Supporting Information

Dubrovska *et al*. 10.1073/pnas.0810956106

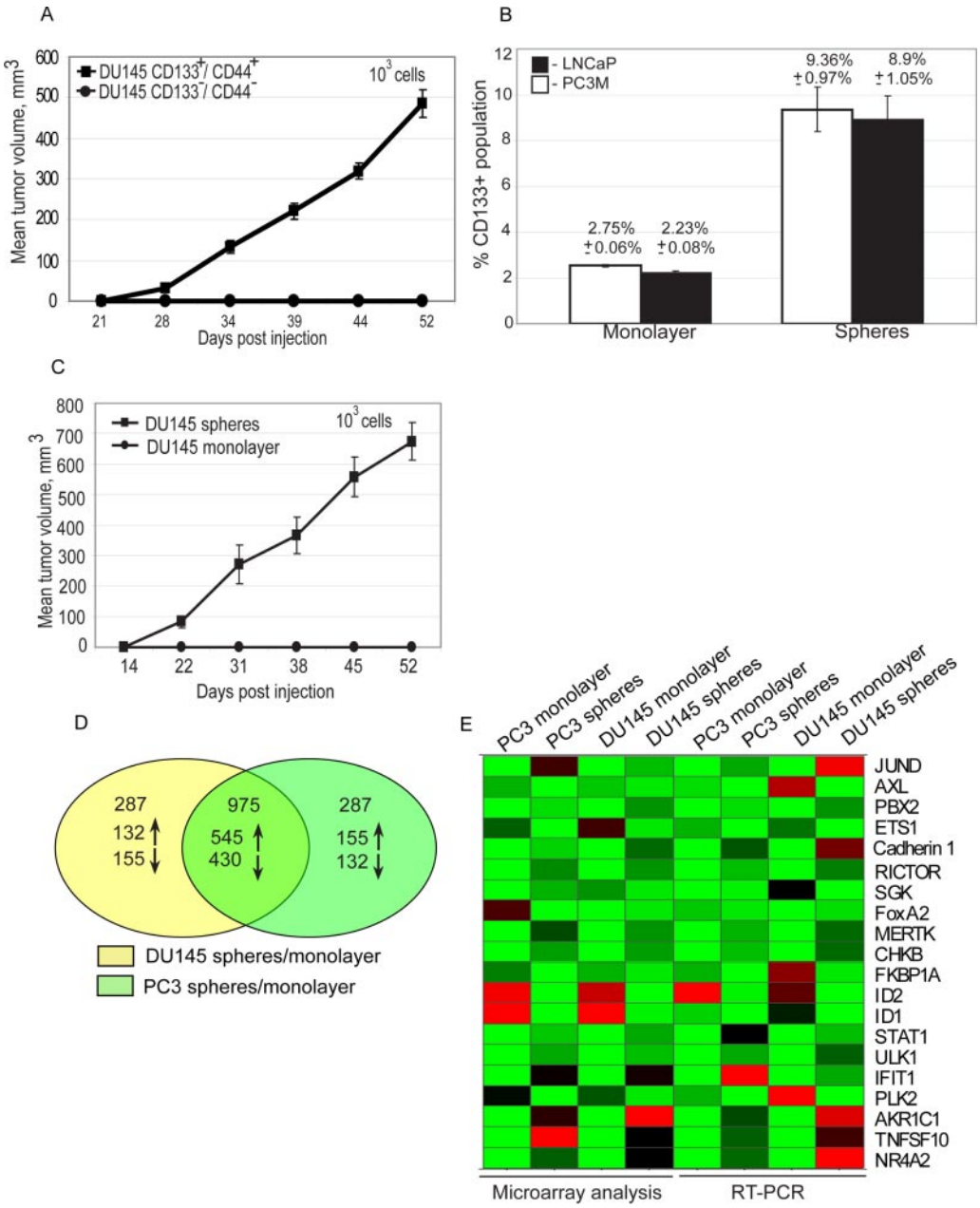

Fig. S1. Characterization of sphere-derived tumor initiating cells. (*A*) FACS-sorted $CD133^+/CD44^+$ cells produced tumors in NOD/SCID mice with s.c. injection of 1,000 cells embedded in matrigel. $CD133^-/CD44^-$ cells did not develop tumors when injected into NOD/SCID mice. (*B*) Percent $CD133^+$ cells in LnCaP and PC3M prostate cancer cell lines grown under sphere and monolayer conditions. (*C*) Secondary tumor formation. Tumors derived from DU145 cells grown under sphere-forming and monolayer conditions were subjected to enzymatic dissociation, and 1,000 cells were reinjected s.c. into NOD/SCID mice. (*D*) Venn diagram representing gene expression sets for the 2 analyzed cell lines. The circles represent the number of differentially regulated genes in both cell lines (DU145 and PC3) grown under sphere-forming and monolayer conditions. The overlapping area represents the number of genes that had similar differential regulation for the 2 cell lines. The arrows indicate the change in gene expression for the cells grown under sphere-forming conditions as compared with the cells grown under monolayer conditions. (*E*) RT-PCR confirmation of differential expression data obtained from microarray analysis of cells grown under sphere-forming conditions compared with cells grown under monolayer conditions. (*F*) Functional clustering of the differentially regulated genes: 9% of identified proteins have been previously described as stem cell regulators. Overlay of canonical networks showing the PI3K pathway as a top network represented by 25 differentially regulated genes.

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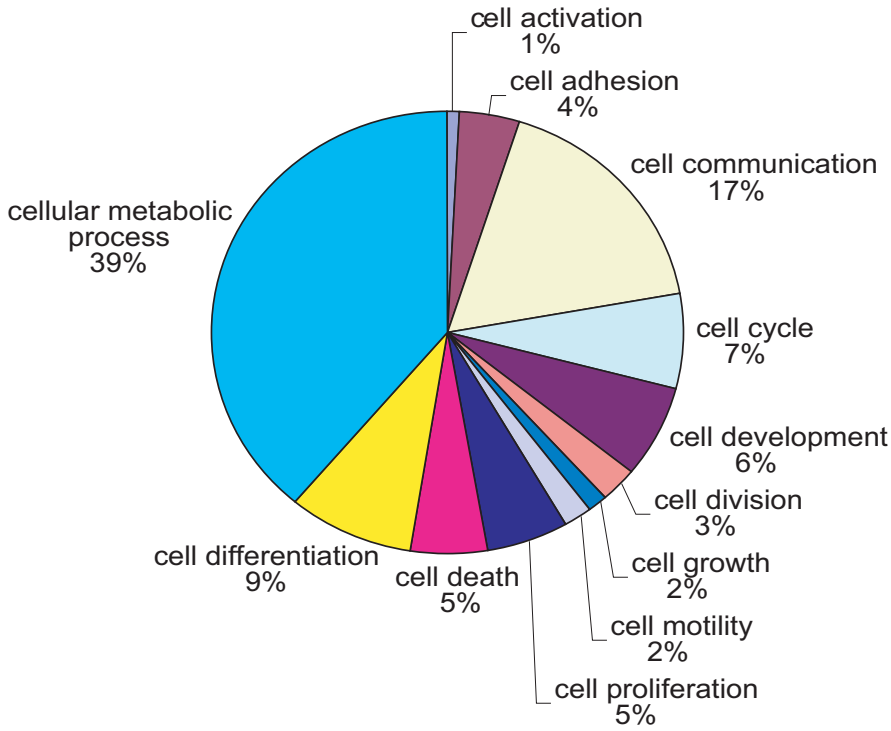

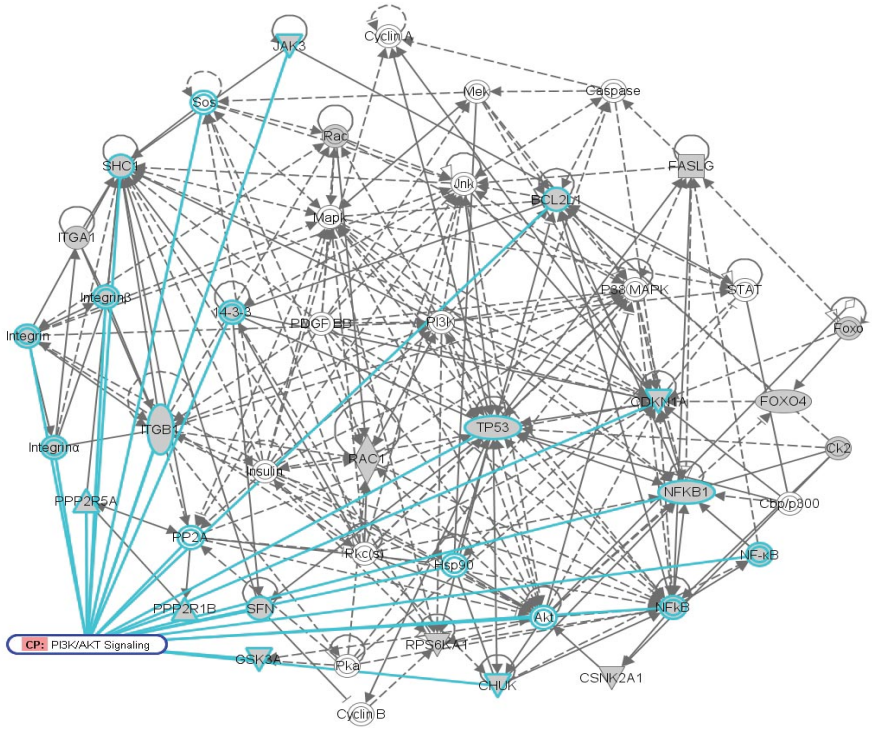

Fig. S1 Continued.

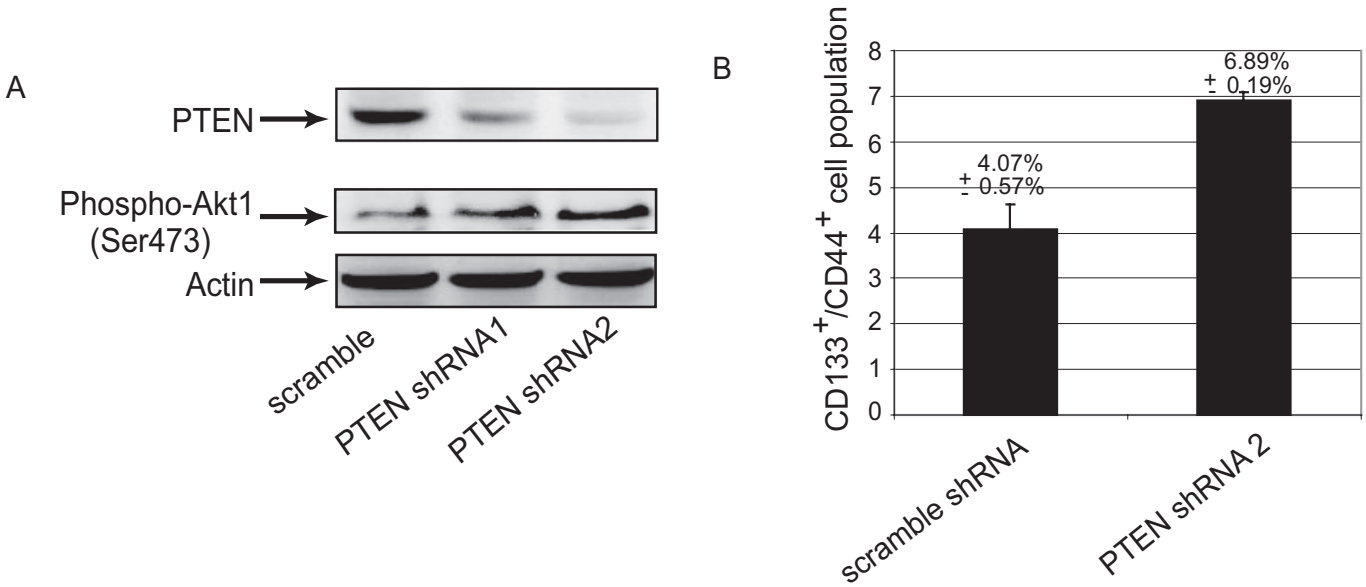

Fig. S2. Effects of PTEN knockdown in DU145 cells. (*A*) PTEN knockdown in DU145 cells resulted in up-regulation of Akt1 phosphorylation. Two different PTEN shRNAs showed different levels of gene silencing. The level of PTEN knockdown negatively correlates with the level of Akt1 phosphorylation. PTEN shRNA2 was used for further experiments. (*B*) PTEN knockdown DU145 cells grown under sphere-forming conditions showed an increase in the $CD133^{+}/CD44^{+}$-enriched PCaP population compared with scramble shRNA-transduced control DU145 cells ($P < 0.03$).

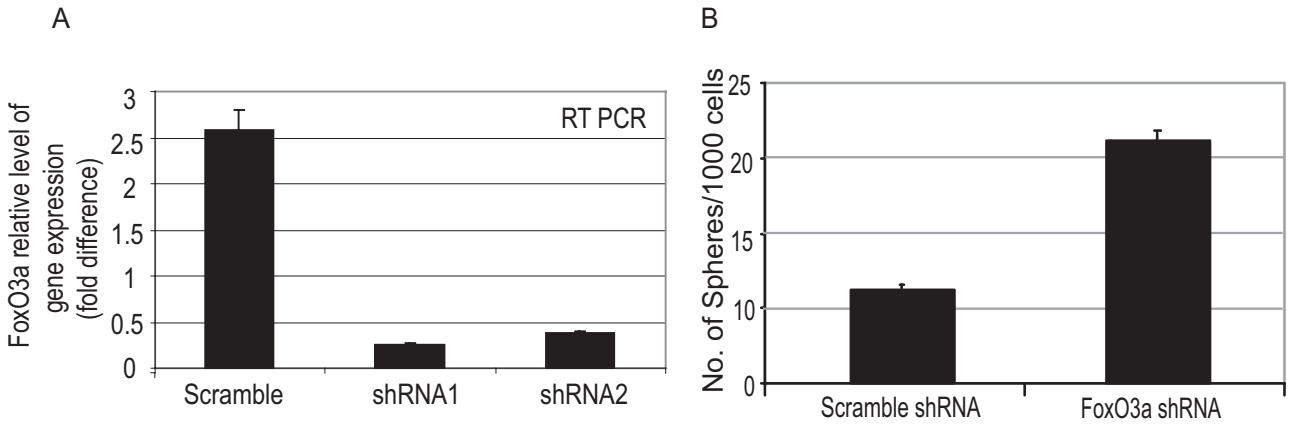

Fig. S3. Effects of FoxO3a knockdown in DU145 cells. (*A*) Validation of FoxO3a knockdown in DU145 cells by RT-PCR analysis. Two different FoxO3a shRNAs showed different levels of gene knockdown; FoxO3a shRNA1 was used for further experiments. (*B*) Increase in clonogenic capacity of FoxO3a knockdown DU145 cells as compared with control DU145 cells ($P < 0.003$). FoxO3a knockdown DU145 cells and control DU145 cells were plated in 6-well low-attachment plates at 5,000 cells per well in serum-free epithelial basal medium with supplements. The sphere numbers were calculated relative to 1,000 plated cells after 1 week.

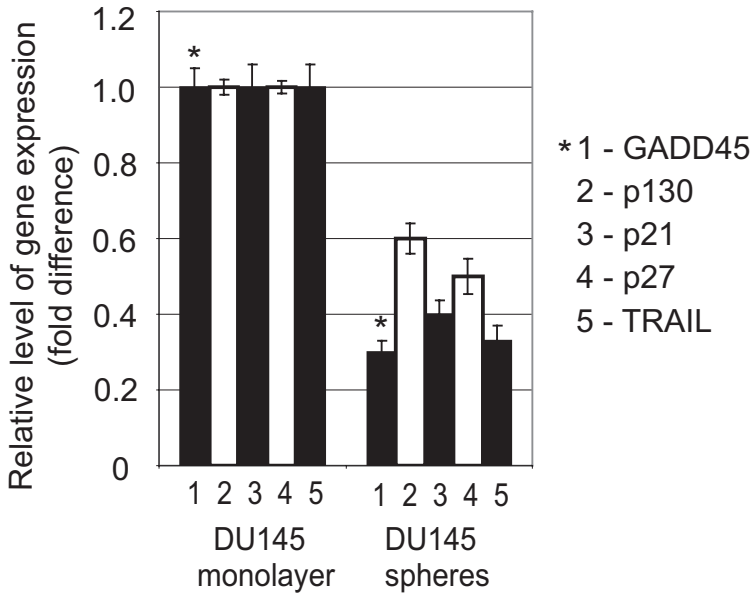

Fig. S4. RT-PCR analyses of transcriptional targets of FoxO3a in DU145 cells grown under sphere-forming and monolayer conditions.

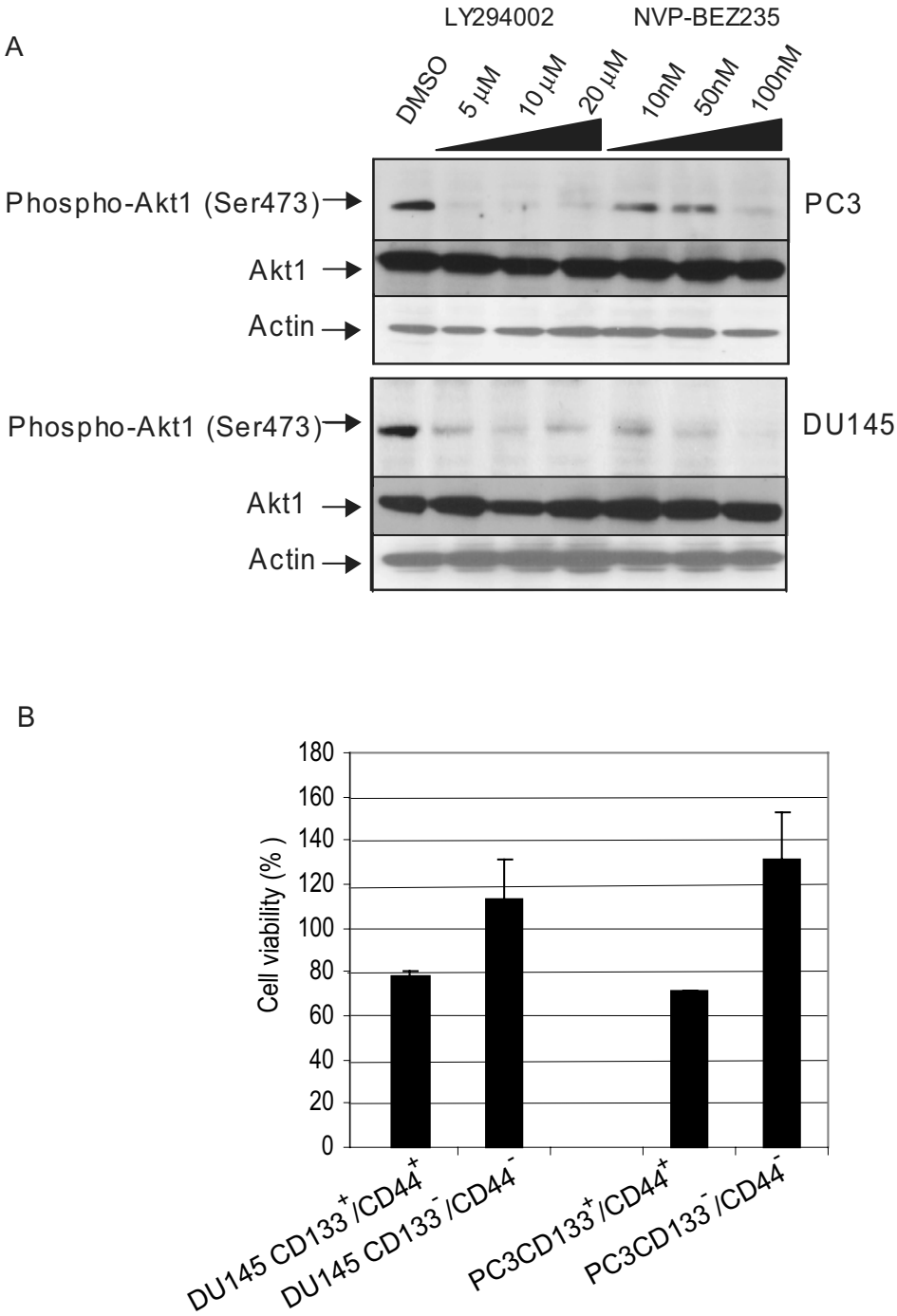

Fig. S5. Effects of PI3K inhibitors on DU145 and PC3 cells. (*A*) Inhibition of Akt1 phosphorylation in DU145 and PC3 cells treated with the PI3K inhibitors LY294002 and NVP-BEZ235. DU145 and PC3 cells were grown in serum-free epithelial basal medium with supplements and treated with the indicated concentrations of LY294002, NVP-BEZ235, or DMSO. On the third day, the cells were subjected to Western blot analysis. (*B*) Effect of LY294002 on viability of $CD133^{+}/CD44^{+}$ and $CD133^{-}/CD44^{-}$ prostate cancer cells. FACS-sorted $CD133^{+}/CD44^{+}$ and $CD133^{-}/CD44^{-}$ DU145 and PC3 cells were treated with LY294002 at concentration 5 μM for 48 h, and cell viability was measured by Cell Titer Glo assay (Promega).

Other Supporting Information Files

Table S1 (XLS)

Table S2 (XLS)