Supplementary Materials and Methods

Cell lines, primary patient prostate cells and cell culture. LNCaP cells are from late passage of cells originally obtained from American Type Culture Collection (Manassas, VA). Cells are maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 2mM L-glutamine and 50 U/ml penicillin/streptomycin. PCSCs were maintained using media and flasks purchased from CelProgen where they grow as an a mixed population of adherent and non-adherent cells.

Antibodies, proteins and peptides. Blocking antibodies for integrins were obtained from Millipore, anti-integrin αVβ3 (MAB1976), anti-integrin β1 (MAB2253), and anti-integrin αVβ5 (MAB1961). The RGD peptide, *cyclo*(Arg-Gly-Asp-d-Phe-Lys), and RAD peptide, *cyclo*(Arg-Ala-Asp-d-Phe-Lys) were purchased from Peptides International (Louisville, KY) and were dissolved in DMSO. Vitronectin-coated plates were purchased from R&D Systems (Minneapolis, MN).

Fluorescence assisted cell sorting (FACs). LNCaP cells were sorted by FACs as previously described [1]. Following sorting, cells were maintained in serum-replacement stem cell media (SCM) as previously described to preserve the CSC state[1].

Fractionation of human serum. Human serum (250 µl) was desalted with pH 7.2 sodium phosphate using a 50 KDa membrane cutoff filter. The sample was fractionated with weak anion exchange (WAX) chromatography using a 5 µm, 1000-Å PolyWAX LP column (PolyLC Inc, Columbia, MD). Mobile phase A was 10 mM sodium phosphate (pH 7.2) and B was 10 mM sodium phosphate (pH 7.2) containing 0.6 M sodium chloride. The mobile phases were delivered by a 1090 LC system (Agilent Technologies, Palo Alto, CA) at a flow rate of 1 ml/min. Proteins were eluted using multistep gradient (0-1 min/15% B, 85 min/55% B, 86-96 min/100% B), and detected by fluorescence (280 nm excitation/350 nm emission) and absorption (280 nm). A total of 96 fractions were collected off the column at one minute intervals. Weak anion exchange fractions (100µl) were pooled into four subfractions of various time intervals (0-10 min, 11-40 min, 41-70 min, 71-96 min) for use in the cell activity assay. To retain cell viability and maximize assay sensitivity, the sub-fractions were buffer exchanged and concentrated into 10 mM sodium phosphate, pH 7.2 buffer. Since initial results indicated that the 71-96 min sub-fraction was active, the cell activity assays were repeated with six subfractions (A-F) of narrower time interval pooled from the 71-96 min WAX fractions. The final cell based assay results showed that the desired activity was present in fractions encompassing the 90-96 min range.

Tryptic digestion. Each WAX fraction from 90-96 min interval was desalted and concentrated in 50 mM ammonium bicarbonate (pH 8.2). Protein quantity was determined using a bicinchoninic acid assay. Dithiothreitol was added at a final concentration of 5 mM and proteins were denatured by boiling in water for 10 min. After cooling to room temperature, trypsin was added (protein:enzyme ratio 25) and the mixture was incubated overnight at 37 ^{o}C. The tryptic peptides were desalted using ZipTips (Millipore, Bedford, MA), and reconstituted in 0.1% formic acid for

microcapillary reversed-phase liquid chromatography-tandem mass spectrometry (RPLC-MS/MS) analysis.

Microcapillary reversed-phase liquid chromatography-tandem mass spectrometry analysis. Microcapillary RPLC-MS/MS was performed using an Agilent 1100 capillary LC system (Agilent Technologies, Palo Alto, CA) coupled to a linear ion-trap mass spectrometer (LTQ, ThermoElectron, Waltham, MA). Peptides were separated using a 75 µm i.d. x 360 µm o.d x 10 cm long fused-silica capillary column (Polymicro Technologies, Phoenix, AZ) packed with 3 µm, 300 Å pore size C-18 silica (Vydac, Hysperia, CA). Mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in acetonitrile. Peptides were injected and eluted under the following conditions: 2% B from 0-20 min at 500 nl/min; linear increase to 42%B from 20-60 min at 250 nl/min; linear increase to 98%B from 60-70 min at 250 nl/min; 98%B from 70-88 min at 500 nl/min. The mass spectrometer was operated in a data-dependent MS/MS mode with each full MS scan being followed by seven MS/MS scans in which the seven most intense peptide molecular ions in the MS scan were sequentially and dynamically selected for collision-induced dissociation (CID) using a normalized collision energy of 35%. Dynamic exclusion was employed to minimize redundant acquisition of tandem mass spectra. Tandem mass spectra were searched against the Uniprot human proteomic database (European Bioinformatics Institute) using TurboSEQUEST operating on a 20 node Beowulf computer cluster (Dell, Inc., Round Rock, TX). For a fully tryptic peptide to be considered legitimately identified, it had to achieve stringent charge state and proteolytic cleavage-dependent cross correlation (X_{corr}) scores of 2.1 for $[M + H]^{1+}$, 2.4 for $[M + 2H]^{2+}$, and 3.2 for $[M + 3H]^{3+}$, and a minimum delta correlation (ΔCn) of 0.08.

Quantitative real time RT-PCR. Taqman primers/probes are as follows: BMI1 Hs00180411_m1, Oct-3/4 Hs01895061_u1, CTNNB1 Hs00170025_m1, SMO Hs00170665_m1, Nanog Hs02387400_g1, KRT14 Hs00559328_m1, and KRT8 Hs01630795_s1. Relative quantification was determined by the comparative Ct method ($\Delta Ct_{sample} - \Delta Ct_{reference} = \Delta\Delta Ct$; relative quantity = $2^{-\Delta\Delta Ct}$). For mouse genes: VCAM Mm01320970_m1, CD34 Mm00519283_m1 and GAPDH Mm99999915_g1.

Microarrays and analysis. Total RNA was extracted from cells using Trizol (Invitrogen). Two rounds of amplification were performed on all samples using Ambion's MessageAmp II kit, following manufacturer's recommendations. The resulting cDNA was labeled and hybridized to Agilent Technologies' Whole Genome 4x44k arrays, as previously described[2]. Arrays were washed and scanned on an Axon GenePix 4000B scanner and analyzed as previously described[3].

In cell western for phosphorylation of FAK and src. Cells were seeded at 1000 cells/well in a 96-well plate overnight. Cells were then fixed in cold methanol for 15 minutes on ice, blocked with 2% fish gelatin in PBS, and incubated with antibodies against phosphorylated FAK (pFAK Y397, BD Biosciences, San Jose, CA)and phosphorylated Src (pSrc Y416, Cell Signaling Technology, Danvers, MA)overnight at 4°C. Following incubation, cells were washed and incubated with secondary conjugated to IRDye 800 and was normalized by DNA staining with TO-PRO-3 iodide (LiCor,

Lincoln, NE). Staining was detected and analyzed using an Odyssey Infrared Imaging System and software (LiCor).

Beta-catenin immunocytochemistry. Following blocking with 1% BSA in PBST, cells were incubated with anti-β-catenin (abcam, Cambridge, MA, cat# ab6302) overnight at 4°C, washed, incubated with mouse-anti-human Alexa-488 secondary, washed and mounted with DAPI-containing Vectashield (Vector Labs, Burlingame, CA).

Mouse xenograft studies. For LNCaP cell injections, $CD44^{+}CD24^{-}$ cells were separated by flow cytometry, placed in serum-replacement medium overnight at a concentration of 1000 cells/ml. Cells were trypsinized, washed once with PBS, then resuspended in serum-replacement medium at 2000 cells/ 50 µl, mixed with an equal volume of matrigel (BD Biosciences, San Jose, CA) and injected subcutaneously into the rear flank of male NOD/SCID mice (Jackson labs). For PCSC2, cells were grown in sphere forming conditions (SCM supplemented with 1% knock-out serum replacement), trypsinized, counted and resuspended at 100 cells/50 µl and injected in the same manner as the LNCaP cells. Mice were monitored once weekly for palpable tumors and measured with calipers. Tumor volume was calculated as $V=W^2 \times L/2$, where w= smallest reading and l=largest reading. For PCSC tumors, tumors were excised, digested with collagenase IV (200 units/ml) in DMEM:F12, washed and passed through a 40 µM cell-strainer, and put over a Ficoll gradient. Cells in the interface were removed, washed and pelleted. Total RNA was extracted and cDNA was generated using an oligo-dT primer as previously described [1].

shRNA studies. SureSilencing GFP shRNAs against FAK (KH02827G) and src (KH00103G) were purchased from SABiosciences (Frederick, MD). All four shRNA constructs were tested for knock-down in LNCaP cells and the shRNAs with greatest knock-down (CloneID3 and CloneID2 for FAK and src, respectively, were chosen). LNCaP cells were FACs sorted, placed in SCM and transfected with shRNAs. Forty-eight hours later, transduction was checked by fluorescence and cells were plated in either SCM alone or supplemented with 1% human serum.

Reference List

1. Hurt, E. M., Kawasaki, B. T., Klarmann, G. J., Thomas, S. B. & Farrar, W. L. CD44(+)CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. *Br. J. Cancer* **98**, 756-765 (2008).

2. Klarmann, G. J. *et al.* Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. *Clin. Exp. Metastasis* (2009).

3. Kawasaki, B. T. *et al.* Effects of the sesquiterpene lactone parthenolide on prostate tumor-initiating cells: An integrated molecular profiling approach. *Prostate* (2009).