Supplemental Data

Supplemental Figure 1. Expression of CXCR4 or CXCR7 in NSFs following over expression of CXCL12 or PGK1.

(A) Expression of CXCR4 or CXCR7 in NSFs as determined by QRT-PCR following overexpression of CXCL12 or PGK1. RNA was purified from cultured cells by homogenization in TRIzol (Invitrogen) and additional processing using the RNeasy (Qiagen) cleanup procedure. 10 μg of RNA was used to obtain labeled cDNA following the Affymetrix Standard protocol. The cDNAs were amplified in TaqMan universal PCR master mix by the ABI Prism 7000 sequence detection system (Applied Biosystems) with primers and probe sets as shown in Fig 1C. Human β-actin endogenous control primers and probe set were provided by Applied Biosystems. Sequences of primers and probe were designed using primer express software provided by Applied Biosystems, and all probes were labeled with FAM/TAMRA by ABI Custom Services. The number of copies of the gene of interest in each sample was calculated from the corresponding standard curve. For each sample, the gene copy number was normalized by the value of β-actin on the same sample. Real-time PCR reactions were done in triplicate and each experiment repeated twice. The geometric mean values were calculated for each gene and condition.

(B) Western bots assay of expression of CXCR4 or CXCR7 in NSFs with over expression of CXCL12 or PGK1. The experimental protocol was similarly performed as described in Figure 4A. For detection of CXCR4 and CXCR7 (Abcam), the membranes were either blocked in 3% BSA in PBS-0.1% Tween-20, and a rabbit anti-human monoclonal antibody (1 μg/ml) (1:1000, Abcam Inc, MA) or a mouse anti-human monoclonal antibody (1 μg/ml) (1:1000, Sigma Chemical Corp., St. Louis, M) was used in conjunction with anti-species conjugated horse radish peroxidase (1:1000, Upstate, Lake Placid, NY) and detected by chemiluminescence (Amersham Pharmacia Inc. Piscataway, NJ). The results indicate that expression of CXCL12 and PGK1 in NSFs dramatically decreased CXCR4 or CXCR7 levels compared to the controls, expression of CXCR4 or CXCR7 level in CAF as positive control.

Supplemental Figure 2. Phenotype of NSFs with overexpression of CXCL12 or PGK1. Representative images of *in vitro* cultured NSFs with over expression of CXCL12 or PGK1 were first visualized under a phase contrast microscope. The data show that overexpression of CXCL12 in NSFs induced cell morphology similar to that of CAF cells, as did over expression of PGK1 in NSFs.

Supplemental Figure 3. Expression of PGK1 in NSFs induces PCa cell proliferation and invasion by CXCL12/CXCR4 axis. (A) Blocking of CXCL12/CXCR4 axis reduced the effect of PGK1 expression in NSFs on PCa cell proliferation. After a 24 h serum starvation, PCa cells (PC3) were washed and $1x10^4$ cells were plated. CM derived from NSF^{PGK1} cells were either treated with CXCL12 neutralizing antibody (30 µg/ml) (R&D Systems, MAB310), or isotype IgG control antibody, and overlaid onto the PC3 cells. Meanwhile, before performing the proliferation assay, PC3 cells were also pretreated for 45 min with different concentrations of inhibitors, including the CXCR4 neutralizing antibody (10 µg/ml) (R&D Systems, MAB173), isotype IgG control antibody, or AMD3100 (500 ng/ml). Then CM derived from NSF^{PGK1} cells was overlaid onto the PC3 cells. Proliferation was evaluated by XTT staining over a five day period. *Denotes significant difference from the treated group and its respective controls ($p<0.05$, ANOVA) for mean ± SE of n=5 samples per condition.

(**B**) Blocking of CXCL12/CXCR4 axis reduced the effect of PGK1 expression in NSFs on PCa invasion. PC3 cells were placed in the top chamber of invasion plates containing a reconstituted extracellular matrix in serum-free RPMI medium, and CM derived from NSF^{PGK1} cells were either treated with CXCL12 neutralizing antibody (30 µg/ml) (R&D Systems, MAB310), or isotype IgG control antibody, were added to the lower chambers. Meanwhile, before performing the invasion assay, PC3 cells were also pretreated for 45 min with different concentrations of inhibitors, including the CXCR4 neutralizing antibody (10 µg/ml) (R&D Systems, MAB173), isotype IgG control antibody, or AMD3100 (500 ng/ml). Then CM derived from

NSF^{PGK1} cells was overlaid onto the PC3 cells. Invasion was determined at 48 h by MTT staining and the data are presented as % invasion ± standard deviation for n=5. *Denotes significant difference from invasion between alterations of from the treated group vs. controls ($p<0.05$, ANOVA).

Supplemental Figure 4. Immunofluorensence staining for CAF markers FAP. FAP in tumor stroma from $NSF^{Control}$, NSF^{CXCL12}, and NSF^{PGK1} mixed with $PC3^{luc}$. Nuclei were identified by DAPI. All images were captured on Zeiss LSM 510 meta confocal laser scanning microscope. Original magnification 60×, where the bars represent 30 μM.

Supplemental Table 1. Summary of gene profiles induced by overexpression of CXCL12 and PGK1 in NSFs.

The GeneChip® Human Genome Focus Array represents over 8,500 verified human sequences from the NCBI database were utilized to identify gene profiles. Top7 pathways were enriched by over expression of CXCL12 and PGK1 in NSFs.

Materials and Methods

Cell lines.

Cell lines used in this study were derived from two human cancerous glands obtained from PCa patients at the University of Michigan in 2008. The glands were dissociated, and the various cell types were then separated to obtain CAFs. NSFs were taken from a histologically noncancerous region of the prostate at least 2 cm away from the outer tumor margin. Their identities were then characterized by immunohistochemical stainings **(IHCs)** for CAF markers α-SMA and vimentin (25). The authors had not conducted a validation test in these cells. The PC-3 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD) in 2000 and cultured in RPMI Medium 1640 supplemented with 10% fetal bovine serum (FBS) (Invitrogen Corp, Carlsbad, CA) at 37 °C , 5% CO_2 as previously described (27). The identity was

then characterized by cytogenetic analysis. The authors had not conducted a validation test in PC3 cells.

LCM and cDNA microarrays.

LCM was performed from frozen tissue sections with the SL Microtest device using CUT software (MMI). Approximately 5,000-10,000 cells from frozen prostate tissue specimens from separate cases of Gleason 3 + 3 tumors. In addition, epithelia from cancerous glands were isolated from three samples, stroma was isolated from cancerous glands from two samples, epithelia from benign glands were isolated from five samples, and stroma from the peripheral zone of a nodule of BPH was isolated from a single sample. The detailed protocol is provided in the supplemental section. Exponential RNA amplification was performed using a TransPlex Whole-Transcriptome Amplification kit (Rubicon Genomics) as described (23), and complete details are provided in the MIAME checklist. Microarrays (20K chip) containing sequence verified PCR-amplified human cDNAs representing 15,495 Unigene clusters were manufactured as described previously with minor modifications (23). cDNA microarray analysis of gene expression was done essentially as described previously (17). Data were median-centered by arrays and only genes that had expression values in at least 80% of the samples were used in the analysis. The normalized data set from each experiment was log transformed, filtered for bad spots, and median centered before it was subjected to statistical analysis of microarrays (SAM) as described previously (24).

Invasion assays.

Cell invasion was examined using a reconstituted extracellular matrix membrane (BD Biosciences, San Jose, CA). PC3 cells were placed in the upper chamber ($1x10^5$ cells / well) in serum-free medium, and CM derived from altered expression of CXCL12 and PGK1 in NSFs, CAFs and the respective controls was added to the bottom chambers. Invasion into the matrix was assayed after 48 h when the invasion chambers were removed and 40 µl of MTT (5 mg/ml, Sigma, St. Louis, MS) was added to the top

well and 80 µl of MTT into the bottom well, and further incubated for 4 h at 37 °C. After complete removal of residual cells or medium, the purple residues attached to the bottom or top chambers were released with 1 ml isopropanol (Sigma, St. Louis, MS). The invasion chambers were rocked for 30 min at a medium speed and then 100 µl from each well transferred into 96 wells and read on a multi-well scanning spectrophotometer (Molecular Devices Corp. Sunnyvale, CA.) at $OD_{450.}$

Western- blot analyses.

PCa, NSFs or CAFs were cultured to confluence, washed and then serum-starved in RPMI with 0.1% BSA for 24 h. The cells were lysed in ice- cold RIPA buffer. Cell lysates were clarified by centrifugation at 14,000 rpm for 10 min and protein concentrations were determined (Bio-Rad Laboratories, Hercules CA). Normalized lysates (30 µg) in loading buffer were electrophoresed on 10% polyacyrlamide gels under reducing conditions and transferred to polyvinylidene difluoride membranes (PVDF) membranes. For detection of MMP-2, 3 (Santa Cruz Biotech Inc, CA) , α-SMA, CXCR4 and CXCR7 (Abcam), the membranes were either blocked in 3% BSA in PBS-0.1% Tween-20, and a rabbit anti-human monoclonal antibody (1 µg/ml) (1:1000, Abcam Inc, MA) or a mouse anti-human monoclonal antibody (1 µg/ml) (1:1000, Sigma Chemical Corp.) was used in conjunction with anti-species conjugated horse radish peroxidase (1:1000, Upstate, Lake Placid, NY) and detected by chemiluminescence (Amersham Pharmacia Inc. Piscataway, NJ). ERK and AKT detection were similarly performed in 5% dry milk in PBS-0.1% Tween-20 with a rabbit monoclonal reactive to dually phosphorylated ERK (Thr202/Tyr-204) and total ERK1 and ERK2, and phospho-AKT (Ser473) and total AKT (Cell Signaling Technology, Beverly, MA).

Proliferation assays.

After a 24 h serum withdrawl, PC3 cells were digested and washed three times in PBS and $1x10^4$ cells were incubated with a 1:1 mixture of CM derived from $CAF^{\beta\text{-gal}}$, CAF^{siPGK1}, $CAF^{siCXCL12}$, $NSF^{control}$, NSF^{PGK1}, or NSF^{CXCL12} and fresh growth medium

in triplicate 96 well flat-bottomed tissue culture plates in a total of 0.1 ml. The cultures were incubated in an atmosphere of 5% CO_2 and 95% O_2 at 37°C for 5 days. Proliferation was quantified by colorimetric assay using sodium 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate (XTT) (Sigma, St. Louis, MS) and read on a multiwell scanning spectrophotometer at OD_{450} (Molecular Devices Corp. Sunnyvale, CA.), OD56O as background calibration.

Tissue microarray construction, immunohistochemistry, digital image capture and analysis.

High-density tissue microarrays were constructed from clinical samples obtained from a cohort of over 120 patients, who underwent radical retro pubic prostatectomy at the University of Michigan as a primary therapy (i.e, no preceding hormonal or radiation therapy) for PCa. These were provided from the University of Michigan Comprehensive Cancer Center Histology and Immunoperoxidase Core as detail previously (25). Tumors were graded using the Gleason grading system and examined to identify areas of benign prostate, PIN, and localized prostate cancer. The formalin-fixed, paraffin-embedded tissues were dewaxed and placed in a pressure cooker containing 0.01 M buffered sodium citrate solution (pH 6.0), boiled and chilled to room temperature for antigen retrieval. The slides were then incubated overnight at room temperature with anti-human PGK1 antibody diluted 1:2000. A standard streptavidin/biotin detection method with 3,3′-diaminobenzidine tetrahydrochloride (DAB) was employed for signal detection and Harris' hematoxylin was used as a counter-stain. PGK1 expression was blindly scored as negative (1), weak (2), moderate (3), or strong (4) on basis of intensity of staining and the percentage of tumors cells using a telepathology system without knowledge of overall Gleason score (e.g., tumor grade), tumor size, or clinical outcome.

IHC assays .

For immunostaining with α-SMA, vimentin and FAP (Abcam), tissue sections were blocked with Sniper for 5 minutes, and incubated overnight at 4° C with 2.8 μg/ml rabbit anti-human α-SMA, vimentin ，and FAP diluted in PBS. The sections were incubated with appropriate secondary antibodies for 30 min, followed by processing with a Lincoln Label 41 Detection System (Biocare Medical). The HRP-AEC Chromogen System (R&D Systems) or a solution of HSS-HRP and AEC chromogen in chromogen buffer were used to visualize bound antibodies. The numbers of stained positive tissue sections were blindly counted in 6 random fields per implant at 630× magnification.

Three or four implants were analyzed per condition and for each time point. All images were generated on Zeiss LSM 510 meta confocal laser scanning microscope. For immunostaining with Ki-67 antigen (Dako), and vimentin (Abcam), NSFs and CAFs were cultured in Lab-Tek II 4- chamber slides (Nalge Nunc International, Naperville, IL, USA) at 5×10^4 cells/chamber. After 24 h the cells were rinsed three times with ice cold-PBS, fixed in 4% paraformaldehyde for 25 min at room temperature, washed and endogenous peroxidase activity quenched with 75 mM NH4Cl and 20 mM glycine in PBS. Primary antibody was incubated at a 1: 50 dilution in PBS for 1 h at room temperature. Antibody detection was performed using an HRP-AEC staining kit using anti-mouse biotinylated antibodies (R&D Systems), and counter stained with hematoxylin (Sigma).

For immunostaining with α-SMA (Abcam) in NSFs and CAFs similar protocol was described above except for the process that antibody detection was performed using a FITC conjugate (goat anti-rabbit IgG as the secondary (R&D Systems). Nuclei were identified by DAPI (Vector Labs, Burlingame, CA).