**SUPPLEMENTAL MATERIALS AND METHODS:**

**Quantitative real-time PCR**

Quantitative Real-time PCR was performed as described previously (1). Briefly, total RNA was purified using the RNeasy Minikit (Qiagen). First-strand cDNA was synthesized using random hexamer primers (Applied Biosystems) and Ready-To-Go You-Prime First-Strand Beads (GE Healthcare), according to the manufacturers’ instructions. Quantitative PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems) and TaqMan primers specific to mouse *Sparcl1* (Applied Biosystems), human *SPARCL1* (Applied Biosystems), and human *GSTP1* (Applied Biosystems). Applied Biosystems software was used to calculate threshold cycle values for *SPARCL1*, *GSTP1* and the reference gene *HPRT*.

**Johns Hopkins University prostate cancer anti-androgen therapy tissue microarray**

The design of the anti-androgen therapy tissues microarray has been described previously (2). The TMA contained ADT-treated localized tumor samples from 52 patients treated briefly (usually 1-6 months) with a luteinizing hormone-releasing hormone agonist before sampling at radical prostatectomy. On evaluation by an expert genitourinary pathologist, all cases showed histologic features of ADT. One 5-µm section cut from the TMA was stained for SPARCL1 as described above. The extent and intensity of SPARCL1 staining was determined by urologic pathologists using digitized TMAJ software. A single score, called the H-score, which integrated both the extent and intensity of SPARCL1 staining was digitally computed by TMAJ software for each core. After exclusion of technically inadequate TMA cores, the final analysis included 46 cases and 18 controls.

**Androgen regulation of *SPARCL1***

LNCaP cells were grown in 10% FCS serum (Gibco) for 48 hours or washed three times for 1 hour each in serum-free media and then grown in 10% charcoal:dextrane stripped serum (Gibco) for 48 hours. Additionally, cells were washed three times for 1 hour each in serum-free media, grown in 10% charcoal:dextrane stripped serum for 48 hours and then grown in 10% charcoal:dextran stripped serum with 100nM DHT for 48 hours. RNA, cDNA, and quantitative RT-PCR was performed as described above.

**Chromatin immunoprecipitation assay (ChIP)**

ChIP was performed similarly as described previously (3). LNCaP and LAPC4 cells were washed three times for 1 hour with serum free media and then grown in media containing 10% charcoal:dextran stripped FBS (Gibco) for 48 hours. Cells were treated with 100nM DHT or vehicle for 12 hours. DNA was cross-linked with 1% formaldehyde in PBS for 10 minutes, treated with 2.5M glycine, lysed in lysis buffer (1% SDS, 5mM EDTA, 50mM Tris HCl, pH8.1), sonicated to a fragment size of 300-800bp, pre-cleared with Dynabeads protein G (Life Technologies), and then incubated with primary antibody (AR [Millipore], and H3K27Ac [Abcam] over-night at 4ºC. Dynabeads were blocked with yeast tRNA and then incubated with lysates/primary antibody for 4 hours at 4ºC. Beads were sequentially washed with TSEI (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris HCl, pH 8.1, 150mM NaCl), TSEII (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris HCl, pH 8.1, 500mM NaCl) and TSEIII (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1mM EDTA, 10mM Tris HCl, pH 8.1). DNA was eluted with IP Elution buffer (1% SDS, 0.1M NaHCO3, proteinase K) and incubated at 56ºC for 15 minutes. Quantitative RT-PCR primers for AR ChIP at the *SPARCL1* locus: Forward: 5’ TGGCCTATCATTTCCTGGAG 3’ and Reverse: 5’ TAGCAGTCTACAGGCTTCCTCTG 3’ (amplicon size 100 bp). Quantitative RT-PCR primers for ChIP at a site upstream of *SPARCL1*: Forward: 5’ CCTGGGCTGTCATAACAAAT 3’ and Reverse: 5’ GGTGCCTGCTGACACCTTA 3’ (amplicon site 111 bp). Quantitative RT-PCR primers for H3K27Ac ChIP: Forward: 5’ GCCACACATTTAGGCTCCTT 3’ and Reverse: 5’ ATGTGAGATCATGCGGACAG 3’ (amplicon 246 bp).

**Immunohistochemistry**

Immunohistochemistry (IHC) was performed as described previously (1). Briefly, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, deparaffinized, steamed in Target Retrieval Solution Ready to Use (Dako) for 40 minutes, blocked with Protein Block Serum-Free (Dako), incubated with antibodies directed against SPARCL1 (Abcam), p63 (Millipore), CK8 (Covance), Ki67 (Abcam), and CD31 (Millipore) in Antibody Diluent (Invitrogen). Primary antibodies were followed by HRP linked secondary antibodies (Vector Laboratories). IHC was detected with 3,3’-Diaminobenzidine kit (Vector Laboratories).

**Cell proliferation assay**

Cell proliferation of Myc-CaP Neo and mSPARCL1 clones was assayed on type I collagen plates using the tetrazolium dye MTT as described previously (1).

**Live cell micromechanical methods**

Using *spontaneous* and *forced* motions of RGD-coated ferrimagnetic microbeads anchored to the cytoskeleton through cell surface integrin receptors, we detected the rate of cytoskeletal remodeling and cell mechanical properties using Magnetic Twisting Cytometry (MTC), respectively. These methods are described in detail elsewhere (4-7). For these studies, PC3 cells were plated at 30,000 cells/cm2 on collagen-coated or collagen+rSPARCL1-coated plastic wells (96-well Removawell, Immulon II, Dynetech). We applied *forced* bead motions using Magnetic Twisting Cytometry (MTC) (5). In brief, an RGD-coated ferrimagnetic bead bound on the surface of the cell was magnetized horizontally with a brief 1,000-Gauss pulse and twisted in a vertically aligned homogeneous magnetic field (20 Gauss) that was varying sinusoidally in time. The sinusoidal twisting magnetic field causes both a rotation and a pivoting displacement of the bead (5): as the bead moves, the cell develops internal stress that bead resist bead motion. Lateral bead displacements in response to the resulting oscillatory torque were detected via a CCD camera (Orca II-ER, Hamamatsu, Japan) attached to an inverted optical microscope (Leica Microsystems, Bannockburn, IL), with an accuracy of 5 nm using an intensity-weighted centre-of-mass algorithm (5). We defined the ratio of specific applied torque to lateral bead displacements as the complex elastic modulus (g\*) of the cell,, where g’ is the elastic/storage modulus (cell stiffness), g” is the loss modulus (cell friction), and i2=-1 (2). Cell stiffness and friction were measured over a physiologic range of frequency (*f*) and are expressed in units of Pascal per nm (Pa/nm).

We then visualized *spontaneous* nanoscale movements of an individual bead (~4.5 µm in diameter) bound on adherent PC3 cells (~50-100 beads per field of view) and recorded its positions every 83 ms. The trajectories of bead motions in two dimensions were then characterized by computing the mean square displacement of all beads as function of time [MSD(t)] (nm2), as previously described (4). Herein, we analyzed MSD data for times greater than 10s and up to 300s. In addition, diffusion coefficient D\* and the exponent α of the bead motion were estimated from a least-square fit of a power-law to the ensemble average of MSD data versus time (4).

**Fourier transform traction microscopy**

Using traction microscopy, we measured the distribution of traction fields arising at the interface between each adherent cell and its substrate (collagen *vs.* collagen+rSPARCL1). As described in detail elsewhere (6-9), we prepared inert elastic polyacrylamide gel blocks and coated them with collagen type I (0.2 mg/ml) using a photo-activating cross-linker sulfo-SANPAH (Pierce, Rockford, IL). For the preparation of collagen+rSPARCL1 substrate, recombinant human SPARCL1 (R&D Systems; 2728-SL) was incubated for additional 24 hours in PBS (10 µg/ml) on collagen-coated gel blocks. In brief, PC3 cells were plated sparsely on the respective gel blocks, and allowed to adhere and stabilize for 24 hours. For each adherent cell, images of fluorescent microbeads (0.2 µm in diameter, Molecular Probes, Eugene, OR) embedded near the gel apical surface were taken at different times; the fluorescent image of the same region of the gel after cell detachment with trypsin was used as the reference (traction-free) image. The displacement field between a pair of images was then obtained by identifying the coordinates of the peak of the cross-correlation function (8). From the displacement field and known elastic properties of the gel (Young’s modulus of 8,000 Pa with a Poisson’ ratio of 0.48), the traction field was computed using both constrained and unconstrained Fourier transform traction cytometry (8). The computed traction field was used to obtain net contractile moment, which is a scalar measure of the cell’s contractile strength (8). Net contractile moment is expressed in units of pico-Newton meters (pNm).

**Immunofluorescence**

Type I Collagen Coated Transwell Slides (Corning) were incubated with vehicle or 10µg/ml rhSPARCL1 overnight at 4ºC. PC3 cells were plated on Type I Collagen/vehicle coated and Type I Collagen/SPARCL1 coated transwell slides overnight in RPMI media (Gibco) containing 10% FCS (Gibco) and penicillin and streptomycin (Gibco). Cells were washed twice with PBS, fixed with 10% neutral buffered formalin in PBS for 10 minutes, washed twice with PBS, incubated in 0.1% Triton X-100 in PBS for 5 minutes, washed twice with PBS, blocked with 1% BSA in PBS for 30 minutes, incubated with Paxillin (Cell Signaling Technology) or Vinculin (Cell Signaling Technology) for 1 hour, washed three times with PBS, incubated with a secondary Alexa Fluor labelled antibody (Invitrogen) and Alexa Fluor labelled Phallodin (Invitrogen) for 1 hour in the dark, and then coversliped using Hard Set Mounting Media with DAPI (Vectashield). Cells were imaged using the Nikon EZ C1 confocal microscope with 4 lasers at 600x magnification.

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**SUPPLEMENTARY FIGURE LEGENDS:**

**Supplementary Figure S1.** Androgen suppresses SPARCL1 expression. A, Relative *SPARCL1* expression in untreated men compared to men who received ADT (1). B, SPARCL1 expression in prostates from castrated mice compared to castrated mice followed by 3 days of DHT as examined by IHC (400x magnification).

**Supplementary Figure S2.** AR directly represses *SPARCL1* expression in prostate cancer. A, B, ChIP seq analysis of the *SPARCL1* locus in vehicle and R1881 treated LNCaP, VCaP, and human prostate tissue (2). C, Schematic of AR binding sites in the enhancer and promoter of *PSA* and enhancer of *TMPRSS2*. Black bars represent the location of the primers following AR ChIP (3). D, ChIP of AR in LNCaP cells treated with vehicle or 100nM DHT followed by qRT-PCR of the *KLK3* enhancer, middle and promoter and the *TMPRSS2* enhancer. E, *GSTP1* expression as determined by qRT-PCR in LNCaP and CWR22RV1 cells treated with vehicle or 1µM 5-Aza-2’-deoxycytidine (DNA methyltransferase inhibitor) for 3 days (n=3). F, *SPARCL1* expression as determined by qRT-PCR in LNCaP cells treated with vehicle or HDAC inhibitor (Panobinostat). G, ChIP of H3K27Ac at *SPARCL1* normalized to the absence of DHT (C/D). Statistical analyses performed by Student’s *t* test (F) and One-way ANOVA (G) (mean ± SEM; n=3; \**P*≤0.05 and \*\**P*≤0.01).

**Supplementary Figure S3.** The tumor microenvironment in *Sparcl1*-/- models.A, Representative images (400x magnification) of immune infiltration and vascularity as measured by IHC of PAX5 (B cells), CD3 (T cells), F4/80 (macrophages) and CD31 (vasculature) in Hi-Myc and Hi-Myc/*Sparcl1*-/- prostates with cancer at 4.5 months (n≥3). B,Representative images (400x magnification) of cellular proliferation and vascularity as measured by IHC of Ki67 (proliferation) and CD31 (vasculature) in Myc-CaP orthotopic allografts in WT and *Sparcl1*-/- prostates (n≥3).

**Supplementary Figure S4.** SPARCL1 inhibits cytoskeletal remodeling. A, Myc-CaP-EV and Myc-CaP-mSPARCL1 stable clones as examined by light microscopy. B, SPARCL1 significantly decreased the speed of cytoskeletal remodeling as determined by computed mean square displacement (MSD) at 300s in Myc-CaP parental (n=376), Myc-CaP-EV (A) (n=484), Myc-CaP-EV (B) (n=804), Myc-CaP-mSPARCL1 (A) (n=988), and Myc-CaP-mSPARCL1 (B) (n=663). Statistical analyses performed by One-way ANOVA, multiple comparisons using Natural Log transformed data compared to the Myc-CaP parental cells (B) (mean ± SEM; \**P*≤0.05).

**Supplementary Figure S5.** SPARCL1-coated beads do not reinforce cellular traction. A, Representative phase contrast and deformation of field of cells adhered to collagen through RGD-coated beads or rSPARCL1 coated beads. B, Compared to PC3 cells adhered to collagen through RGD-coated beads, cells adhered to collagen through rSPARCL1 coated beads tended to decrease cell spreading as measured by cell traction microscopy. C, Compared to PC3 cells adhered to collagen through RGD-coated beads, cells adhered to collagen through rSPARCL1 coated beads did not reinforce cellular traction. D, Representative bright field and traction field images of cells adhered to Type I Collagen through RGD-coated beads or rSPARCL1 coated beads. E, Representative RMS traction images of cells adhered to Type I Collagen through RGD-coated beads or rSPARCL1 coated beads. F, SPARCL1 in the ECM inhibits focal adhesion assembly**.** Focal adhesion assembly measured by immunofluorescence of Vinculin (focal adhesion), Phallodin (actin fibers), and DAPI (nuclei) of PC3 cells on a matrix of collagen (n=35) or collagen+rSPARCL1 (n=59).

**Supplementary Figure S6.** SPARCL1 engages cell-ECM interactions. A, SPARCL1 tethers to the cytoskeleton as measured by the binding kinetics of microbeads coated with RGD or rSPARCL1 to the cell surface. B-C, computed mean square displacement (MSD) (B) and MSD up to 300s (C) incubation time of RGD-coated and rSPARCL1-coated microbeads to PC3 cells.

**Supplementary Figure S7.** Responses to SPARCL1-coated beads are concentration dependent. A-C, The exponent α (A), Diffusion coefficient (B), and MSD (C) of PC3 cells adhered to collagen through rSPARCL1-coated beads were concentration dependent on rSPARCL1.

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