Supplemental Figure Legends

Figure S1. Synergy of Akt signaling with AR promotes the progression of prostate cancer.

A) Schematic representation of the method used for obtaining prostate epithelial cells from Bl6 mice, lentiviral infection of mAkt (with the fluorescent marker RFP), and/or AR (with the fluorescent marker GFP), and combined with UGSM for prostate regeneration.

B) Histological analysis of regenerated tissues by H&E and IHC for Akt, AR and E-cadherin (red) and vimentin (green). Regenerated tissues were derived from primary prostate cells transformed by mAkt or AR+mAkt. Scale bar, 50 μm

Figure S2. IHC analysis of regenerated tissues for (A) CK5 (red), CK8 (green), and p63; (B) EZH2, histone H3 lysine 27 tri-methylation (H3K27me3), phospho-AR, and cyclin D1. Regenerated tissues were derived from primary prostate cells transformed by mAkt or activation of Kras (G12D). The double arrow shows the expression level of H3K27me3 in Kras(G12D) or mAkt transformed cells, while the single arrow shows the similar expression level of H3K27me3 in normal regenerated tubules. Scale bar, 50 μm.

Figure S3. IHC staining of Kras(G12D)+AR tumor tissues for CK5 (red), p63 (green), and the merged image. Scale bar, 50 μm.

Figure S4. Synergy of Kras(G12D) and AR in generating advanced prostate tumors from FACS sorted primary cells. FACS sorted prostate epithelial cells (Lin-CD49f+Sca-1+) from Kras(G12D)-Loxp mice were either infected with FUCRW-Cre, or FUCRW-Cre and FUCGW-AR lentiviruses. Kras(G12D)+AR regenerated larger tumors in size (A) and in weight (B). (C) Histology of Kras(G12D)+AR and Kras(G12D) tumors. Scale bar, 50 μm.

Figure S5. Dividing prostate spheres in Kras(G12D)+AR transduced cells. Dissociated tumorigenic cells derived from Kras(G12D)+AR primary tumors were mixed with matrigel. Spheres were formed after 10 days incubation. As shown in dashed lines, some spheres were in the process of division during the sphere formation.

Figure S6. Sphere formation of normal primary prostate cells is suppressed by DZNep. Murine normal primary prostate tissues were dissociated into single cells and mixed with Matrigel for prostate sphere formation. The cells were incubated in the PreGM medium with DMSO or 5 μM DZNep. After 10 days incubation, the number **(A)** and diameter **(B)** of prostate spheres were counted, or measured by micro-scale. Images of spheres were also taken **(C).**