Supplementary Figure S1. Myc overexpression in primary prostate cancers. Level of Myc mRNA in tumors and normal prostate samples were derived from two prostate cancer gene expression datasets. Myc expression values are shown as log2, z-scores (mean=0; standard deviation=1) for the Kunderfranco (A) and Taylor (B) dataset, respectively. Fold change and significance p-values are shown above the box plots. Numbers of normal and prostate cancer samples in each dataset are indicated below the plots.

Supplementary Figure S2. Tumor-initiating capability of sphere-forming and adherent growing PC3 cells. PC3.Luc cells cultured as spheres (A) or in adherent conditions (B) were injected subcutaneously in athymic nude mice using from 10^2 to 10^5 cells per injection. Tumor growth was determined after 4 weeks by measuring Luciferase reporter levels with IVIS Spectrum.

Supplementary Figure S3. Tumor-initiating capability of sorted subpopulations of PC3 cells. (A) PC3.Luc cells cultured in adherent conditions were stained with antibodies specific for CD44 and CD24. The main subpopulations of CD44+/CD24- and CD44+/CD24+ were sorted with a FACS ARIA and the purity of the sorted populations was controlled with a FACS Fortessa. (B) Sorted CD44+/CD24- and CD44+/CD24+ cells were injected subcutaneously in athymic nude mice (10^5 cells per injection/site) and tumor growth monitored after 4 weeks using IVIS Spectrum.

Supplementary Figure S4. Expression of the cell surface markers CD44 and CD24 determined by qRT-PCR in PC3 and DU145 cells grown in adherent (black bars) or sphere forming (white bars) conditions.

Supplementary Figure S5. PC3 cells cultured in sphere forming conditions (bulk) and sorted subpopulations (CD44+/CD24- and CD44+/CD24+) were transfected with Myc13-Cy3 siRNA and the intracellular level of Cy3-labeled siRNA was determined after 4 h by flow cytometry.

Supplementary Figure S6. Expression of Myc in normal prostate epithelial cells (PrECs) and ESE3kd-PrECs determined by qRT-PCR.

Supplementary Figure S7. Normal prostate epithelial cells (PrECs) were transfected with GL3 and Myc13 siRNA in 12-well plates and counted with a Neubauer haemocytometer chamber over a 9-day period. Trypan blue was used to assess cell viability.

Supplementary Figure S8. Tumors generated by PC3.Luc xenografts as described in Figure 5 were dissociated in single cell suspensions and cells (10^5 cells/injection) were injected subcutaneously in the right flank of nude mice
(n = 5). (A-B) Formation of secondary tumors by GL3 and and Myc13 treated PC3.Luc cells detected by in vivo bioluminescence. (C) Tumor formation efficiency of secondary xenografts by GL3 and and Myc13 treated PC3.Luc cells. (D) Growth of established secondary xenografts (GL3, n = 5; Myc13, n = 3) over a 6-week period from implantation.