Supplementary Information

Supplementary materials and methods

DNA isolation and genomic PCR

DNA was isolated using the Qiagen AllPrep DNA/RNA Micro Kit (Valencia, CA, USA). Primer sequences are as follows: *Pb-Cre* F, CGTATAGCCGAAATTGCCAG, R, CAAAACAGG TAGTTATTCGG; *Pten* F, TCCCAGAGTTCATACCAGGA, R1, GCAATGGCCAGTACTAGTGAAC, R2, AATCTGTGCATGAAGGGAAC; *Kras* F, GTCTTTCCCCAGCACAGTGC, R1, CTCTTGCCCTACGCCACCAGCTC, R2, AGCTAGCCACCATGGCTTGAGTAAGTCTGC.

Real time PCR

Total RNA from *PKV* cells or *CPKV* prostates, as well as epithelial, EMT, and MES-like tumor cell populations sorted from *PKV* cells or *CPKV* prostates, was isolated using the Qiagen AllPrep DNA/RNA Micro Kit and reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit with Multiscribe Reverse Transcriptase (Applied Biosystems, Carlsbad, CA, USA). Transcript levels were assessed with quantitative real-time PCR with mouse gene specific RT-PCR primers and iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) using the CFX Real-Time PCR detection System (Bio-Rad) and normalized against β-actin expression. The relative expression levels were
derived from the delta-delta Ct values using the CFX software (Bio-Rad) and compared to expression levels from epithelial tumor cells. Primer sequences are as follows: 

- **B-Actin** F, GGCTGTATTCCCCTCCATCG, R, CCAGTTGGTAACAATGCCATGT; **Hmg2** F, GTACCGGTAGAGGCGAGTGG, R, GGGTCTTCTCTGGGTTCTCT; **Vim** F, CCGCTGAGAGAAATTGC, R, CCACATTCCGTAAGTCAAG; **Snai1** F, AAGATGCACATCCAAGGC, R, ATCTCTTCACACCGAGTGG; **Zeb1** F, CATGTGACCTGTGTGACAAG, R, GCGGTGATTCATGTGTTG; **Cdh2** F, CAGGTCTCTCTCATGGCTTTGC, R, CTTCCGAAAGAGGCTGTCC; **Mmp2** F, CACCTACACCAAGAACTTCC, R, GAACACAGCCTTCTCCTCCT; **Cdhl1** F, AATGGCGGCAATGCAATCCCAAGA, R, TGCCACAGACCGATTGGAGAGATA; **Oct4** F, CACGAGTGAAAGCAACTCA, R, CCAAGGTGATCCTCTTCTGC; **Sox2** F, AAGAAAGGAGAAGGTGGAGGC, R, GAGATCTGGGCGGGAATAGTTGG; **Sox9** F, GACAAGCGGAGGCGGAA, R, CCAGCTTGCACGTGGTT; **Klf4** F, GTGCCCGACTAACCGGTG, R, GTCGTGAACTCTCGGTCT; **Nestin** F, AGCAGGAGAGCAGGTCTA, R, CTGGGAACCTCTTGAGGTG; **Bmi1** F, AATCCCCACCTGTGTGT, R, GCTGGTCTCAGTTACGGA; **Ezh2** F, ATCTGGTCAGTGGTATT, R, CAGGGAGCGCAGTGGATT; **Tmprss2** F, TACGGGAAAGTGGGCTGTT, R, CAGGGGACAGTCAACCAA; **Nkx3-1** F, CCACCAAGTATCCGTCAG, R, CTACCAGAAGATGGATGCC; **Fkbp5** F, AGATCTCCATGTGCGAGG, R, CTTCCTCCATGCGCTGACT; **Slc45a3** F,
TGGTGGTAGTGACAGCCTCA, R, AGAGAGCTCCTGGCTTAGGG; Mdm2 F, AGGTCCCTGTCTTTTGATCC, R, ATCCTGATCCAGGCAATCAC

**Tissue dissociation and single cell suspension**

Single cell suspensions were prepared from prostates of CPKV mice at the indicated time points. Prostates were minced in sterile tissue culture dishes, and subjected to collagenase I (1 mg/ml; Invitrogen) digestion overnight at 37°C with constant agitation in Dulbecco's modified eagle medium (DMEM) (Sigma-Aldrich) containing 1% Pen/Strep, 10% Fetal Bovine Serum (FBS) (Omega Scientific), 4 mM L-glutamine (Fisher Scientific, Pittsburgh, PA, USA), 10 mM Hepes, 25 µg/mL bovine pituitary extract, 5 µg/mL insulin (Invitrogen), 6 ng/mL recombinant human EGF (BD Biosciences), and 10 µM Rocki (Abcam, Cambridge, MA, USA). Undigested tissue was trypsinized for 5 minutes at 37°C, passed through a 18G and 21G syringe 5 times, and filtered through a 40 µm filter (Fischer Scientific) to facilitate dissociation, followed by washes in PBS and resuspension in DMEM media plus 10% FBS. Total cell numbers were assessed using trypan blue exclusion.

**FACS analysis and cell sorting**

Single-cell suspensions were stained with the following directly conjugated antibodies: CD45-PE (eBioscience, San Diego, CA, USA; 12-0451-81), CD31-PE (Biolegend, San Diego, CA, USA; 102407), Ter119-PE (Biolegend; 116207), and
EpCAM-APC-cy7 (Biolegend; 118218). 7-AAD (BD Biosciences) was used to gate out dead or apoptotic cells.

Flow cytometric analysis was performed on a FACS Canto II (BD Biosciences) and data were analyzed using BD FACS Diva software (BD Biosciences). For isolation and analysis of epithelial, EMT, and MES-like tumor cells from the prostate and blood of CPKV mice, single-cell suspensions were stained with directly conjugated antibodies against CD45, CD31, Ter119, and EpCAM, and sorted on a FACS Aria (BD Biosciences) as 7AAD<sup>−</sup>CD45<sup>−</sup>CD31<sup>−</sup>Ter119<sup>−</sup>EpCAM<sup>+</sup>GFP<sup>−</sup>, 7AAD<sup>−</sup>CD45<sup>−</sup>CD31<sup>−</sup>Ter119<sup>−</sup>EpCAM<sup>−</sup>GFP<sup>+</sup>, and 7AAD<sup>−</sup>CD45<sup>−</sup>CD31<sup>−</sup>Ter119<sup>−</sup>EpCAM<sup>−</sup>GFP<sup>−</sup> cell fractions, respectively. Cells were collected in DMEM plus 50% FBS. Epithelial, EMT, and MES-like tumor cell populations within the PKV cell line were analyzed as 7AAD<sup>−</sup>EpCAM<sup>−</sup>GFP<sup>−</sup>, 7AAD<sup>−</sup>EpCAM<sup>−</sup>GFP<sup>+</sup>, and 7AAD<sup>−</sup>EpCAM<sup>−</sup>GFP<sup>−</sup> cell fractions, respectively.

**Histology and immunohistochemistry**

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissues. Antigen retrieval was performed by boiling sections in 10 mM citrate buffer (pH6) for 30 minutes. The following primary antibodies were used: HMGA2 (Biocheck, Foster City, CA, USA; 59170AP), p53 (Vector Laboratories, Burlingame, CA, USA; VP-P956), Ki67 (Vector Laboratories; VP-RM04), H3K27Ac (Abcam; ab4729), GFP (Cell Signaling, Danvers, MA, USA; 2955), Pan-Cytokeratin (Sigma-Aldrich; C1801), and AR (Santa Cruz, Santa Cruz, CA, USA; SC-816).
**Ki67 proliferation index**

The Ki67 proliferation index was calculated by quantifying the percentage of Ki67+ cells in 10 fields for each epithelial and stromal region at 40x magnification for every sample.

**Western analysis and immunoprecipitation**

Protein extracts were prepared by lysing cells in RIPA buffer (Cell Signaling) containing phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich), 1 mM PMSF, and complete protease inhibitor cocktails (Roche, San Francisco, CA, USA), followed by brief sonication and 10 minute centrifugation at 20 000 x g at 4°C. 30-50 µg of cleared lysates were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with the following antibodies: β-actin (Sigma-Aldrich; A5441), HMG2 (Abcam; ab52039), H3K27Ac (Abcam; ab4729), p53 (CalBiochem, San Diego, CA, USA; OPO3), AR (Santa Cruz; SC-816), and Acetyl Lysine (Ac-Lys) (Millipore, Billerica, MA, USA; 05-515). Anti-rabbit and anti-mouse HRP-conjugated secondary antibodies and ECL Plus Western Blotting Detection Reagents were used to detect the protein signals (GE Healthcare Amersham, Piscataway, NJ, USA). Band densities were quantified using a ChemiDoc XRS+ imager and Image Lab software (Bio-Rad).

To assess AR and p53 acetylation levels, 50 µg of protein was precleared using Protein A/G Plus agarose beads (Santa Cruz; SC-2003) and incubated
overnight with AR or p53 antibodies. Samples were washed three times with RIPA buffer, and proteins were eluted by boiling in Laemmli buffer containing β-mercaptoethanol followed by Western blot analysis using an Acetyl Lysine primary antibody.

**Matrigel sphere assay**

The Matrigel sphere assays were carried out as previously described. 5 X 10^3 cells from each cell line were plated in triplicate per experimental condition.

**Matrigel invasion assay**

8µm transwell inserts (BD Biosciences) were coated with Matrigel (BD Biosciences) at a concentration of 300 µg/ml and placed into 24-well culture plates. 5 X 10^4 sorted cells per population were resuspended in serum free media in the top chamber, while full serum media (Dulbecco’s modified eagle medium (DMEM) with 10% Fetal Bovine Serum (FBS)) was used in the bottom chamber. 24 hours later, invaded cells were fixed with methanol, stained with 0.2% crystal violet, and counted using a light microscope at 10x magnification.

**Proliferation assay**

Cell proliferation was assessed by measuring the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Life Technologies) to
formazan. Cells were seeded in 24-well dishes 24 hours before addition of MTT reagent. MTT reagent was added as specified in the manufacturer's protocol and absorbance was read using a Bio-Rad Benchmark microplate spectrophotometer.

**CTC isolation**

For isolation of CTCs by FACS, 100 µl of peripheral blood was extracted from mice through retro-orbital bleeding and incubated in RBC lysis buffer (Biolegend) for 10 minutes. Cells were then washed with PBS, passaged through a 40 µm filter (Fisher Scientific), and resuspended in DMEM media plus 10% FBS prior to FACS analysis.

**Surgical castration**

Castration of 6-week old CPKV mice was performed as previously described.²

**RNA sequencing and library construction**

RNA was extracted from pooled epithelial, EMT and MES-like tumor cells isolated from the prostates of 10-12 week old CPKV mice (n=17) and separated into 2 technical replicates per sample. Paired-end sequencing data with read lengths of 100 bp were generated by the UCLA Clinical Microarray Core using the Illumina HiSeq2000 system (San Diego, CA, USA). An average of 75.5 million read pairs per replicate were generated. Total RNA was extracted using the Qiagen AllPrep DNA/RNA Micro Kit. The NEBNext Poly(A) mRNA kit
(Ipswich, MA, USA) was then used to isolate mRNA, and the KAPA stranded RNA-Seq library preparation kit (Wilmington, MA, USA) for library preparation. Reads were mapped to the reference genome mm9 by TopHat (v.2.0.4), allowing 2 mismatches per seed. The differentially expressed genes were calculated using Cuffdiff (v2.2.0), with a false discovery rate less than 0.01.

**Laser capture microdissection (LCM) and microarray analysis**

LCM and downstream microarray analysis was carried out as previously described. Briefly, well-differentiated (epithelial) (n=3) and poorly differentiated (EMT) (n=3) tumor regions were isolated from the dorsolateral and anterior lobes of a prostate from a 15-week old CPK mouse, and gene expression values were compared to age-matched WT prostates (n=3) following microarray analysis. H&E stained frozen sections were used to confirm pathological lesions and define well-differentiated tumor regions as cancerous morphology confined to epithelial glandular structures and poorly differentiated tumor regions as EMT regions in the stromal compartment.

**Unsupervised hierarchical clustering and gene ontology pathway analysis**

Unsupervised hierarchical clustering was carried out on both genes and samples. Complete approach was used to stratify genes and samples based on the Euclidean distance. The Gene Ontology analysis was conducted with DAVID GO, in which Fisher’s exact test was used to assess enrichment of gene sets in
differentially expressed genes. Fisher’s exact test was also used to assess enrichment of HMGA2-regulated genes using genes in the dataset derived from the study of.\textsuperscript{7} P-values were adjusted for multiple tests by using the Benjamini-Hochberg procedure\textsuperscript{8} to obtain FDR.

**Rank-rank analysis**

Rank–rank hypergeometric overlap analysis (RRHO) as carried out as previously described\textsuperscript{9} using genes in human datasets derived from the studies of Taylor \textit{et al}.\textsuperscript{10} and Grasso \textit{et al}.\textsuperscript{11} The ranks of genes are ordered by the signed log\textsubscript{2} p-values, where the sign is determined by whether a gene is upregulated or downregulated.
Supplementary Figure Legends

Supplementary Figure 1. EMT and mesenchymal-like tumor cells isolated from the PKV cell line have undergone Cre recombination and display epithelial-mesenchymal plasticity. (a) Genomic PCR confirms that Cre recombination occurred in all cell populations isolated from PKV cells. Δ5, deletion of exon 5 of Pten; -, Cre" control; +, Cre" control. (b) Brightfield (BF) and fluorescent images (GFP) of FACS isolated epithelial, EMT, and MES-like tumor cells 24 hrs (Left Panel) and 14 days (Right Panel) after being plated separately in culture. Scale bar, 50 µm.

Supplementary Figure 2. HMG2 knockdown inhibits epithelial-mesenchymal plasticity. (a) FACS sorted epithelial and EMT tumor cell populations from PKV-shHmga2 cells plated separately in culture have an increased percentage of epithelial tumor cells and a decreased percentage of MES-like tumor cells compared to control PKV-shScramble (Scramble) cells after 7 days in culture. Data were pooled from 2 independent experiments done in triplicate. (b) PKV-shScramble and PKV-shHmga2 cells were treated with vehicle alone (DMSO), PKI-587 (100nM), or PD0325901 (100nM) for 7 days. After 7 days, the relative percent growth of each tumor cell population in PKV-shHmga2 as compared to control PKV-shScramble cells following each treatment was determined. % growth is relative to PKV-shScramble cells given the same
treatment. Data in a and b are represented as mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001.

Supplementary Figure 3. Differential expression of epigenetic regulators during early and late stages of EMT. (a) A large number of epigenetic regulatory genes from Gu et al.\textsuperscript{12} are differentially expressed in MES-like (M) tumor cells as compared to EMT tumor cells (See Table S2). (b) p53 and Mdm2 mRNA expression levels are relatively unaltered in PKV cells after LBH589 treatment (24 hr). Expression levels are relative to expression levels found in vehicle-treated PKV cells. Data in b is represented as mean ± SEM from 2 independent experiments done in triplicate.

Supplementary Figure 4. Effects of LBH589 treatment in CPKV mice. (a) H&E stained CPKV prostate sections reveal empty cysts in the anterior lobes (Top Panel) and degenerated glandular structures in the dorsolateral lobes (Bottom Panel) in LBH589-treated mice. Scale bar, top panel, 500 µm; Scale bar, bottom panel, 100 µm. (b) LBH589 treatment induces increased levels of H3K27 acetylation in both the epithelium and stroma of CPKV mice compared to vehicle alone, validating that LBH589 is able to modulate its molecular target in vivo. Scale bar, 25 µm. (c) Peripheral blood was collected from LBH589 (n=9) and vehicle-treated (n=4) CPKV mice after 2 weeks of treatment. FACS analysis of Lineage depleted (Lin\textsuperscript−) EpCAM\textsuperscript+GFP\textsuperscript− epithelial (E) and EpCAM\textsuperscript+GFP\textsuperscript+
mesenchymal-like (M) populations revealed that LBH589-treated mice had a dramatically reduced number of MES-like (M) circulating tumor cells (CTCs) compared to vehicle-treated mice. Data in c is represented as mean ± SEM from 2 independent experiments. *, p<0.05; **, p<0.01; ***, p<0.001.

**Supplementary Figure 5.** LBH589 treatment in combination with castration reduces the castration-resistant tumor burden and sensitizes all tumor cell populations to androgen withdrawal-induced apoptosis. (a) H&E stained prostate sections reveal that castration-resistant tumor regrowth in the anterior lobes occurs as early as 2 wks post-castration (Cx). Cx + LBH589 impede the onset of CRPC. Black Circle, Anterior Lobe. Scale bar, 500 µm. (b) CPKV mice treated with LBH589 in combination with castration (n=6) had significantly reduced prostate tumor weights compared to intact (n=3) or castrated (n=4) CPKV mice that were similar to those of WT prostates (n=7). (c) LBH589 treatment (6 hr) increases AR acetylation levels in PKV cells in a dose-dependent manner. LNCaP cells were used as a positive control for AR acetylation. Relative AR acetylation levels were quantified by comparing the AR acetylation (Ac-Lys) band intensity to that of the AR band by densitometry. IP, immunoprecipitation; WB, Western Blot; Ac-Lys, acetylated lysine antibody. (d) While neither 1nM LBH589 treatment nor ADT alone induced apoptosis in any of the tumor cell populations, LBH589 treatment in combination with ADT induced significantly higher levels of apoptosis in all PKV cell populations, including castration-resistant MES-like (M) tumor cells, as measured by the percentage of 7AAD⁺
cells. Data were combined from 2 independent experiments done in triplicate. Data in b and d are represented as mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001.
Supplementary Table Legends

Supplementary Table 1. Gene expression levels of differentially expressed genes in epithelial (E), EMT, and mesenchymal-like (M) tumor cell populations from CPKV mice.

Supplementary Table 2. Relative gene expression values in laser capture microdissected prostate tissues from CPK mice (Log₂ ratio to WT samples).

Supplementary Table 3. Differentially expressed epigenetic regulators between the epithelial (E), EMT, and mesenchymal-like (M) tumor cell populations from CPKV mice.
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