Supplemental Materials and Methods

Cells and tissues. Human BMEC-60 (HBMEC-60), from Dr. C. Ellen van der Schoot (1) [1], and HUVECs were obtained from the Vascular Biology Core Facility, Brigham and Women's Hospital, and maintained in endothelial cell medium (ScienCell Research Laboratories, Carlsbad, CA). Human primary and metastatic PCa tissues were obtained as described (2,3) [2, 3].

Antibodies. Anti-sLe^X HECA-452 (BD Biosciences (BD), San Jose, CA), anti-Le^X 80H5 (Beckman Coulter, Indianapolis, IN), anti-CD44 A3D8 (Sigma-Aldrich, St. Louis, MO), anti-E-selectin BBIG-E4 (5D11) (R&D Systems, Minneapolis, MN), anti-VCAM-1 IG11 (Beckman Coulter), anti-ICAM-1 HA58 (BD Biosciences), anti-β1 4B4 (Beckman Coulter), anti-β2 L130 ((BD), anti-β3 SZ21 (Beckman Coulter), anti-β4 450-9D (AbD Serotec, Raleigh, NC), anti-β5 KN52 (eBioscience, San Diego, CA), anti-β6 437211 (R&D, Minneapolis, MN), anti-β7 Fib504 (eBioscience), anti-β8 416922 (R&D), anti-αVβ3 LM609 (EMD Millipore, Billerica, MA), anti-α2 12F1-H6 ((BD), anti-α4 9F10 (BioLegend, San Diego, CA), anti-α5 IIA1 ((BD), anti-α6 GoH3 ((BD), anti-αIIb HIP8 (BioLegend) and anti-αV NKI-M9 (BioLegend); N29, HUTS-21, 9EG7, anti-β-Actin C4, isotype and fluorophore-conjugated Abs were from BD.

Proteins. Human E-selectin/Fc and P-selectin/Fc, human ICAM-1/Fc and mouse PECAM-1 were from R&D Systems. Human VCAM-1 was obtained from Dr. Deane F. Mosher (University of Wisconsin-Madison, Madison, WI). Human fibronectin, human laminin 1, rat collagen type I, human vitronectin and human osteopontin were from Sigma-Aldrich.

Real-time PCR. Primers validating FT3 and FT7 upregulation were described (2) [2], and FT6 primers were forward, 5'-GGACGTGGCCTTTTAACAAA-3' and reverse, 5'-CTGCCTGTGGATACACCTTG-3'.

Generating PCa cells stably expressing FT3, 6 or 7 and luciferase. Human FT3, 6 or 7 (3) [3] were subcloned into pLNCX2 (Dr. Hans R. Widlund, Brigham and Women's Hospital), packaged into retroviruses, transduced into PCa cells and selected (200-400 μg/ml G418) (Life Technologies). PCa cells expressing firefly luciferase were generated from lentiviruses encoding pFUW-Luc-Cherry-puro plasmid (Dr. Widlund and Dr. Andrew L. Kung) and selected in 1 μg/ml puromycin (Fisher Scientific).

Silencing β1 integrins. Short-hairpin RNA (shRNA) against human β1 or scrambled shRNA were from the RNAi Consortium (TRC), Broad Institute (MIT, Cambridge, MA) or Addgene (Cambridge, MA), respectively, packaged into lentivirus, transduced into PCa cells and selected in 1 μg/ml puromycin (Fisher Scientific). The target 21mer for β1 knockdown was (5'-3'): GCCTTGCATTACTGCTGATAT.

PCa static adhesion. PCa cell adhesion to protein (10 μg/ml) was conducted as described (2) [2] and integrin-mediated cell adhesion was assessed at 37°C. For adhesion to HBMEC-60 and HUVECs, PCa cells were incubated 30 min at 37°C with calcein AM in RPMI/0.2%, added at $6x10^4$ cells/well to confluent monolayers of HBMEC-60 or HUVEC grown on fibronectin-coated 96-wells (20 μg/ml), washed thrice and fluorescence measured as described (2) [2].

PCa TEM in Transwells. HBMEC-60 and HUVECs were seeded ($1.2x10^5$ cells/well) atop fibronectin-coated (20 μg/ml) Millicell Transwell Cell Culture Inserts (8 μm pores, EMD Millipore) and cultured 24hr. EC confluence was verified with 0.1% crystal violet/2% ethanol (J.T. Baker Chemical Co., Phillipsburg, NJ) and, where indicated, stimulated with 50 ng/ml IL-1β (Sigma-Aldrich) 4-6 hrs. PCa cells ($6x10^4$) labeled in 0.5 μM CellTracker Green (Life Technologies) were added to Transwells in RPMI/1% Pen/Strep and incubated at 37°C, 16 hrs. PCa cells that migrated to the Transwell underside were released with Trypsin/EDTA, transferred to 96-well plates, photographed under fluorescence at 40X and quantified digitally by NIS-Elements AR software (Nikon Instruments, Melville, NY). Where indicated, PCa cells were pre-treated 1hr at 37°C with CXCR4 antagonist AMD3100 (5 μg/ml) (Sigma-Aldrich), CCR2 antagonist sc-202525 (50 nM) (Santa Cruz Biotechnology, Santa Cruz, CA), Pertussis Toxin from *Bordetella pertussis* (250 ng/ml) (EMD Millipore), Rac1 inhibitor NSC23766 (50 μM) (EMD Millipore) or Rap1 inhibitor GGTI-298 (10 μM) (EMD Millipore).

Analysis of PCa formation in TRAMP mice lacking α1,3 FTs. TRAMP mice carrying the TRAMP transgene in an FVB background [$FVB^{TRAMPT/T}$] (4) [4] were crossed with mice homozygous null for FT4 and FT7 in a C57BL/6 background [$C57^{KO/KO}$] (The Jackson Laboratory, Bar Harbor, ME). The F1 offspring were crossed four times with $FVB^{TRAMPT/T}$ to generate F5 progeny with genotype $FVB^{KO/+;TRAMPT/T}$, yielding

mice that were 97% FVB. The $FVB^{KO/+;TRAMPT/T}$ mice were crossed with $C57^{KO/KO;+/+}$ to generate experimental animals either KO/KO or KO/+ in a C57:FVB (~50%/50%) background hemizygous for TRAMP transgene (T/+). This hybrid background C57:FVB (~50%/50%) was generated to minimize strain background variability in PCa invasion and localization as described (4,5) [4, 5]. At 18 and 23 weeks, mice were euthanized, prostates were microdissected into individual prostatic lobes using a dissecting microscope, examined for the presence of a solid tumor mass and prostatic tissue weighed. All procedures were performed in accordance with IACUC approval.

PCa cell trafficking. Homing analysis was performed as described (3) [3] and luciferase$^+$ PCa cell burden in bone was quantified by TaqMan probe and primers against firefly luciferase (Biosearch Technologies, Novato, CA): forward, 5'-TTCCGGGTGGTGCTGATGT-3', reverse, 5'-GGCGCTCTGGATCTTGTAGTC-3', TaqMan probe: FAM-5'-ACCGGTTCGAGGAAGAGCTGTTC-3'-BHQ-1.

Analysis of E-selectin binding glycolipids on PC-R1 cells. Gangliosides were extracted according to Schnaar (6) [6]. Briefly, cells were suspended in cold water (W) and methanol (M) at 8:3 (v/v), vigorously mixed and chloroform (C) added to a final ratio C/M/W; 4:8:3. After 2 hrs under stirring, the suspension was centrifuged to remove precipitated protein, and water was added to the supernatant to a final ratio 4:8:5,6; C/M/H. The upper phase, enriched in polar lipids, was applied to pre-washed reverse-phase cartridges (tC18 SepPak Plus, Waters, Milford, MA). After washing with M/W 1:3 and M/W 1:1, gangliosides were eluted with methanol and dried under N_2. An immune-overlay assay was performed to investigate if GSL from FT cells support E-selectin binding (7) [7]. Equal amounts of GSL from Empty and FT cells were resolved using TLC plates (Merck, Gibbstown, NJ). Solvent system was C/M/0.25% aqueous KCl (60:35:8 v/v/v). Dried plates were immersed in a mixture of hexanes and then transferred to polyisobutylmethacrylate (1 mg/ml) in hexanes. After drying at RT, plates were immersed in PBS and blocked in PBS-BSA 0.1% and Tween 20 (0.05%) 1 hr at RT. Plates were incubated with E-selectin/Fc (5 μg/ml in blocking buffer) 1 hr at RT, followed by alkaline phosphatase conjugated anti-human IgG under identical conditions. Plates were washed with PBS, washed once in TBS, immersed in nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution (Sigma–Aldrich) and the reaction stopped with water. A standard mixture of gangliosides (GM2, GM3, GM1,

GD3, GD1a, GD1b, and GT1b from Matreya, Pleasant Gap, PA), lacking glycans that support E-selectin binding, was used as a negative control. GSL bearing sialic acid were visualized in replicate plates after spraying with resorcinol followed by heating .

Analysis of chemokine receptor expression on PCa cells and PCa tissue. Flow cytometry was performed as described (2) [2] utilizing PE-anti-CXCR4 12G5, PE-anti-CXCR7 8F11-M16, PerCP/Cy5.5-anti-CCR2 TG5/CCR2, PE-mouse IgG2a, PE-mouse IgG2b, PerCP/Cy5.5-mouse IgG2b, all from BioLegend.

Supplemental Figures

Table S1. Primers for real-time RT-PCR of integrins and E-selectin binding glycoproteins.

Fig. S1. PCa cells prominently express β1 and αVβ3 integrins. (**A-B**) qRT-PCR of β expression in PCa cell lines or PCa tissue relative to level of lowest expressed β6 transcript (n=3 ± SEM). (**C**) Anti-integrin Ab (*Open histogram*) or isotype (*Shaded histogram*) of NPE, PCa or positive control KG1a cells; representative of n=3.

Fig. S2. β1 and αVβ3 are constitutively active and mediate PCa cell adhesion. (**A**) % PCa cell adhesion to integrin ligands (n=9 ± SEM). (**B**) FACS analysis of anti-β1 activation-sensitive Abs (*Open histogram*) or isotype control on PCa cell lines (*Shaded histogram*); (n=3). (**C-E**) PC-E1 cell adhesion to integrin ligands or HBMEC-60 cells in the presence of blocking anti-β1 or αVβ3 mAbs or isotype Abs (n=9 ± SEM. *, $P<0.05$; **, $P<0.01$, vs. isotype; one-way ANOVA with Dunnett post test).

Fig. S3. Modulating FT expression does not alter PCa cell integrin expression or function. (**A-C**) % PC-R1 FT cell adhesion to integrin ligands (n=3 ± SEM). (**D-E**) Real time RT-PCR of α and β integrin subunits and candidate E-selectin-binding glycoproteins in PC-R1 FT cells relative to expression in empty cells (n=3 ± SEM).

Fig. S4. FT expression generates E-selectin-binding determinants on PCa cell glycoproteins and glycolipids. (**A**) FACS analysis of sLe^X and Le^X (*Open histograms*) or isotype (*Shaded histograms*); n=3. (**B**) Western blot analysis of sLe^X on PC R1 FT cell glycoproteins. (**C**) TLC of GSLs on PC-R1 FT cells, as detected with resorcinol or developed after incubation with E-selectin/Fc. (**D**) FACS analysis of CD44,

sLe^X and E-selectin ligands on untreated or bromelain-digested PC-R1 FT cells. Mean fluorescence intensities were normalized to untreated controls and presented as % untreated control (n=3 ± SEM; **,P<0.01;***, P<0.001, *t*-test). (**E-F**) Analysis of rolling or velocity (at 1 Dyne/cm^2) of bromelain-digested PC-R1 cells on E-selectin/Fc 1 Dyne/cm^2. (**G**) FACS analysis of E-selectin, VCAM and ICAM-1 on IL-1β-stimulated HUVEC and HBMEC-60 cells (n=3).

Fig. S5. Chemokine receptor expression is variably expressed on human PCa cell lines and on localized and metastatic PCa tissue. (**A**) FACS analysis of CXCR4, CXCR7 and CCR2 on PCa cell lines and on KG1a and HUVEC controls; primary Abs (*Open histogram*) or isotype Ab control (*Shaded histogram*) (n=3). (**B-E**) Real time RT-PCR of CXCR4, CXCR7, CCR2 and CCR4 expression in human PCa tissue relative to NPE tissue after normalizing to GAPDH in the same sample (n=3 ± SEM).

Fig. S6. E-selectin ligand$^+$ PC-R1 FT7 cells breach HUVEC under shear flow without exogenous chemokines. (**A**) % PC-R1 FT7 rolling cells that breached IL-1β-stimulated HUVEC in RPMI media devoid of serum and exogenous growth factors/chemokines (n=3 ± SEM). (**B**) Confocal fluorescence imaging projected in 3-dimensions of breached PC-R1 FT7 cells (green) within confluent HUVEC monolayers ((red) at 100X magnification.

Movies S1-S2. E-selectin ligand$^+$ RC-R1 FT7 cells roll, adhere and breach on HBMEC-60 cell monolayers under physiologic flow. (Movie S1) E-selectin ligand$^+$ PC-R1 FT7 cells or (**Movie S2**) E-selectin ligand$^-$ PC-R1 empty cells were infused into the parallel-plate flow chamber over IL-1β-stimulated E-selectin$^+$ HBMEC-60 monolayers at 1.5 ml/min for 35s, and a physiologic shear stress of 0.5 Dynes/cm^2 was maintained. Cell rolling, firm adhesion and breaching was recorded over 250 minutes.

Supplemental Tables

Table S1

Gene	Primer sequences 5'-3'	Product size (BP)
α2	F: GCAACTGGTTACTGGTTGGTT	167
	R: GAGGCTCATGTTGGTTTTCATCT	
α4	F: GTCCTTGTTTAATGCTGGAGATGAT	101
	R: GCTTCTCTTCCAGCTCTAAAATCTT	
α5	F: TTCTGGAGTATGCACCCTGC	116
	R: TGGTCCACCTAAAACCACACG	
α6	F: GCTCGAGGTTATGGAACAGC	97
	R: GCAGCAGCAGTCACATCAAT	
αV	F: GTCCCCAAGTCACTCCAAGA	88
	R: AGATTCATCCCGCAGATACG	
αIIb	F: GGGACAAGCGTTACTGTGAAG	105
	R: GGGCCAGGAGACCTAAGAAATA	
β1	F: GTAACCAACCGTAGCAAAGGAACAGC	109
	R: ATGTCTGTGGCTCCCCTGATCTTA	
β2	F: ACGAATTCGACTACCCATCG	85
	R: CTACTGGTCACCGCGAAGAT	
β3	F: AAGGAGGAGTCAGGGAGAGC	85
	R: CCTTGCGAGAGAACCATAGC	
β4	F: GGCAACCGGGACTACATCC	113
	R: CGCAGGAGGGAGTCAACTT	
β5	F: AAGTGCCACCTCATGTGAAGA	135
	R: CCACAGCCATTTTTGACAAGG	
β6	F: TCCATCTGGAGTTGGCGAAAG	134
	R: TCTGTCTGCCTACACTGAGAG	
β7	F: ATGGTGGCTTTGCCAATGGT	120
	R: GGACAGGTGAGGATTCCGC	
β8	F: CAGCACTGTGTCAATTCAAAGG	132
	R: GCAGGCTGTATAACAGGTGGG	
CD44	F: GACCTCTGCAAGGCTTTCAA	69
	R: TCCGATGCTCAGAGCTTTCTC	
PSGL-1	F: ATGATTTCCTGCCAGAAACG	93
	R: TGGTAGACTCAGGGGTTCCA	
PCLP	F: CAGAGGAGCTGCAGACAGTG	103
	R: TTGAGGCTGACCACCTTCTT	
GAPDH	F: CAGCCTCAAGATCATCAGCA	138
	R: ACAGTCTTCTGGGTGGCAGT	

Supplemental References

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