SUPPLEMENTARY METHODS

Cell Lines: The human CRPC cell lines, C4-2 and PC3, were maintained in phenol red-free Roswell Park Memorial Institute (RPMI) 1640 media (Life Technologies, Carlsbad, CA) supplemented with 5% fetal bovine serum (FBS, Life Technologies) and Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) with 10% FBS respectively in a 5% CO_2/37°C incubator. All experiments were performed within 10 passages of thawing. Cell line authentication was performed by short tandem repeat profiling (John's Hopkins University) and mycoplasma testing was performed routinely using a PlasmoTest™ kit (InvivoGen, San Diego, CA).

HDL-Cholesterol Uptake: Cells were incubated in standard media containing 0.5% BSA and DiI-HDL (10 μg HDL protein per mL) for 2 h and suspended in PBS prior to being subjected to flow cytometry analysis. Mean fluorescent intensity was normalized to the mean fluorescent intensity of non-treated cells incubated with DiI-HDL.

Quantitative PCR: RNA was isolated from siRNA transfected cells using TRIzol (Life Technologies) and cDNA was synthesized using SuperScript® II Reverse Transcriptase kit (Life Technologies) following manufacturer instructions. qPCR was performed on an ABI 7900HT (Life Technologies) using FastStart Universal SyberGreen Master mix (Roche, Indianapolis, IN).

Western Blotting: Whole cell lysates were prepared using modified radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitors (Roche). Protein concentrations of cell lysates were measured using the Pierce BCA Protein Assay (Fisher, Hampton, NH). Samples were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretic transfer to

polyvinylidene difluoride membranes (Millipore, Burlington, MA). Membranes probed with the described antibodies were imaged either by enhanced chemiluminescence detection (Supersignal West Pico, Thermo Fisher, Waltham, MA) or on an Odyssey CLx infrared imaging system using Odyssey software version 3.0 (LI-COR, Lincoln, NE).

Steroid analysis: Samples containing internal standard (deuterated testosterone/DHT) were extracted with 60/40 hexane/ethyl acetate, dried, reconstituted in 50 mM hydroxylamine and incubated at 65˚C. The resulting steroid-oxime derivatives were analyzed using a Waters Acquity UPLC Separations Module coupled to a Waters Quattro Premier XE Mass Spectrometer (Waters, Milford, MA) using a 2.1x100 mm BEH 1.7 µM C18 column and a water and acetonitrile mobile phase gradient. Data was collected in ES+ mode by multiple reaction monitoring. Quantification was by AUC ratio of standard to internal standard.

Pharmacokinetics: Athymic nude mice (Crl:NU-Foxn1nu; Harlan) were dosed by oral gavage with either 25 mg/kg BLT-1 in propylene glycol. Blood samples were obtained via capillary collection of tail vein bleeds at the following time points: 0, 0.5, 1, 1.5, 2, 4, 8 and 24 h post dosing. Capillaries were spun by centrifuge at 3000 g for 10 min to isolate serum for analytical. Following the final tail vein bleed mice were exsanguinated by cardiac puncture to collect blood from which serum was prepared and flash frozen prior to analysis by LCMS.

BLT-1 analysis: Serum samples for each of the time points collected were subjected to LC-MS analysis. Sample extraction was performed in which 2 µL of 1 µM VPC-13226 internal standard was combined with 8 µL of serum and 22 µL of acetonitrile (ACN) prior to vortexing for 10 sec. Precipitate was pelleted by centrifugation at 20,000 g for 5 min

and supernatant transferred to LC vials for analysis. Standards were prepared in a similar fashion with standard/IS spiked mouse serum (2 µL standard/2µl IS/20µl ACN) while parallel standards made up in 67% ACN were used to characterize matrix effects. Samples and standards were analyzed using a Waters Acquity UPLC Separations Module coupled to a Waters Quattro Premier XE Mass Spectrometer (Waters). Separations were carried out with a 2.1x100 mm BEH 1.7 µM C18 column (Waters) and a 50-98% acetonitrile (ACN) gradient from 0.2 - 3 min (0.3 mL/min) used for separation (with 1 min 98% ACN flush and a 2 min re-equilibration; 6 min run length; 0.1% formic acid throughout). Data was collected in ES+ mode and compounds were detected by multiple reaction monitoring with mass to charge (m/z) transitions of 242.1>166 and 242.1>95.9 for BLT1 and m/z 256.1>110.8 for IS. Retention times for BLT-1 and IS were 3.16 min and 2.83 min respectively. Data analysis was performed with Quanlynx (Waters) with AUC of BLT-1 normalized to IS AUC. Comparisons with acetonitrile based and serum based standard extracts indicated high extraction efficiency (>90%) but substantial ion suppression with BLT-1 (~50%), therefore serum-based standards were used for the generation of the standard curve with 7 calibration standards (4 nM to 4 µM, R2>0.99) for the determination of BLT-1 concentration in test samples.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1: Post-hoc multiple comparisons of IHC scoring of samples from the UWRA program.

Comparison	Corrected p-value*
Normal vs PCa	1.92E-25
Normal vs Bone	1.71E-12
Normal vs Liver	9.90E-23
Normal vs LN	1.75E-24
Normal vs Lung	6.67E-17
PCa vs Bone	0.0419
PCa vs Liver	>1.00
PCa vs LN	0.9633
PCa vs Lung	3.0314
Bone vs Liver	0.0018
Bone vs LN	0.0003
Bone vs Lung	0.1033
Liver vs LN	>1.00
Liver vs Lung	>1.00
LN vs Lung	>1.00

* Chi squared p-value

Supplementary Table 2: Assessment of common serum toxicity markers following repeated BLT-1 dosing in mice.

		BLT-1 Dose	
Marker (units measured)	**Vehicle ± SEM**	**25 mg/kg ± SEM (p-value vs Vehicle)***	**50 mg/kg ± SEM (p-value vs Vehicle)**
ALB (g/L)	45.2 ± 1.07	43.5 ± 1.19 (0.657)	50.3 ± 2.18 (0.047)
ALP (U/L)	42.7 ± 3.49	53.3 ± 8.07 (0.651)	86.1 ± 17.51 (0.005)
ALT (U/L)	47.8 ± 4.45	43.3 ± 9.46 (0.989)	131.6 ± 57.75 (0.062)
AMY (U/L)	1161.9 ± 141.44	962.7 ± 34.94 (0.484)	1243.0 ± 68.75 (0.894)
TBIL (mmol/L)	4.8 ± 0.21	5.0 ± 0.31 (0.906)	6.3 ± 0.42 (0.004)
BUN (mmol/L)	6.8 ± 0.27	7.3 ± 0.57 (0.692)	6.1 ± 0.52 (0.495)
CA (mmol/L)	2.9 ± 0.07	2.9 ± 0.07 (0.952)	2.9 ± 0.07 (0.997)
PHOS (mmol/L)	2.8 ± 0.19	3.6 ± 0.21 (0.051)	2.5 ± 0.31 (0.613)
CRE (mmol/L)	18.7 ± 0.75*	20.71 ± 1.76** (0.163)	18.0 ± 0.00** (0.751)
GLU (mmol/L)	13.4 ± 0.70	11.9 ± 0.74 (0.372)	8.4 ± 0.82 (<0.001)
NA+ (mmol/L)	167.4 ± 1.72	170.71 ± 2.58 (0.438)	169.3 ± 0.42 (0.771)
K+ (mmol/L)	8.5 ± 0.00**	8.5 ± 0.00*** (----)	8.5 ± 0.00*** (----)
TP (g/L)	58.8 ± 1.39	60.8 ± 1.72 (0.660)	67.6 ± 2.15 (0.004)
GLOB (g/L)	14.0 ± 0.93	17.43 ± 0.87 (0.086)	17.3 ± 1.65 (0.118)

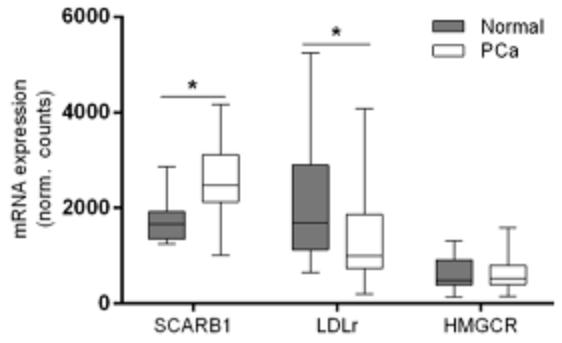
* Tukey's adjusted p-values post one-way ANOVA as describe in Materials and Methods.

** CRE levels were only detectable at >18.0 mmol/L, as such 18 mmol/L was used to represent all values less than or equal to 18 mmol/L.

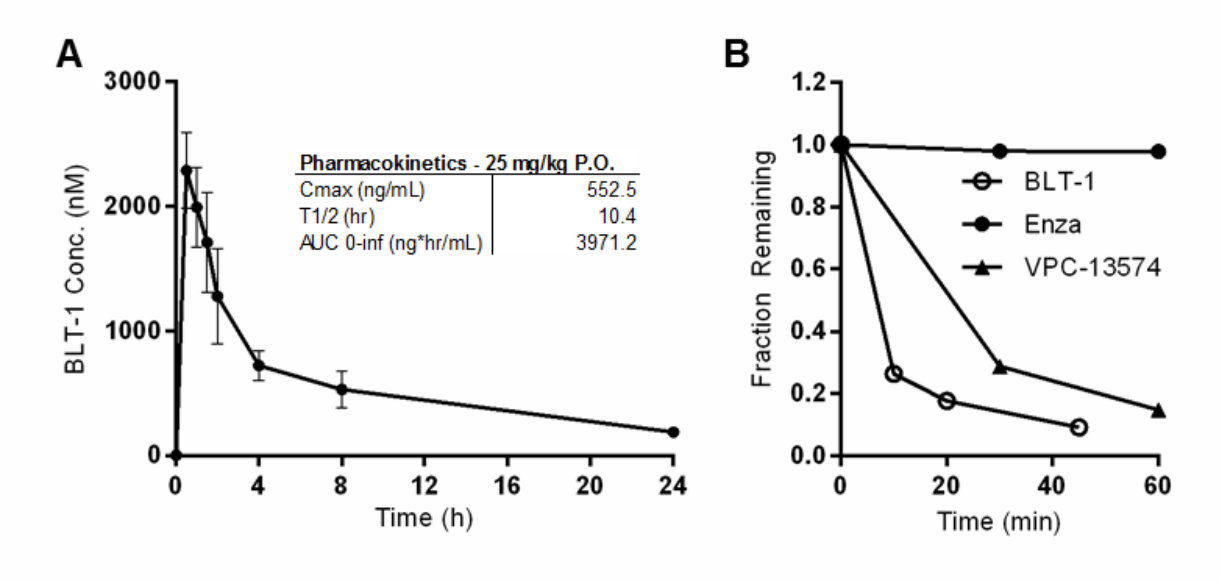
*** K+ levels were only quantifiable at < 8.5 mmol/L, as such 8.5 mmol/L was used to represent all values greater than or equal to 8.5 mmol/L.

Supplementary Table 3: Individual mouse tumor growth rates with vehicle or 25 mg/kg BLT-1 treatment.

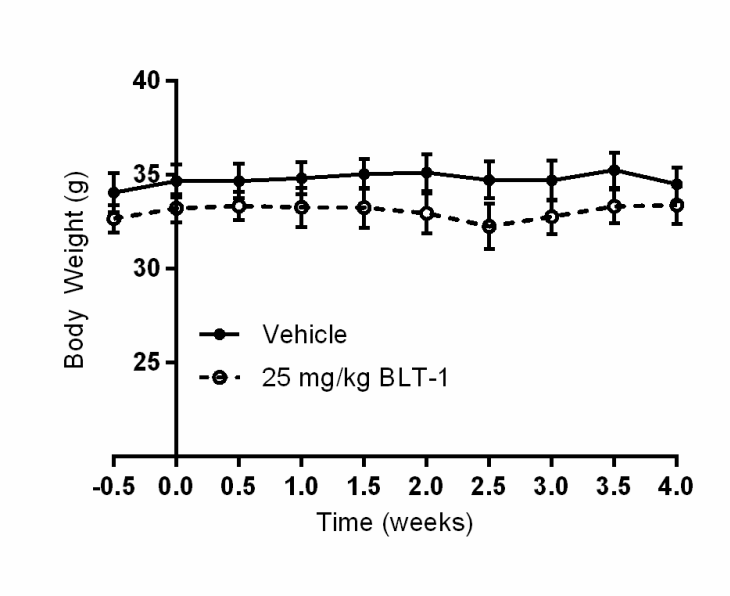
	Tumor growth rate (mm^3/week)	
Mouse	Vehicle	BLT-1
1	106.1	232.6
2	137.2	30.9
3	197.6	135
4	236.2	43.2
5	195.8	85.3
6	218.4	79.5
7	136.9	187.4
8	99.8	137.5
9	282.7	143.5
10	214.3	30.3
11	43.0	116.1
12	139.8	21.4
13		106.1
Average	167.3	103.8
SEM	19.6	17.8



Supplementary Figure 1: SR-B1 expression is increased in prostate cancer
The mRNA expression levels of important cholesterol metabolism proteins were assessed from samples available in The Cancer Genome Atlas (TCGA) database. The expression of SR-B1 ($p < 0.001$) was increased in cancerous prostatic tissue (PCa) and LDLr ($p < 0.001$) expression decreased when compared to normal prostatic tissue (Normal)(Cancer: n = 375, Normal: n = 48, ANOVA with Sidak's Test).



Supplementary Figure 2: Pharmacokinetic assessment of BLT-1. (A) Serum samples from mice dosed with 25 mg/kg BLT-1 were assessed for BLT-1 concentration over time and used to calculate basic pharmacokinetic parameters (n = 3). **(B)** BLT-1 was incubated in the presence of mouse liver microsomes and concentration measured over time. Enzalutamide (Enza) and VPC-13574 were used non-labile and labile controls respectively (n = 1).



Supplementary Figure 3: Mice receiving BLT-1 maintain body weight. Twice weekly body weight measurements of mice receiving either vehicle or 25 mg/kg BLT-1 by oral gavage daily.