**Supplementary Materials and Methods:**

**Bioinformatic analysis:**

Comparison of *CYP27A1* mRNA levels between different stages of PC: Data from seven independent studies (Tomlins-GSE6099, Chandran-GSE6752, Tamura-GSE6811, Yu-GSE6919, Wallace-GSE6956, Taylor-GSE21032, and Brase-GSE29079) were downloaded from GEO and processed with R software. Clinical information *was obtained from GEO, the original publication, or by contacting the authors. Box plots showing CYP27A1* expression and annotated sample types were generated using ggplot2 in R. All P-values reported are calculated using a t-test.

**CYP27A1 IHC analysis:** We used tissue microarrays (TMAs) that included duplicate 1 mm cores of formalin fixed, paraffin embedded PC and benign prostate tissue from men treated with radical prostatectomy. The TMAs included paired PC samples and benign prostate tissue. A total of 101 cancer cases and 119 benign samples were evaluable for CYP27A1 expression. TMA sections were deparaffinized, treated with sub-boiling antigen retrieval buffer (citrate, pH 6) for 20 minutes, and then reacted with an anti-CYP27A1 rabbit monoclonal antibody (ab126785 from Abcam, Cambridge, MA) at 1:300 for 2 hours. The detection reaction utilized the rabbit Envision kit from Dako-Agilent Technologies (Santa Clara, CA). Diaminobenzidine (DAB) was used as chromogen, with hematoxylin as counterstain. The IHC experiments were performed on an automated immunostainer (Intellipath from Biocare, Concord, CA). Positive cells showed granular cytoplasmic reactivity. All analysis including cell type identification and staining intensity was performed by a board certified pathologist (JG). Staining intensity in tumor cells was scored prospectively as 0 (absent), 0.5 (borderline), 1 (weak), 2 (moderate) or 3 (strong) by a board certified pathologist (JG) blinded to clinical information. For statistical analysis, the tumors were categorized as negative (0 and 0.5) or positive (1, 2 and 3). A chi-square test was used to test association between expression of CYP27A1 (treated as a binary variable) and prostate adenocarcinoma vs. benign tissue.

**Cell culture and *in vitro* assays:**

Chemicals, reagents and antibodies: Media, Annexin V and Sytox antibodies were purchased from Invitrogen (Carlsbad, CA, USA). The synthetic androgen, R1881, was purchased from PerkinElmer (Waltham, MA, USA). Enzalutamide, doxycycline, puromycin, 27-hydroxycholesterol, cholesterol and (2-Hydroxypropyl)-β-cyclodextrin were purchased from Sigma (St Louis, MO). The LXR agonist TO901317 (T1317) was purchased from Tocris Bioscience (Ellisville, MO). CYP27A1 and LDLR antibodies were purchased from Abcam (Cambridge, MA). GAPDH, cleaved PARP, p27 andβ-actin antibodies were purchased from Cell signaling (Danvers, MA). β-tubulin antibody was purchased from Santa Cruz Biotechnology (Burlingame, CA). Custom oligonucleotide primers were from IDT (San Diego, CA, USA). Human lipoprotein deficient serum and human lipoprotein LDL were both purchased from INTRACEL (Frederick, MD). Small interfering RNA (siRNA) (siLDLR: SAS1\_Hs02\_00302732, SAS\_Hs02\_00302733; siLXR α/β: SAS\_Hs01\_\_00207735, SAS\_Hs01\_00229530) were purchased from Sigma to transiently silence LDLR and LXRs (α and β). Negative control siRNAs targeting either luciferase (siLuc) or a nonspecific siRNA with a medium GC content (MED-GC) were purchased from Invitrogen.

Plasmids: The pLenti CMV TRE3G puro Gal4-DBD (control) and pLenti CMV TRE3G puro CYP27A1 plasmids were generated by subcloning Gal4-DBD and CYP27A1 cDNA into pENTR1a (Invitrogen) and recombining into pLenti CMV TRE3G puro DEST (Addgene, Cambridge MA). pQCXIP (retroviral vector) was purchased from Clontech (Mountain View, CA). pQCXIP-LDLR was generated by PCR amplification of insert followed by subcloning into pQCXIP.

Caspase 3/7 activity assay: The assay was performed as described in Fritz et al.([60](#_ENREF_60)) with modifications. Briefly, LNCaP and 22RV1 cells were seeded at a density of 10,000 cells/well and treated with different concentrations of 27HC, in combination with cholesterol (dissolved in 40% 2-hydroxypropyl-β-cyclodextrin) or vehicle control. Twenty-four hours later, 50µl of the following buffer ((50mM HEPES pH 7.5, 100mM KCl, 5mM EDTA, 10mM MgCl2, 10mM CHAPS, 20% Sucrose, 10mM DTT, 10µM of (Z-DEVD)2-Rh110 (Santa Cruz Biotech) and complete protease inhibitor (Roche)) was added to each well. Following an incubation period of 18 hours at 37°C, fluorescence was measured at Excitation/Emission: 485/535nm.

27HC and cholesterol measurements: Cell pellets, tumors following cryo-grinding, and spent media were saponified with 0.9M ethanolic NaOH containing d6-27HC and d7-cholesterol internal standards (Avanti Polar Lipids), then liquid/liquid extracted into hexanes and dried. 27HC was measured against a calibration curve, while cholesterol was measured using single-point calibration to the internal standard. Calibration standards were prepared alongside the samples, using 50 mg/ml bovine serum albumin as matrix. Hydroxycholesterols and cholesterol were derivatized with dimethylaminophenylisocyanate (DMAPI) in dimethylformamide (DMF) and triethylamine (TEA), and quenched with phosphate buffer. Analytes were again extracted into hexanes, dried, and finally resuspended in 4/3/1 v/v/v/ IPA/MeCN/water, then measured using a reversed-phase targeted LC-MS/MS assay (Acquity UPLC and Xevo TQ-S mass spectrometer) which separates the isoforms of hydroxycholesterol based retention time and MS/MS fragmentation.