Supplementary Materials and Methods

siRNA and stable miRNA expression systems

For siRNA experiments, ON-TARGETplus SMARTpools or the Non-targeting Pool (D-001810-10-20) were transfected using the Dharmafect 1 transfection reagent (Dharmacon, Lafayette, CO). The PTP1B (L-0035029-00-20) and AR targeting sequences (L003400-00-20) are described in Table S1.

Stable PTP1B-knockdown cells were derived from parental LNCaP cells transfected with the pcDNA tm 6.2-GW/EmGFP-miR vector (Invitrogen) containing a miR-155-based PTP1B-targeting short hairpin RNA (shRNA) sequence. Two different PTP1B-targeting vectors were constructed, shPTP1B1 and shPTP1B2 (Table S1). Control non-targeting clones (shCTRL) were transfected with the pcDNA6.2-GW/EmGFP-miR-neg control plasmid (Invitrogen). Transfections were performed using Lipofectamine 2000, and stables clones were selected according to the manufacturer's instructions in 5mg/ml (shCTRL1a and shPTP1B1a) or 2.5mg/ml (shCTRL1b, shPTP1B1b and shPTP1B2) blasticidin.

Immunohistochemistry

The TMA used in this study was previously described (1). Briefly, the TMA contained normal adjacent, PIN, and tumor tissues from 62 radical prostatectomy patients that were not subjected to neo-adjuvant therapy. Informed consent was obtained from all patients and the protocol was approved by the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) institutional review board.

PTP1B expression in prostate tumor sections was assessed using the polyclonal Rabbit Anti-human PTP1B Antibody AF1366 (R&D Systems, Minneapolis, MN). Immunostaining was performed using the biotin-streptavidin-peroxydase method. Formalin-fixed paraffin-embedded sections were deparaffinized in toluene and rehydrated through graded ethanol followed by distilled water. Antigen retrieval was done heating slides in the microwave for 15min in TET buffer (10mM Tris, 1mM EDTA, 0.05% Tween 20, pH 9.0). Non-specific sites were blocked with 10% goat serum and slides were then incubated with the primary antibody at a 1/750 dilution for 2hrs at room temperature. Endogenous peroxydase was quenched in 3% hydrogen peroxide. A biotinylated goat anti-rabbit secondary antibody, followed by streptavidin-HRP (Lab Vision, Fremont, CA), were applied for 10min each. All rinsing steps between antibodies were done in PBS-T for 3 times 5min. Chromogen reaction using a 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution kit (Lab Vision, Fremont, CA) was carried out for 5min. Slides were counterstained with Harris Hematoxylin for 10sec, dehydrated in ethanol and toluene and then mounted. Epithelial zones were scored by an expert uro-pathologist (L.R.B.) according to their cytoplasmic staining intensities (value of 0 for absence, 1 for weak, 2 for moderate, 3 for high intensity). To account for the heterogeneity in staining intensities, each core was given a primary and a secondary score. The resulting scoring scale is shown in Table S2. The detection and scoring of AR and Ki-67 have already been described (1).

Migration and invasion assays

LNCaP, C4-2, and DU145 cells were treated for siRNA experiments as described in the cell culture section, except for DU145 cells that were not androgen-deprived prior to the experiment. Transwell® (8mm pore polycarbonate membrane insert; Costar, Corning, NY) bottom filters were precoated with 10mg/ml fibronectin/PBS solution (LNCaP, C4-2) or 0.15% gelatin/PBS solution (DU145) overnight at 4°C. Transwell® were then washed with PBS and assembled into 24-well plates. The upper chamber of each Transwell® was filled with 100ml of cells (5.0×10^5 cells/ml) and LNCaP were allowed to adhere for 45min. 600ml of phenol-free RPMI 1640 with or without 10% (LNCaP, C4-2)

or 0.1% (DU145) FBS was added into the lower chamber as chemoattractant and the plate was placed in the incubator (37°C, 5% CO_2/95% air) for 4hrs. Cells that had migrated to the lower surface of the filters were fixed with 10% formalin phosphate and stained with 0.1% crystal violet/20% methanol (vol/vol). Cell migration was quantified with ImageJ and data are expressed as the average density of migrated cells per five fields (magnification x100). For the invasion assay, the migration protocol was modified as followed: Transwell® upper filters were precoated with 100ml of a 0.5mg/ml phenol-red free Matrigel™ (BD Biosciences, Bedford, MA)/PBS solution overnight at room temperature, washed with PBS and cells were allowed to invade for 24hrs.

Microarray hybridization
R1881-supplemented (1nM) or ethanol-treated (vehicle) cells were washed with PBS and RNA was extracted using Trizol (Invitrogen) following the company's protocol. RNA was then purified (Ambion, PN AM1928) and RNA quality was assessed on the Bioanalyzer (Agilent). A total of 250ng of RNA was used as starting material to generate purified sense-strand cDNA with incorporated dUTP (Ambion WT Expression Kit, Ambion, PN 4425209C). cDNA was then fragmented, labeled (Affymetrix GeneChiP WT Terminal Labeling Kit, Affymetrix, PN 900671) and hybridized on the Affymetrix GeneChip Gene 1.0 ST Array (Affymetrix, PN 901086).

PTPN1 transcript stability
LNCaP and C4-2 cells were incubated in androgen-deprived phenol-free RPMI 1640 (5% charcoal-stripped FBS, L-glutamine, and 50μg/ml gentamycin) for 24hrs and then treated with actinomycin D (5mg/ml) or the vehicle. Copy number standard curve was done following qPCR amplification of human cDNA using PTPN1 Forward and Reverse primers (Table S1). Obtained amplicon was purified on gel, quantified (NanoDrop, ThermoScientific) and copy number was determined using the following formula: (amount in ng * 6.022×10^{23}) / (length in bp * 1×10^9 * 650). PTPN1 copy number in LNCaP and C4-2 cells at different time points (0, 2, 4, 6, 8, 12 and 24hrs) was estimated by comparison to a standard curve ranging from 10 to 1,000,000 copies.

Substrate-trapping assay
LNCaP cells were cultured in androgen-deprived medium for 48hrs, transfected with either GST, GST-PTP1B, or GST-PTP1B-D/A vectors using Lipofectamine 2000 (Invitrogen), and subsequently treated with 1nM R1881 for 36hrs. At this point, cell lysis, GST pull-down, and phosphotyrosine detection were performed as already described (2).

Proliferation assay
LNCaP cells were transfected with the indicated siRNA and plated 24hrs later with 50,000 cells/wells in phenol-free RPMI 1640 (5% charcoal-stripped FBS, L-glutamine, and 50μg/ml gentamycin) on interdigitated gold micro-electrodes integrated on the bottom of tissue culture plates (E plates, xCELLigence system, Roche, Laval, Qc). Eighteen hours later, cells were treated with 20μM BIC or vehicle for 30min and then with 10nM R1881 or vehicule. Cellular proliferation, mirrored by the increase in electrode impedance in each well, was displayed as cell index and recorded in real time.

Supplementary References

1. Gannon PO, Koumakpayi IH, Le Page C, Karakiewicz PI, Mes-Masson AM, Saad F. Ebp1 expression in benign and malignant prostate. Cancer Cell Int. 2008;8:18.
2. Stuible M, Dube N, Tremblay ML. PTP1B regulates cortactin tyrosine phosphorylation by targeting Tyr446. J Biol Chem. 2008;283:15740-6.

Supplementary Figures

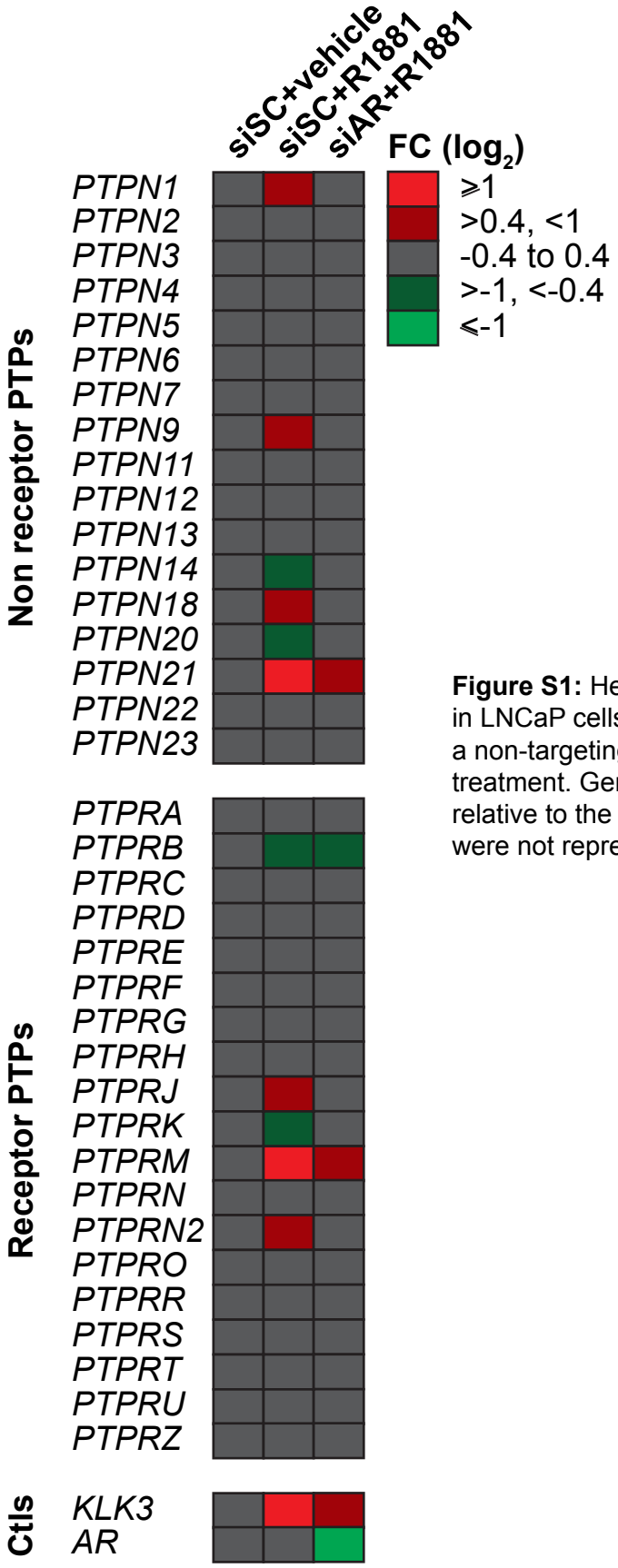

Figure S1: Heat map representing classical PTP gene expression in LNCaP cells transfected with an siRNA against the AR (siAR) or a non-targeting sequence (siSC) prior to a 24hrs R1881 (or vehicle) treatment. Gene expression is represented in fold-change (FC) relative to the siSC+vehicle condition. *PTPRQ* and *PTPRV* were not represented on this microarray.

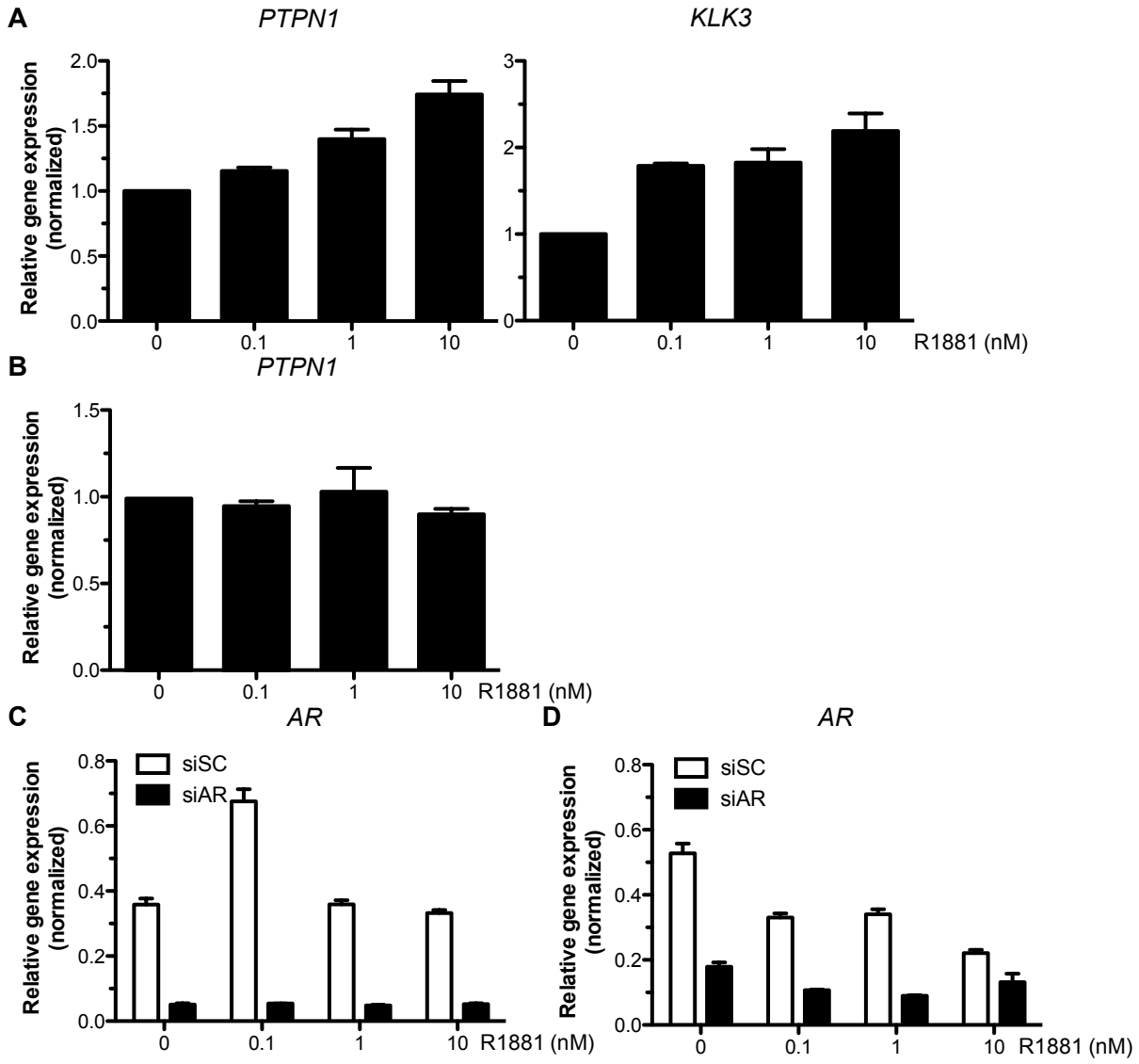

Figure S2: (A) In 22rv1 cells treated with R1881 (24hrs), *PTPN1* mRNA levels were significantly increased in a dose-dependent manner (Kruskal-Wallis test, $p<0.05$; representative experiment, N=3, +/- SEM). Relative *KLK3* expression levels were monitored as a treatment control. **(B)** *PTPN1* mRNA levels does not change in DU145 cells treated for 24hrs with different concentrations of R1881 (Kruskal-Wallis test, p-value is non-significant; representative experiment, N=3, +/- SEM). **(C and D)** *AR* mRNA levels following R1881 treatment in **(C)** C4-2 and **(D)** LNCaP transfected with siRNAs against the AR (siAR) or control sequences (siSC) (matching experiment with Figure 1C and D).

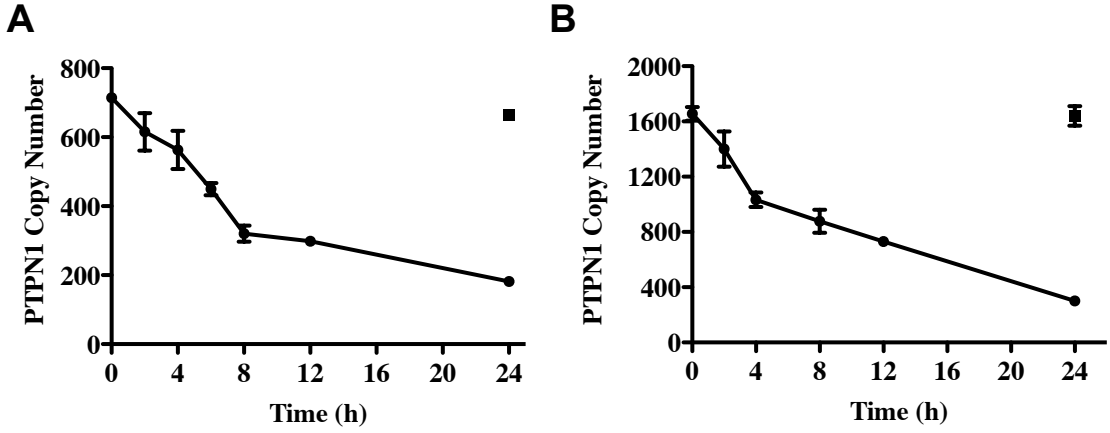

Figure S3: *PTPN1* copy number in **(A)** LNCaP and **(B)** C4-2 cells treated with actinomycin D (5µg/ml; circles) or the vehicle (squares).

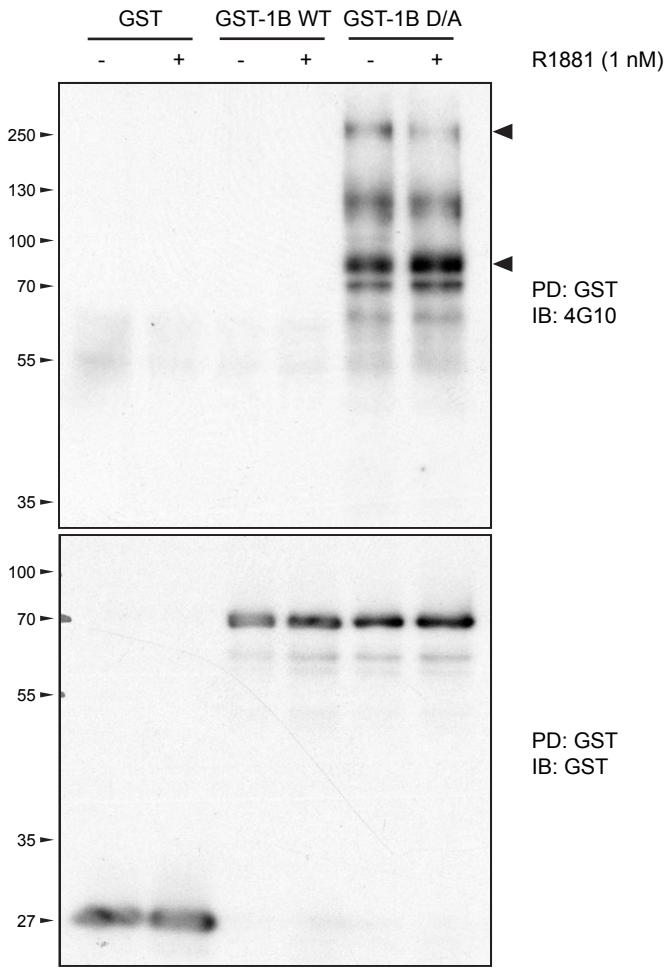

Figure S4: PTP1B substrate-trapping experiment. GST pull-down of GST alone, GST-PTP1B, or GST-PTP1B-D/A expressing cell lysates followed by immunoblot detection of phosphotyrosine proteins using the 4G10 antibody (Millipore). Representative of 3 independent experiments. Arrows represent putative PTP1B substrates differentially modulated by R1881 treatment.

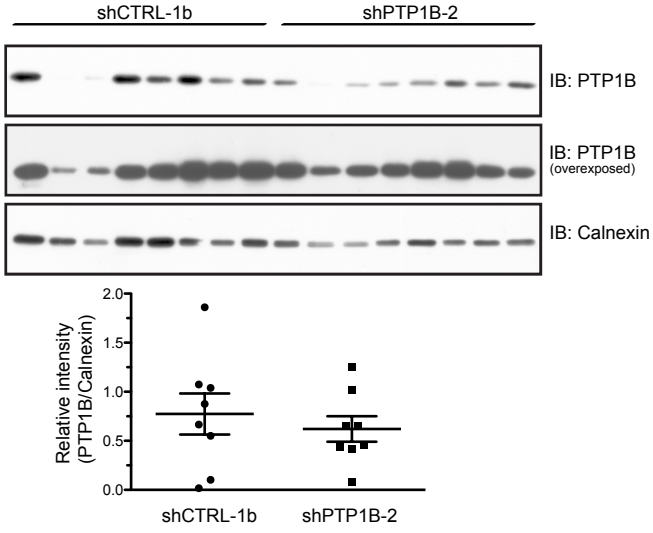

Figure S5: Western immunoblotting showing PTP1B levels in endpoint tumors from stable shCTRL-1b (n=8) and shPTP1B-2 (n=8) clones (*upper panel*). No significant difference was observed between the two groups following quantification (*lower panel*; Mann-Whitney, p=0.5054).

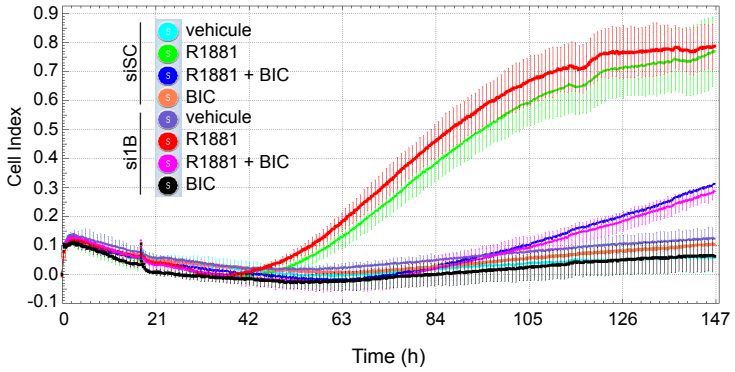

Figure S6: LNCaP cell proliferation following transfection with an siRNA against PTP1B (si1B) or a non-targeting sequence (siSC), pre-treated with BIC (20µM) or the vehicle and then treated with R1881 (10nM) or the vehicle.

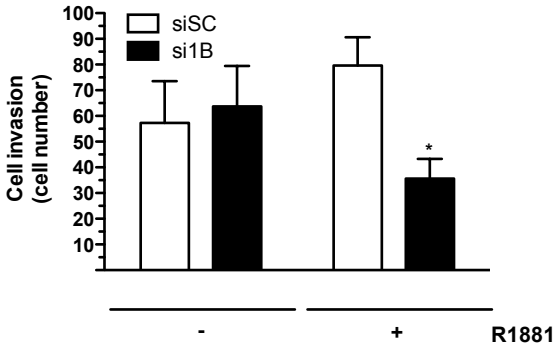

Figure S7: PTP1B down-regulation impedes LNCaP cell invasion on Matrigel *in vitro*. LNCaP cells transiently transfected with an siRNA against PTP1B (si1B) or a non-targeting sequence (siSC) were androgen-deprived for 48hrs. Cells were then treated with R1881 or the vehicle for 36hrs before FBS-induced cell invasion as described in *Materials and Methods*. (Mann-Whitney test; * p<0.05 compared to the corresponding siSC; +/- SEM).

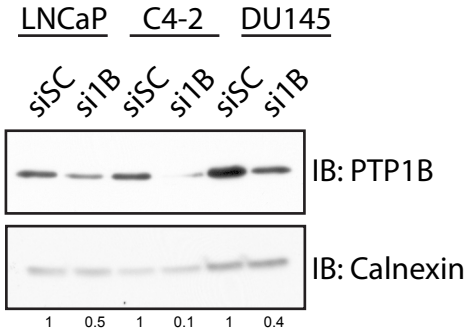

Figure S8: Western immunoblotting showing PTP1B knockdown in LNCaP, C4-2 and DU145 cells transiently transfected with an siRNA against PTP1B (si1B) or a non-targeting sequence (siSC) prior to cell migration experiments. Numbers below represent PTP1B levels relative to Calnexin and normalized to the corresponding siSC.

Supplementary Tables

Table S1. List of the different oligonucleotides used in the experiments.

	Name	Sequence (5' to 3')
Analysis of gene expression	*PTPN1* forward	AGAAGGACGAGGACCATGCAC
	PTPN1 reverse	AGTGGAGGAGGGTCAGGCTAT
	AR forward	CCTGGCTTCCGCAACTTACAC
	AR reverse	GGACTTGTGCATGCGGTACTC
	KLK3 forward	TCTGCGGCGGTGTTCTG
	KLK3 reverse	GCCGACCCAGCAAGATCA
	PPIB forward	TAATGGCACTGGTGGCAAGTC
	PPIB reverse	TTGCCATCCAACCACTCAGTC
	RPLP0 forward	GCAGCAGATCCGCATGTCGCTCCG
	RPLP0 reverse	GAGCTGGCACAGTGACCTCACACGG
siRNA experiments	siPTP1B (1)	GGAGAAAGGUUCGUUAAAA
	siPTP1B (2)	CUACCUGGCUGUGAUCGAA
	siPTP1B (3)	GCCCAAAGGAGUUACAUUC
	siPTP1B (4)	GACCAUAGUCGGAUUAAAC
	siAR (1)	GGAACUCGAUCGUAUCAUUUU
	siAR (2)	CAAGGGAGGUUACACCAAAUU
	siAR (3)	UCAAGGAACUCGAUCGUAUUU
	siAR (4)	GAAAUGAUUGCACUAUUGAUU
miRNA expression systems	shPTP1B-1 forward	TGCTGTCCAGTAACTCAGTGCATGGTGTTTTGG CCACTGACTGACACCATGCAGAGTTACTGGA
	shPTP1B-1 reverse	CCTGTCCAGTAACTCTGCATGGTGTCAGTCAGT GGCCAAAACACCATGCACTGAGTTACTGGAC
	shPTP1B-2 forward	TGCTGAATAACAACTGCTTACAACCGGTTTTGG CCACTGACTGACCGGTTGTACAGTTGTTATT
	shPTP1B-2 reverse	CCTGAATAACAACTGTACAACCGGTCAGTCAGT GGCCAAAACCGGTTGTAAGCAGTTGTTATTC
ChIP experiments	*PTPN1*-TSS forward	TTGGTCCTTCTGCTTCAGGGGCGGAG
	PTPN1-TSS reverse	GCCCTAGCCGCTGCTGCTTCTTCATG
	PTPN1-enh 6kb forward	GTATGGTTCCTGTCTGTGGCTGTCTT
	PTPN1-enh 6kb reverse	GTACGCAACATCTTCAATGACCTAAG
	PTPN1-enh 28kb forward	GTTTGGAGAAGAGTGGGGAAGAGTAGGC
	PTPN1-enh 28kb reverse	TAGAAAACACAAATGTTCCTAAATGCCA
	Control-1 forward	TCACACAGTTCCACCCGCTCCAGAAA
	Control-1 reverse	GGTGTGGTATTGACAAGCCCTCAGGT
	Control-2 forward	ACAAAGGGAATAAGCAATCTGGCATA
	Control-2 reverse	TCGGCTCCAACTCAACTCGTCTATGT

Table S2. TMA scoring scale

Primary Intensity	Secondary Intensity	Intensity Score
0	N/A	0
0	1,2,3	1
1	N/A	2
1	2	3
1	3	4
2	1	5
2	N/A	6
2	3	7
3	2	8
3	N/A	9

Table S3. Correlation between PTP1B expression and clinico-pathological parameters

Clinico-Pathological Parameters	Correlation	p-value	n
Pre-Operative PSA	0,073	0,59	57
Gleason Score	0,009	0,95	61
Pathological Stage	-0,127	0,33	61
Positive Surgical Margins	**-0,272**	**0,03**	61
Extracapsular Extension	-0,171	0,26	45
Seminal Vesicle Invasion	0,034	0,8	59
Lymph Node Metastasis	0,129	0,32	61
Biochemical Recurrence	-0,077	0,55	61