Supporting Information

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SI Materials and Methods

Subcloning. Full-length mouse *Hmga2* (BC052158), *Dkk1* (BC050189) or *Sfrp2* (BC014722), complete coding sequence was PCR amplified with Pfu DNA polymerase (Stratagene) from the related mouse mammalian gene collection (MGC) verified or IMAGE cDNA library clones (Open Biosystems). The PCR products were purified and subcloned into FUCRW or FUCGW lentiviral vectors (1, 2), which carry an *mRFP* or *GFP* reporter gene, respectively. To generate the inducible Hmga2 (High-mobility group AT-hook 2) lentiviral vector, an intermediate plasmid (TRET-CRW) was generated using PacI-XbaI sites by replacing the human ubiquitin-C promoter in FUCRW vector with a TRE-Tight promoter fragment from pLTRET-Luc vector (3), then mouse *Hmga2* full-length complete coding sequence was inserted immediately downstream of the TRE-tight promoter. Cre lentiviral vector has been described previously (4) and modified to link with a GFP marker. The androgen receptor (AR)/GFP lentiviral vector has been described previously (1).

Fluorescent Activated Cell Sorting. Dissociated prostate cells were suspended in DMEM with 10% FBS and incubated with phycoerythrin-conjugated anti-CD49f, allophycocyanin-conjugated anti-mouse Sca-1 and FITC-conjugated lineage antibodies mixture consisting anti-mouse CD31, CD45, and Ter119 monoclonal antibodies (eBioscience) at 4 °C for 30 min. After washing, FACS analysis was carried out on an Aria cell sorter (Becton Dickinson) as described previously (5, 6).

Surgical Castration and Androgen Supplementation. Experimental mice underwent surgical castration using standard techniques. Eight days after castration, one testosterone pellet (12.5 mg per pellet; Innovative Research of America) was implanted s.c. into some castrated mice.

Histones Extraction and Immunocytofluorescent Assay. Sulfuric acid extraction of histones was performed as described previously (7). Ten to twelve micrograms of histones solution were separated by SDS/PAGE, followed by immunoblotting analysis using anti-histone H3 and acetyl-histone H3K9/K14 (Cell Signaling Technology). For immunocytofluorescent assay, infected urogenital sinus mesenchymal (UGSM) cells were cultured in Lab-Tek chamber slides (Thermo Scientific Nalgene) overnight and fixed in 4% paraformaldehyde in PBS for 15 min, then permeabilized with 0.2% Triton X-100 in PBS for 10 min. After blocking with 10% normal goat serum, slides were incubated at 4 °C overnight with acetyl-histone H3K9/K14 antibody, and stained with Alexa 488-conjugated anti-rabbit (Molecular Probes) at room temperature for 1 h. The signals were visualized under fluorescent microscopy after counterstaining with DAPI.

Luciferase Reporter Assay. The 293T cells were seeded into 24-well plates and cultured overnight. For each well, 0.1 μg of the TOPFlash or FOPFlash luciferase construct was cotransfected with 0.06 μg of Wnt3a-expressing plasmid, 0.1 μg of Dkk1, Sfrp2-expressing vector, or FUCRW control vector using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. A Renilla luciferase plasmid was also included as an internal control for transfection efficiency. Cells were lysed at 45–48 h after transfection. Both Firefly and Renilla luciferase activities were measured using a Dual-Luciferase reporter assay kit (Promega) in an LMax II384 luminometer (Molecular Devices). Each sample was tested in duplicate.

1. Xin L, et al. (2006) Progression of prostate cancer by synergy of AKT with genotropic and nongenotropic actions of the androgen receptor. *Proc Natl Acad Sci USA* 103(20):7789–7794.
2. Zong Y, et al. (2009) ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. *Proc Natl Acad Sci USA* 106(30):12465–12470.
3. Huang X, et al. (2011) Regulated expression of microRNAs-126/126* inhibits erythropoiesis from human embryonic stem cells. *Blood* 117(7):2157–2165.
4. Memarzadeh S, et al. (2010) Cell-autonomous activation of the PI3-kinase pathway initiates endometrial cancer from adult uterine epithelium. *Proc Natl Acad Sci USA* 107(40):17298–17303.
5. Lawson DA, et al. (2010) Basal epithelial stem cells are efficient targets for prostate cancer initiation. *Proc Natl Acad Sci USA* 107(6):2610–2615.
6. Lukacs RU, Goldstein AS, Lawson DA, Cheng D, Witte ON (2010) Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat Protoc* 5(4):702–713.
7. Shechter D, Dormann HL, Allis CD, Hake SB (2007) Extraction, purification and analysis of histones. *Nat Protoc* 2(6):1445–1457.

Fig. S1. Expression pattern of endogenous Hmga2 in adult murine prostate. (*A*) Quantitative PCR analysis of Hmga2 transcripts in different FACS-sorted subpopulations of adult murine prostate. (*B*) Immunohistochemistry (IHC) analysis of wild-type murine prostate at 5 mo of age with antibodies against AR, p63, and Hmga2. Arrow, sporadically distributed Hmga2 positive cells. (Scale bars, 50 μm.) (*C* and *D*) Surgical castration resulted in remarkable prostate involution and significant up-regulation of Hmga2 mRNA expression in prostatic stromal cells sorted from adult mice.

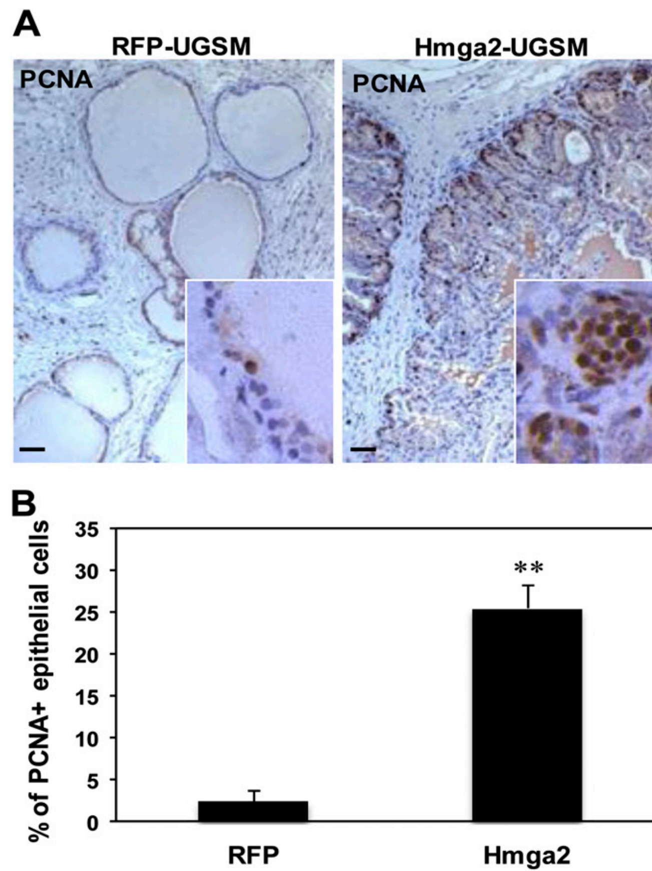
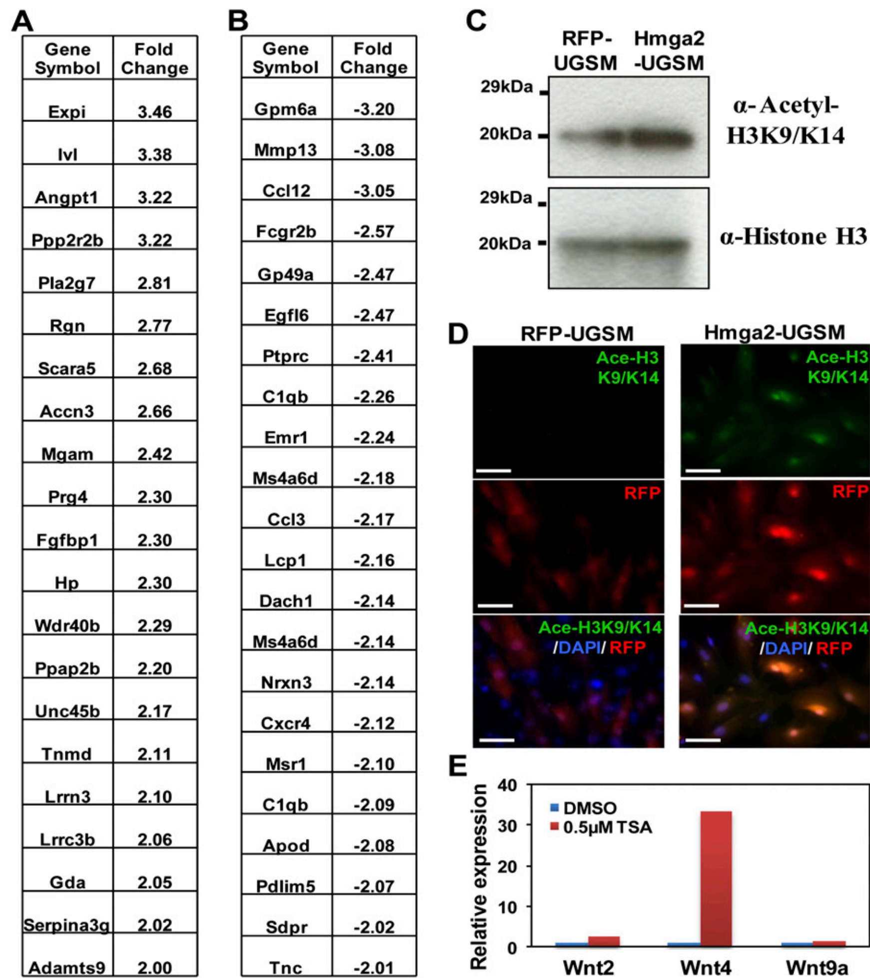


Fig. S2. Remarkably increased cell proliferation index in prostatic intraepithelial neoplasia (PIN) lesions induced by Hmag2-modified stroma. (*A*) IHC analysis of prostate grafts with anti-proliferating cell nuclear antigen (PCNA) antibody. (Scale bars, 50 μm.) (*B*) Percentage of PCNA-positive epithelial cells in regenerated prostate tissue recombinants. Data are presented as mean ± SD. $**P$ value < 0.01.

Fig. S3. Androgen ablation by surgical castration remarkably inhibits the growth of Hmga2-UGSM induced PIN lesions. (*A*–*C*) Gross appearance, growth curve, and weight measurement of Hmga2-UGSM s.c. grafts from intact and castrated recipients. Data are presented as mean ± SD. $^{**}P$ value < 0.01. (*D*) Histological and IHC analyses of Hmga2-UGSM grafts from intact and castrated recipients. (Scale bars, 100 μm.)

Fig. S4. Short-range growth-promoting effects of Hmga2-UGSM cells. (*A*) Photographs and histological sections (H&E staining) of RFP-UGSM graft and Hmga2-UGSM graft grown on the same kidney. (Scale bars, 100 μm.) (*B*) Representative transilluminated (TI), fluorescent (GFP) photographs, and H&E staining of fusion grafts consisting of RFP-UGSM combined with GFP-marked prostate epithelial cells as well as Hmga2-UGSM cells combined with wild-type prostate epithelial cells in each bipartition. (Scale bars, 100 μm.)



A

Gene Symbol	Fold Change
Expi	3.46
Ivl	3.38
Angpt1	3.22
Ppp2r2b	3.22
Pla2g7	2.81
Rgn	2.77
Scara5	2.68
Accn3	2.66
Mgam	2.42
Prg4	2.30
Fgfbp1	2.30
Hp	2.30
Wdr40b	2.29
Ppap2b	2.20
Unc45b	2.17
Tnmd	2.11
Lrrn3	2.10
Lrrc3b	2.06
Gda	2.05
Serpina3g	2.02
Adamts9	2.00

B

Gene Symbol	Fold Change
Gpm6a	-3.20
Mmp13	-3.08
Ccl12	-3.05
Fcgr2b	-2.57
Gp49a	-2.47
Egfl6	-2.47
Ptprc	-2.41
C1qb	-2.26
Emr1	-2.24
Ms4a6d	-2.18
Ccl3	-2.17
Lcp1	-2.16
Dach1	-2.14
Ms4a6d	-2.14
Nrxn3	-2.14
Cxcr4	-2.12
Msr1	-2.10
C1qb	-2.09
Apod	-2.08
Pdlim5	-2.07
Sdpr	-2.02
Tnc	-2.01

Fig. S5. Increased histone acetylation in Hmga2-expressing UGSM cells. (*A*) List of genes with more than twofold up-regulation by Hmga2 overexpression in UGSM cells. (*B*) List of genes with more than twofold down-regulation by Hmga2 overexpression in UGSM cells. (*C*) Immunoblotting of histones extracted from indicated UGSM cells with anti-acetyl-histone H3K9/K14 and histone H3. (*D*) Immunocytofluorescent analysis of in vitro cultured UGSM cells with anti-acetyl-histone H3K9/K14, with corresponding RFP fluorescent and merged images after DAPI counterstaining. (Scale bars, 100 μm.) (*E*) Quantitative PCR assay to compare the expression of Wnt transcripts in trichostatin A (TSA)-treated UGSM cells and the control cells exposed to DMSO for 24 h.

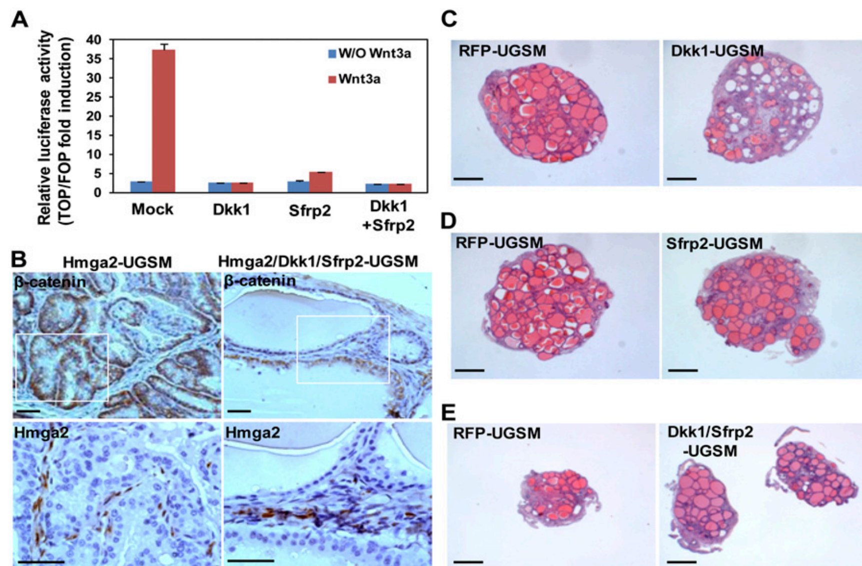


Fig. S6. Normal prostate tubule formation from dissociated adult prostate cells in the presence of high levels of Dkk1 and Sfrp2 in the stroma. (*A*) Functional validation of Dkk1 and Sfrp2 lentiviral vectors by luciferase reporter assay in 293T cells. The results are expressed as TOPFlash over FOPFlash firefly luciferase activity after normalization with Renilla control. Data are presented as mean ± SD. (*B*) β-Catenin and Hmga2 staining of prostate grafts derived from UGSM cells coexpressing Hmga2, Dkk1, and Sfrp2. (Scale bars, 50 μm.) (*C*–*E*) Representative histological sections of prostate grafts derived from various UGSM cells as indicated. (Scale bars, 1 mm.)

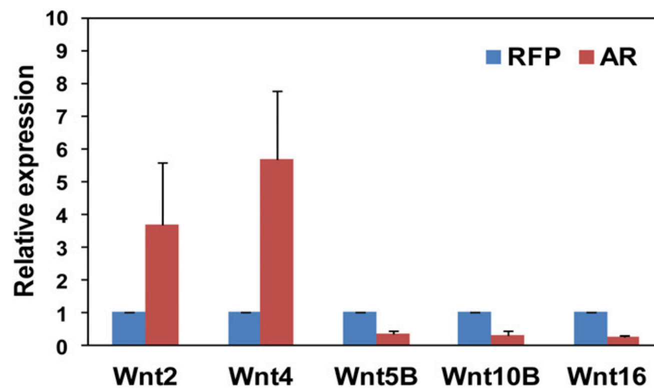


Fig. S7. AR overexpression causes modest changes in Wnt expression in mouse UGSM cells. Quantitative PCR analyses of Wnt ligands differentially expressed between AR-UGSM cells and RFP-UGSM control cells. Data are presented as mean ± SD after normalization with β-actin.

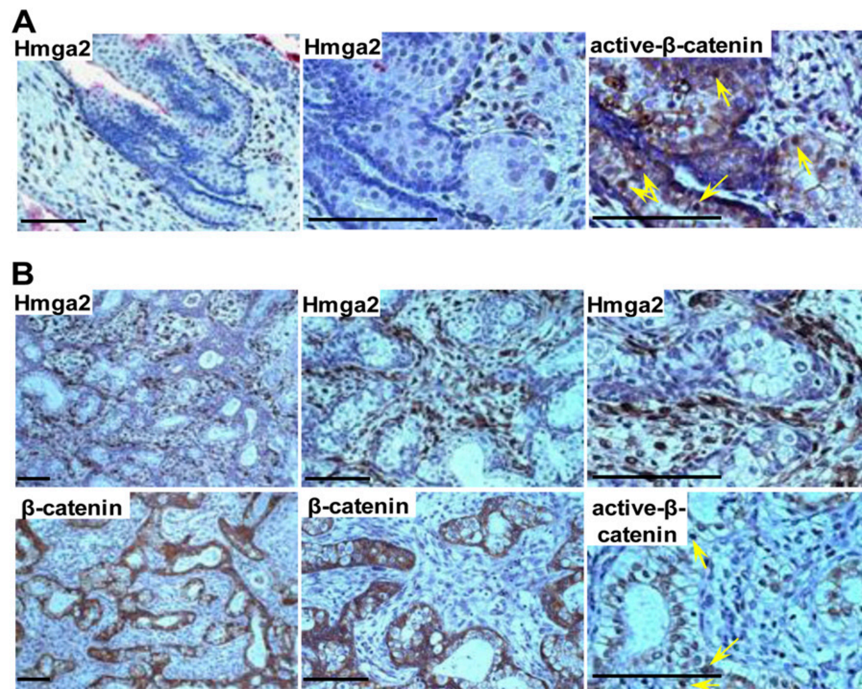


Fig. S8. Stromal-specific overexpression of HMGA2 in human biphasic tumors of the prostate. HMGA2 and β-catenin staining of human prostate tumor tissues of (*A*) stromal tumor of uncertain malignant potential and (*B*) phyllodes tumor of the prostate with exuberant epithelial hyperplasia. Arrows indicate nuclear staining of active-β-catenin in the epithelial compartment. (Scale bars, 100 μm.)

Table S1. Oligos for real-time PCR used in this study

Gene	PCR primer sequence
Wnt1	FWD: TTCCTCATGAACCTTCACAACA
	REV: GCGATTTCTCGAAGTAGACGAG
Wnt2	FWD: CGGGTCCTCCTCCGAAGTAG
	REV: TGGATCACAGGAGCAGGACTTT
Wnt2b	FWD: GCCAAAGAGAAGAGGCTTAA
	REV: TCAGTCCGGGTGGCGTGGCG
Wnt3	FWD: TGGGCCTGTCTTGGACAAA
	REV: GCGATGGCATGCACGAA
Wnt3a	FWD: GCACCACCGTCAGCAACAG
	REV: GGGTGGCTTTGTCCAGAACA
Wnt4	FWD: TGGACTCCCTCCCTGTCTTTG
	REV: CAGCCACACTTCTCCAGTTCTC
Wnt5a	FWD: GGTGCCATGTCTTCCAAGTT
	REV: TGAGAAAGTCCTGCCAGTTG
Wnt5b	FWD: GGCATTGGGATGGGTTGAGG
	REV: GGAGTTGGCGTCAGTCAGCAG
Wnt6	FWD: TGCCCGAGGCGCAAGACTG
	REV: ATTGCAAACACGAAAGCTGTCTCTC
Wnt7a	FWD: GATGCCCGGGAGATCAAGCAGA
	REV: GAGCCTGACACACCATGGCACT
Wnt7b	FWD: TGAGGCGGGCAGAAAGG
	REV: CCTGACACACCGTGACACTTACA
Wnt8a	FWD: GCAGCGACAACGTGGAGTTC
	REV: GCTTCCTGAGATGCCATGAC
Wnt8b	FWD: TTGGGACCGTTGGAATTGCC
	REV: AGTCATCACAGCCACAGTTGTC
Wnt9a	FWD: GCAGCAAGTTTGTCAAGGAGTTCC
	REV: GCAGGAGCCAGACACACCATG
Wnt9b	FWD: AAGTACAGCACCAAGTTCCTCAGC
	REV: GAACAGCACAGGAGCCTGACAC
Wnt10a	FWD: TTCTGGGCGCTCCTGTTCTTCC
	REV: CAGGCACACTGTGTTGGCGTTG
Wnt10b	FWD: GCACCACAGCGCCATCCTCA
	REV: TTGCTCACCACTACCCTTCCATCC
Wnt11	FWD: AACAGTGAAGTGGGGAGACAG
	REV: GTAGCGGGTCTTGAGGTCAG
Wnt16	FWD: AGTAGCGGCACCAAGGAGAC
	REV: GAAACTTTCTGCTGAACCACATGC
β-actin	FWD: GATCTGGCACCACACCTTCT
	REV: GGGGTGTTGAAGGTCTCAAA