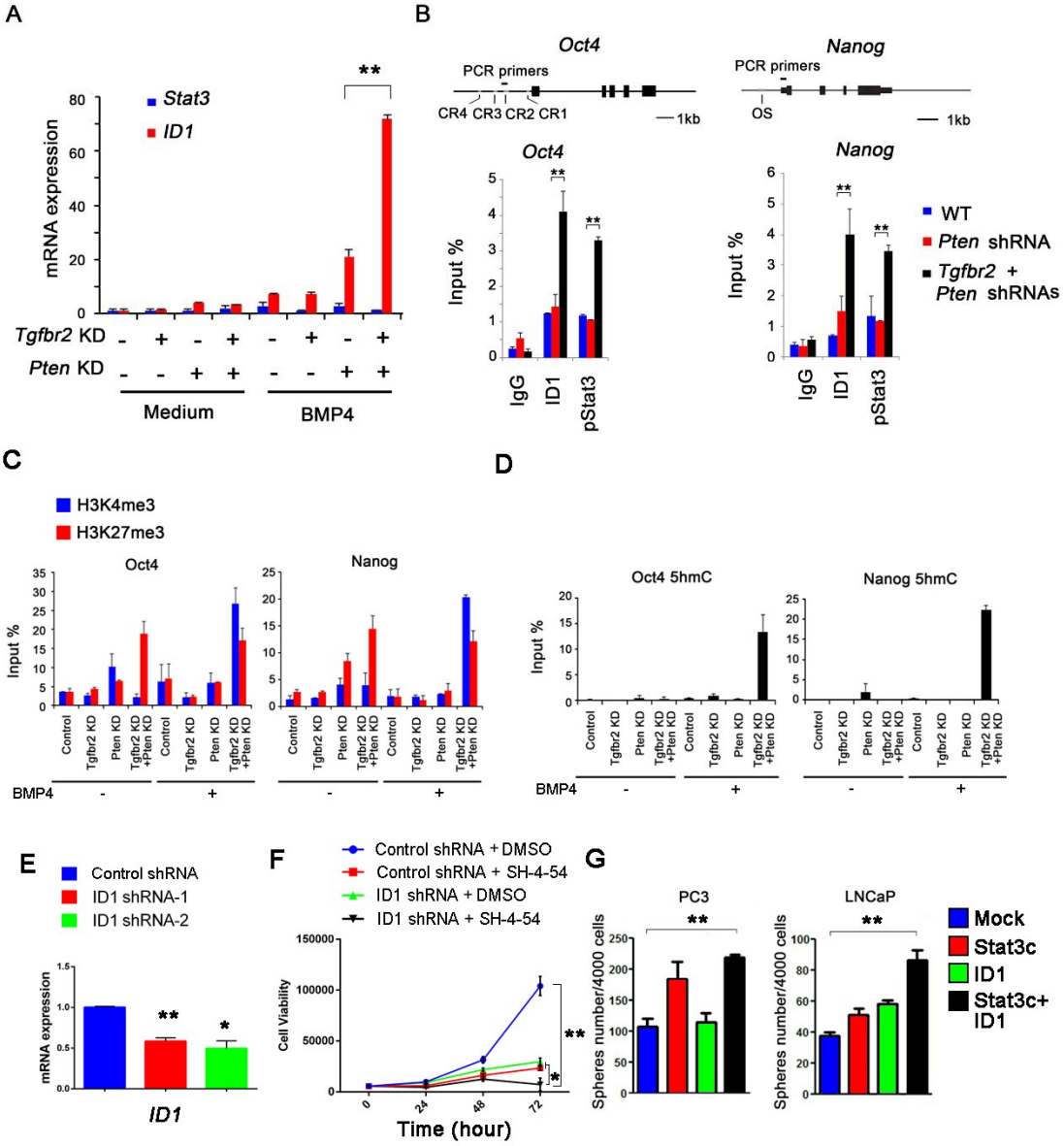


Figure S6. ID1 and Stat3 Occupy Promoter Regions of Pluripotency Genes. Related to Figure 4.

(A) TRAMP-C3 cells were transduced with the indicated shRNA for 48 hours, and subsequently treated with 50 ng/ml BMP4 for 24 hours. Real-time PCR analysis of expression of *Stat3* and *ID1* was performed.

(B) TRAMP-C3 cells were infected with control shRNA, *Pten* shRNA, or combination of *Tgfbr2* and *Pten* shRNAs for 8 days. ChIP-real-time PCR analysis of the binding of ID1 and Stat3 on *Oct4*, *Nanog*, and *Sox2* promoters in transduced prostate epithelial cells. Values are expressed as fold enrichment relative to input DNA. Black bars indicate primer locations.

(C) TRAMP-C3 cells were infected with control shRNA, *Tgfbr2* shRNA, *Pten* shRNA, or combination of *Tgfbr2* and *Pten* shRNAs for 48 hours. Next, the cells were treated with 50 ng/ml BMP4 for another 24 hours. ChIP-real-time PCR analysis of H3K4me3 and H3K27me3 at the *Oct4* and the *Nanog* promoter region of transduced TRAMP-C3 cells. Values are expressed as fold enrichment relative to input DNA.

(D) TRAMP-C3 cells were infected with control shRNA, *Tgfbr2* shRNA, *Pten* shRNA, or combination of *Tgfbr2* and *Pten* shRNAs for 48 hours. Next, the cells were treated with 50 ng/ml BMP4 for another 24 hours. ChIP-real-time PCR analysis of 5hmC at the *Oct4* and the *Nanog* promoter region of transduced TRAMP-C3 cells. Values are expressed as fold enrichment relative to input DNA.

(E) TRAMP-C3 cells were transduced with the lentivirus-mediated *ID1* shRNA for 48 h, and total RNA was extracted for RT-real-time PCR analysis of *ID1*.

(F) Growth of control Tramp-C3 cells versus *ID1* shRNA-2 KD combined with Stat3 inhibitor SH-4-54 treated Tramp-C3 cells.

(G) PC3 cells or LNCaP cells were transduced with indicated genes for 48 hours. Growth of the cells in 3D Matrigel culture. Sphere (>0.1 mm) numbers of each group.

Data are represented as mean ± SD. Student's t test, *$p<0.05$, **$p<0.01$.

Supplemental Figure 7

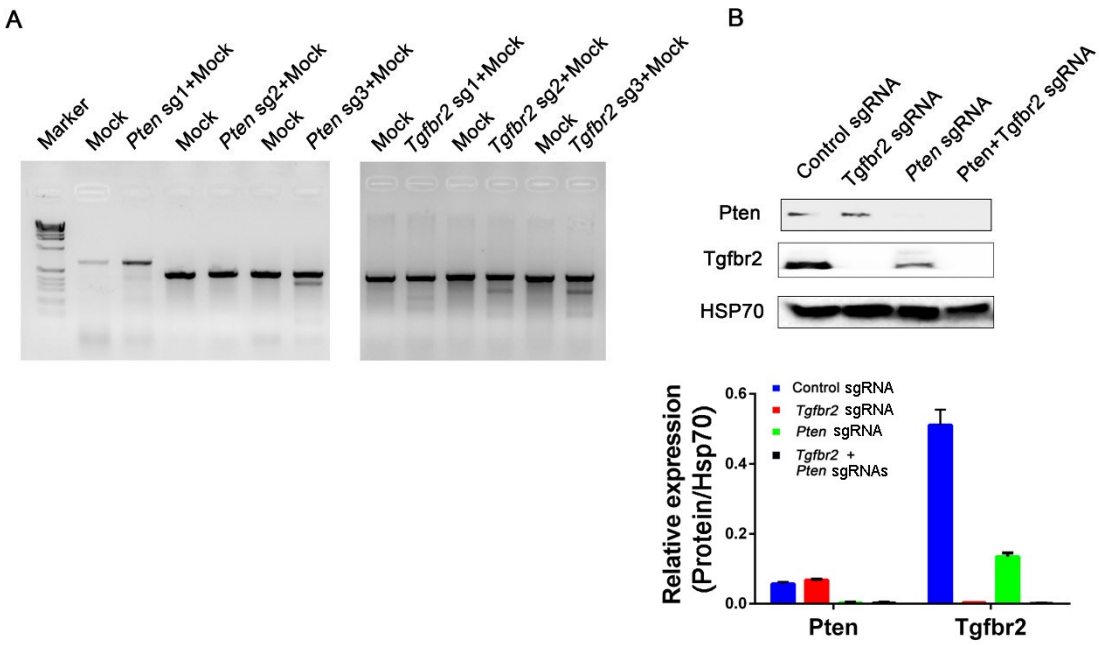


Figure S7. Prostate-specific Depletion of Tgfbr2 Promotes Expression of EMT Genes in *Pten* deficient Mice. Related to Figure 6.

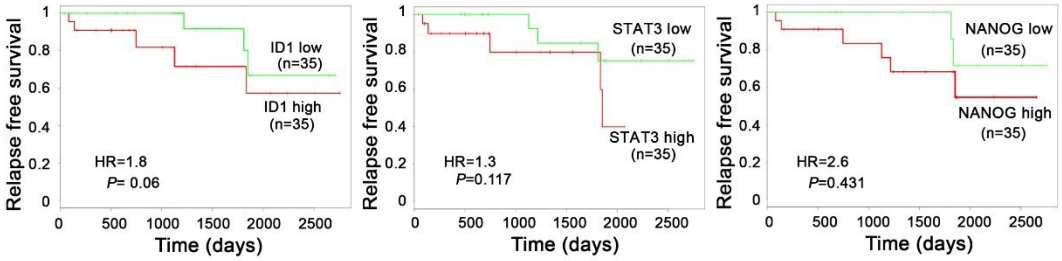
(A) Indels induced by lentivirus-delivered sgRNA targeting the *Pten* and *Tgfbr2* gene in the TRAMP-C3 cells were assayed by surveyor digestion. All the cells were treated with 1 μg/ml puromycin for 5 days after infection before assay as indicated.

(B) TRAMP-C3 cells were infected with control sgRNA, *Tgfbr2* sgRNA, *Pten* sgRNA, or combination of *Tgfbr2* and *Pten* sgRNAs for 2 days. Then, the cells were treated with 1 μg/ml puromycin for another 5 days. Western blot analysis of expression of Pten and Tgfbr2 in TRAMP-C3 cells with indicated genotypes.

Data are represented as mean ± SD. Student's t test, *$p<0.05$, **$p<0.01$.

Supplemental Figure 8

A



B

Sex	Age	Grade	Stage	Gleason Grade	Gleason Score	TNM
M	77	–	–	–	–	–
M	70	–	–	–	–	–
M	27	–	–	–	–	–
M	70	–	–	–	–	–
M	35	–	–	–	–	–
M	77	–	–	–	–	–
M	80	–	–	–	–	–
M	80	–	–	–	–	–
M	35	–	–	–	–	–
M	73	–	–	–	–	–
M	73	–	–	–	–	–
M	27	–	–	–	–	–
M	69	3	II		5	5+5 T2N0M0
M	66	3	II		5	5+5 T2N0M0
M	66	3	II		5	5+5 T2N0M0
M	69	3	II		5	5+5 T2N0M0
M	75	3	IV	4–5	5	4 T2N1M1c
M	75	3	IV	4–5	5	4 T2N1M1c
M	80	3	IV		5	5+4 T4N1M1c
M	60	2	IV		4	3+4 T4N1M1c
M	64	2	IV		4	3+4 T3N0M1
M	80	3	IV		5	5+5 T4N1M1c
M	64	2	IV		3	3+3 T3N0M1
M	60	2	IV		3	3+3 T4N1M1c

C

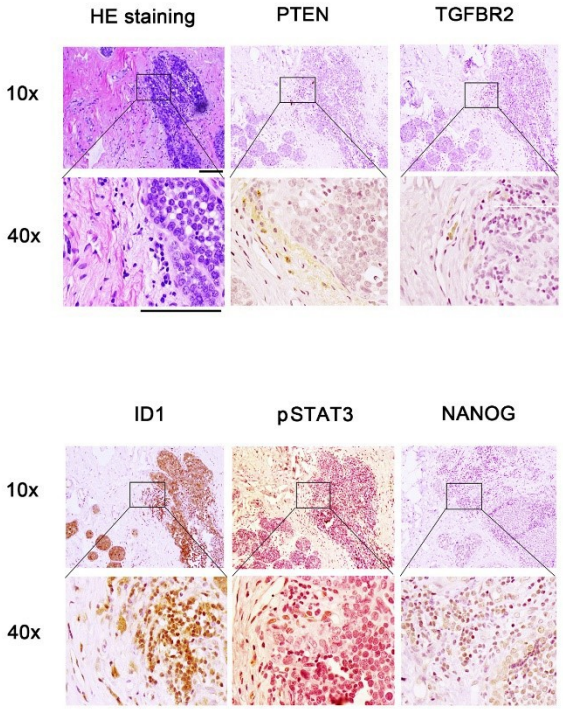


Figure S8. Prognostic Potential of a Three-gene Signature in Human Prostate Cancer. Related to Figure 7.

(A) Kaplan-Meier plots of the recurrence based on the expression of *ID1*, *STAT3*, or *NANOG* in patients. HR, Hazard ratio.

(B) Prostate cancer patient information of tissue microarray.

(C) HE staining and immunohistochemical staining with specific antibody against PTEN, TGFBR2, ID1, pSTAT3 and NANOG in a prostate cancer patient tumor sample. Scale bar, 200 μm.

Table S1. shRNA and sgRNA information.

shRNA number	Gene symbol	Open Biosystems shRNA library clone ID
1	Pten	V2LMM_52491
2	Pten	V2LMM_54320
1	Tgfbr2	V2LMM_188921
2	Tgfbr2	V3LMM_466224
1	Suv39h1	V2LMM_17223
2	Suv39h1	V3LMM_498634
1	Suv39h2	V3LMM_480915
2	Suv39h2	V3LMM_480917
1	Dnmt1	V2LMM_43170
1	Dot1L	V2LMM_193454
1	Smad4	V3LMM_515311
1	Setdb1	V3LMM_494966
2	Setdb1	V3LMM_494967
1	Tmeff1	V3LMM_516733
2	Tmeff1	V3LMM_479434
1	ID1	V3LMM_519695
2	ID1	V3LMM_519694
sgRNA number	Gene symbol	sgRNA target sequence
1	Pten	GCTAACGATCTCTTTGATGA
2	Pten	AAAGACTTGAAGGTGTATAC
3	Pten	ACAATATTGATGATGTAGTA
1	Tgfbr2	AACTTTACCCGGGCGCCGAG
2	Tgfbr2	ACTTTACCCGGGCGCCGAGA
3	Tgfbr2	GCGCGGGGGGTGTCGTCGGT

Table S2. Primers used for this study.

Real-time PCR primers

mouse BMP4-forward	GCCCTGCAGTCCTTCGCTGG
mouse BMP4-reverse	CTGACGTGCTGGCCCTGGTG
mouse Oct4 (endogenous)-forward	TCTTTCCACCAGGCCCCCGGCTC
mouse Oct4 (endogenous)-reverse	TGCGGGCGGACATGGGGAGATCC
mouse Sox2 (endogenous)-forward	GTATCAGGAGTTGTCAAGGCAGAG
mouse Sox2 (endogenous)-reverse	GTATCAGGAGTTGTCAAGGCAGAG
mouse Nanog-forward	AAGCAGAAGATGCGGACTGT
mouse Nanog-reverse	ATCTGCTGGAGGCTGAGGTA
mouse Pten-forward	TGGATTCGACTTAGACTTGACCT
mouse Pten-reverse	GCGGTGTCATAATGTCTCTCAG
mouse Tgfbr2-forward	ACGTTCCCAAGTCGGATGTG
mouse Tgfbr2-reverse	GCTGGCCATGACATCACTGT
mouse Tmeff1-forward	GGGGACACCTACCAGAATGAG
mouse Tmeff1-reverse	AGATCCAGAGCCGTTATCAGAG
mouse IL6-forward	CTGCAAGAGACTTCCATCCAG
mouse IL6-reverse	AGTGGTATAGACAGGTCTGTTGG
mouse Smad1-forward	CGTGAAGGGTTGGGGAGCCG
mouse Smad1-reverse	TCCAGCCACTGGAGAGGGCC
mouse Smad5-forward	CCAGCCGTGAAGCGATTGT

mouse Smad5-reverse	CTCCTCCATAGCACCCTTCT
mouse ID1-forward	CCTAGCTGTTCGCTGAAGGC
mouse ID1-reverse	GTAGAGCAGGACGTTCACCT
mouse Stat3-forward	GCCGACCCAGGTAGTGCTGC
mouse Stat3-reverse	GCCCTCCTGCTGAGGGCTCA
mouse Snail-forward	CACACGCTGCCTTGTGTCT
mouse Snail-reverse	GGTCAGCAAAAGCACGGTT
mouse Slug-forward	CAGCGAACTGGACACACACA
mouse Slug-reverse	ATAGGGCTGTATGCTCCCGAG
mouse Suv39h1-forward	GAGAGCTTGTCCGACGACAC
mouse Suv39h1-reverse	CTTCTGCACCAGGTAATTGGC
mouse Suv39h2-forward	GTGCCTTGCCTAGTTTCACTT
mouse Suv39h2-reverse	CCTTTGCTACCTTGTAGTCACAC
mouse Smad4-forward	ACACCAACAAGTAACGATGCC
mouse Smad4-reverse	GCAAAGGTTTCACTTTCCCCA
mouse Dnmt1-forward	GGTCAACGAGGCAGACATCA
mouse Dnmt1-reverse	TTCACCACAGCTTCCTCGTC
mouse Dot1L-forward	GAGGCTCAAGTCGCCTGTG
mouse Dot1L-reverse	GACCCACCGGATAGTCTCAAT
mouse Setdb1-forward	TGGACACCCAGGGTATGAGT

mouse Setdb1-reverse	GGTGAAGGAGGACAAGAGGC
mouse Cyclin D1-forward	GCGTACCCTGACACCAATCTC
mouse Cyclin D1-reverse	ACTTGAAGTAAGATACGGAGGGC
mouse Spp1-forward	ATCTCACCATTCGGATGAGTCT
mouse Spp1-reverse	TGTAGGGACGATTGGAGTGAAA
mouse Rex1-forward	ACGAGTGGCAGTTTCTTCTTGGGA
mouse Rex1-reverse	TATGACTCACTTCCAGGGGGCACT
mouse Cripto-forward	ATGGACGCAACTGTGAACATGATGTTCGCA
mouse Cripto-reverse	CTTTGAGGTCCTGGTCCATCACGTGACCAT
mouse BMP7-forward	CCTGTCCATCTTAGGGTTGCC
mouse BMP7-reverse	GGCCTTGTAGGGGTAGGAGA
mouse TGF-β1-forward	CCACCTGCAAGACCATCGAC
mouse TGF-β1-reverse	CTGGCGAGCCTTAGTTTGGAC
mouse TGF-β2-forward	GTCAAGGCTGAGAACGGGAA
mouse TGF-β2-reverse	AAATGAGCCCCAGCCTTCTC
mouse TGF-β3-forward	GGACTTCGGCCACATCAAGAA
mouse TGF-β3-reverse	TAGGGGACGTGGGTCATCAC
mouse BMPRIa-forward	ACAGGAGGAATCGTGGAGGA
mouse BMPRIa-reverse	GTTCCAGCGGTTAGACACGA
mouse BMPRIb-forward	CCTCGGCCCAAGATCCTAC

mouse BMPRIb-reverse	CCTAGACATCCAGAGGTGACA
mouse BMPRII-forward	GTGTTATGGTCTGTGGGAGAAAT
mouse BMPRII-reverse	AAAGCGGTACGTTCCATTCTG
mouse Gremlin1-forward	AAGCGAGATTGGTGCAAAACT
mouse Gremlin1-reverse	GAAGCGGTTGATGATAGTGCG
mouse Gremlin2-forward	TGTGCTGGTAAAGGTAGCTGA
mouse Gremlin2-reverse	CCACCTCTCTGAGTTGTTGCT
mouse Noggin-forward	GCCAGCACTATCTACACATCC
mouse Noggin-reverse	GCGTCTCGTTCAGATCCTTCTC
ChIP-qPCR primers	
mouse Sox2-pro-1-forward	GAAAAGGCTGGGAACAAGGC
mouse Sox2-pro-1-reverse	GGTTCCCAAACACGAGTCCT
mouse Sox2-pro-2-forward	AGGACTCGTGTTTGGGAACC
mouse Sox2-pro-2-reverse	TCTTCCAACCTTGTCGCCAG
mouse Sox2-pro-3-forward	CTAGTTGGACAGTCGCCCTG
mouse Sox2-pro-3-reverse	CTCCTTTCCCCCATGCTACG
mouse Oct4-pro-1-forward	CCCAATATGGGTGCTAGGAA
mouse Oct4-pro-1-reverse	GTCCACCAGCATGAAAAGGT
mouse Oct4-pro-2-forward	GGTGCGATGGGGCATCCGAG
mouse Oct4-pro-2-reverse	CCCCTGGGTGGGTGGAGGAG
mouse Oct4-pro-3-forward	GCCGTCTTTCCACCAGGCCC

mouse Oct4-pro-3-reverse	CCCACCTGGAGGCCCTTGGA
mouse Oct4-pro-4-forward	AGGGGATGGAGCCTGGGTGC
mouse Oct4-pro-4-reverse	GACCCCGAGCCCCAACCAGA
mouse Oct4-pro-5-forward	GCAGCCTTGGGTGTAGTGGTGAAG
mouse Oct4-pro-5-reverse	TGGAAAGACGGCTCACCTAGGGAC
mouse Nanog-pro-1-forward	CATTCCTTTCCCCACCCACA
mouse Nanog-pro-1-reverse	CTGGTGAAAGATCCGAGCGA
mouse Nanog-pro-2-forward	AAGCAGACTCCTTTGACCCG
mouse Nanog-pro-2-reverse	AAACTTGCCTCTGGGTCCAC
mouse Nanog-pro-3-forward	TGGACCCAGAGGCAAGTTTC
mouse Nanog-pro-3-reverse	GGAGTCCAGAGAACTGGCTG
mouse Nanog-pro-4-forward	CCGGCTTAGAGCTTGAACCA
mouse Nanog-pro-4-reverse	TCCCAAGGGCGACGTAATTT
mouse Snail-pro-1-forward	CAGGAAATCGGACCCCTCAG
mouse Snail-pro-1-reverse	GAAGAAGCACCGAGGGGAAA
mouse Slug-pro-1-forward	GCGCTACAAAGGGAGGAAGT
mouse Slug-pro-1-reverse	CTGGTGCAGACTCCGACAAT

Supplementary Materials and methods

Cell Culture

mESCs and miPSCs were cultured in mESC medium (DMEM with 15% FBS, 1 mM L-glutamine (Life Technologies), 1% nonessential amino acids (Life Technologies), 0.1 mM β-mercaptoethanol (Sigma) and 1,000 U ml−1 LIF (Santa Cruz) on irradiated feeder cells. MEFs were isolated by trypsin digestion of midgestation (E13.5) embryos followed by culture in fibroblast medium (DMEM with 10% FBS, 1 mM L-glutamine, 1% nonessential amino acids and 0.1 mM β-mercaptoethanol). TRAMP-C3 were cultured in Complete Growth medium (4mM L-glutamine, 1.5g/L sodium bicarbonate and 4.5g/L glucose supplemented with 0.005mg/ml bovine insulin and 10nm dehydroisoandrosterone, 90% with 5% fetal bovine serum and Nu-Serum IV 5%.

Lentivirus Transduction

All lentiviruses were generated as prescribed before. Viral supernatants were collected at 48-72 hour after transfection and concentrated by ultracentrifugation at 25,000 rpm for 2 h at 4 °C. The MEF or TRAMP-C3 cells were infected concentrated virus with polybrene (8 ug/ml; Sigma). Typically, more than 95% of cells were successfully transduced using this methodology based on GFP cDNA transduction.

shRNA library screening

shRNA library were purchased from Open Biosystems (GE Dharmacon). *Tet-O-OSKM* transgenic MEF cells were transduced with lentivirus-based shRNAs specific for 8 transcription

factors or epigenetic factors. Then the cells were reseeded on feeder cells at the desired density. 2 mg/ml Dox was added on day 2, and the iPSC colonies were stained for AP on days 12.

Mammosphere formation of prostate cancer cells

Single-cell suspensions were resuspended at a density of 10,000–20,000 cells/ml in ultralow attachment 6 well plate (Corning) in mammosphere culture medium (Stemcell). Cultures were changed bi-weekly and mammosphere formation was monitored weekly.

3D culture prostate epithelial cells

Viable single cells were plated in 24-well plate in 50% Matrigel (BD Biosciences) containing TRAMP-C3 medium. Cells were allowed to grow for the indicated time and fresh medium with or without cytokine was added every two days. Images were taken with a CCD camera-equipped Olympus microscope.

Alkaline Phosphatase (AP) Staining

We used Alkaline Phosphatase Detection Kit (Vector lab) to determine alkaline phosphatase activity of reprogrammed cells, according to the manufacturer's instructions.

Immunoblotting Analysis

Cells were lysed in RIPA buffer containing protease inhibitors. The supernatants were added to loading buffer and boiled for western blot as previously described.

Sox2 and Oct4 antibodies were purchased from life technologies, Nanog, Snail and Slug antibodies were from Abcam. Sma4, p-Smad2, p-Smad3, Smad1, Smad5, p-JNK, p-ERK, p-p38,

Cyclin-D1, Spp1, Stat3, p-Stat3 Y705, Bcl-xl, Bcl-2 antibodies were purchased from Cell Signaling Technology. Vimentin, SKP2, p27, ID1, HSP70 antibodies were came from Santa Cruz.

Immunohistochemistry

Tissues were formalin-fixed, processed, and paraffin-embedded. H&E staining was done by the Cancer Pathology Laboratory (Methodist Hospital). Antigens were retrieved by autoclaving in 0.01 mol/L sodium citrate buffer pH 6.0 at 121°C/20 psi for 3-5 min. The peroxidase activity was blocked in 3% H_2O_2 and nonspecific binding sites blocked in 10% Goat serum. The blocked sections were incubated overnight at 4°C with primary antibody (1% bovine serum albumin [BSA] in phosphate buffer saline with tween 20 [PBST]) followed by incubation with secondary antibody for 1 h. The slides were stained with diaminobenzidine for 2 min, counterstained with hematoxylin and mounted with Immuno-mount (Thermo Scientific).

ChIP Assay

ChIP assay was performed according to the Imprint Ultra Chromatin Immunoprecipitation Kit manual (Sigma). Briefly, cells were grown to final count of 1×10^7 cells for each reaction. Cells were cross-linked with 0.75% formaldehyde solution for 10 min at room temperature with gentle agitation and quenched with 0.125M glycine for 5 min. Cells were rinsed twice with cold PBS, lysed, and sonicated to solubilize and shear cross-linked DNA. The resulting chromatin extract was incubated overnight at 4°C with 5 g antibody. Next day, each sample was added 20 l blocked beads and then incubated at 4°C for 1 h. Beads were washed 5 times with RIPA buffer, once with TE containing 50 mM NaCl and complexes were eluted from beads in elution buffer. Reverse crosslinking was performed overnight at 65°C. Input DNA (reserved from sonication) was concurrently treated for crosslink reversal. DNA were treated with RNaseA, proteinase K

and purified. Primary antibodies used for IP were: ID1 (Santa Cruz), pStat3 Y705 (Cell signaling technology) 5hmC (Abcam), H3K4me3 (Abcam), H3K27me3 (Abcam), and mouse/rabbit IgG (Sigma). Relative Fold enrichments were calculated by determining the immunoprecipitation efficiency (ratios of the amount of immunoprecipitated DNA to that of the input sample).

Real-Time Quantitative PCR (qPCR)

cDNA was generated from the total RNA of cells with SuperScript III Reverse Transcriptase (Life Technologies), using oligo (dT) as a primer. Gene transcripts were quantified by real-time PCR with SYBR Green real-time PCR SuperMix (Life Technologies) in a QuantStudio 6 Flex Real-time PCR system. All of the values of the target gene expression level were normalized to 18s RNA.

Xenograft

All animal procedures listed in this article were performed as per the protocol approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine and Houston Methodist Research Institute. Lentivirus transduced PC3 cells were grown to confluence in 250-ml flasks. Cells were resuspended in PBS to a concentration of $2x10^6$/ml. Cell suspension (50 l with 50ul Matrigel) was injected subcutaneously in 8-week-old NOD/SCID-γ mice. Tumor sizes were measured from days 26. Mice were sacrificed on day 34.