Supplementary Information

Materials and methods

Cell culture

Human PCa cell lines $ARCaP_E$, $ARCaP_M$, PC-3, and C4-2 were routinely maintained in T-medium (ThermoFisher, Carlsbad, CA) with 5% fetal bovine serum (FBS). Genistein was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO).

Quantitative PCR (qPCR)

Total RNA was prepared with Qiagen RNeasy Kit (Valencia, CA). The first-strand cDNA was synthesized using SuperScript ®III First-Strand Synthesis System (ThermoFisher). Quantitative PCR was performed by the LightCycler 480 system (Roche Applied Science) using a Brilliant® SYBR® Green QPCR Master Mix (Stratagene) according to the manufacturer's instructions. The specific primer pairs for human EPLIN-β and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously [62].

Western blot analysis

Total cell lysates were prepared using radioimmunoprecipitation (RIPA) buffer (Santa Cruz Biotechnology). Immunoblotting analysis followed standard procedures. The following antibodies were used: EPLIN (NB100-2305, Novus Biologicals), E-cadherin (610181, BD Transduction Laboratories), and β-actin (A5316, Sigma-Aldrich).

EPLIN report assay

Human EPLIN-β reporter was obtained from SwitchGear Genomics (Menlo Park, CA). pTK-RL plasmid was purchased from Promega (Madison, WI). Plasmid transfection and reporter luciferase were conducted as we described previously [62]. 24 h after the transfection, cells were treated with DMSO or genistein at the indicated concentrations for 48 h, luciferase activities were measured using a Dual-Luciferase Reporter Assay system (Promega). Relative Luciferase Units (RLU) were expressed as firefly luciferase intensity normalized to Renilla luciferase activity.

Animal study

A total of 12 athymic nude mice (Harland; 6-week-old) fed with a standard diet were used. C4-2 cells (2×10^6) per 100 μl per site were mixed with Matrigel (HC) at the ratio of 1:1 and injected subcutaneously using a previously established procedure. One week later, tumor-bearing mice were randomly divided into two groups (n = 6 per group) and treated with vehicle control (100% DMOS) or genistein (50 mg/kg) via the intraperitoneal route, 4 times per week, respectively. The treatment lasted for 4 weeks. Animal protocols were approved by Emory University Institutional Animal Care and Use Committee (IACUC).

Immunohistochemistry (IHC) analysis

EPLIN expression in a human prostate tumor array (TMA), and the expression of EPLIN and E-cadherin in C4-2 xenograft tumor tissues were analyzed by IHC staining following our published procedures [11]. The antibodies used were EPLIN (612115) and E-cadherin (610181, BD Transduction Laboratories).

Statistical analysis

Treatment effects were evaluated using a two-sided Student's *t* test. All data represent three or more experiments. Errors are S.E. values of averaged results, and values of $P \leq 0.05$ were taken as a significant difference between means.

Supplementary Figure 1. Dr. Leland W. K. Chung and his Molecular Urology and Therapeutics team at Emory University (2001-2009).