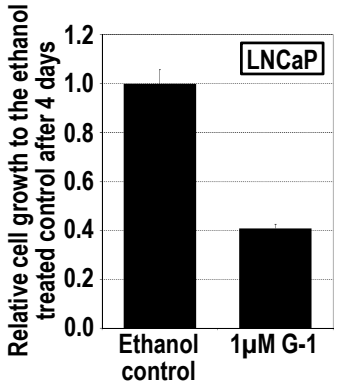


Figure S1. G-1–induced inhibition of cell growth in androgen-dependent PCa cells. LNCaP cells were treated with 1 μM G-1 for 4 days, and control cells were treated with ethanol. Cell growth of the control after treatment for 4 days was set at 1, and relative cell growth of the G-1–treated LNCaP cells to the control is presented. Columns, means; bars, standard deviations; n=3.

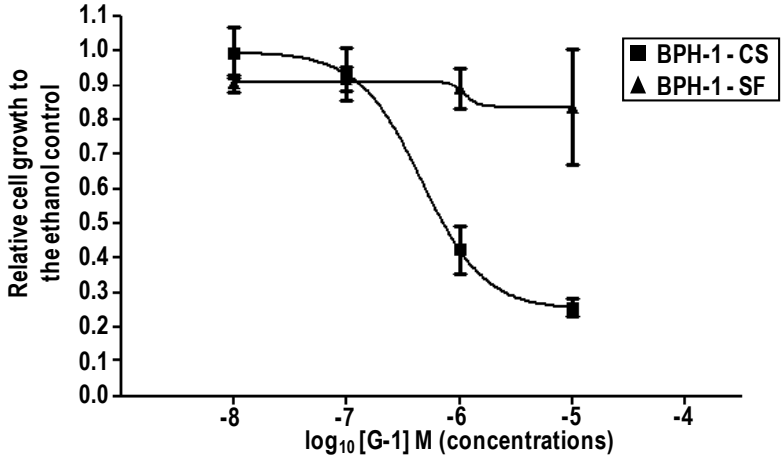


Figure S2. G-1–induced inhibition of cell growth in nonmalignant prostatic epithelial cells. BPH-1 cells grown either in medium supplemented with either charcoal-stripped fetal bovine serum (CS) or in serum-free medium (SF) were treated with 10^{-8}–10^{-5} M G-1 for 4 days, and control cells were treated with vehicle. Cell growth relative to that of the control at day 4 was plotted against the concentrations of G-1.

(A)

	Body weight (g)	Prostate (g)
Control	20.976	0.039
	24.528	0.037
	21.380	0.052
mean	22.295	0.043
SD	1.945	0.008
G-1 treated	22.332	0.063
	23.198	0.047
	22.949	0.053
mean	22.826	0.054
SD	0.446	0.008
p-value	0.668	0.153

(B)

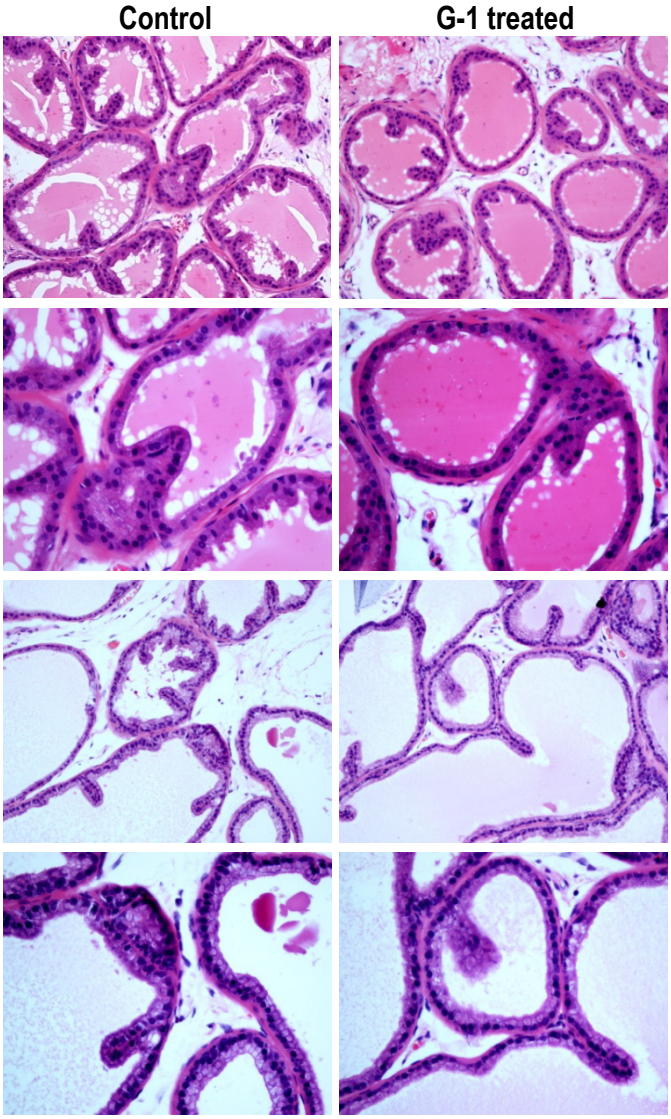


Figure S3. Unresponsiveness of normal prostatic epithelia *in vivo* by G-1. Mice were treated with G-1 at 4 mg/kg/day for consecutive 7 days and compared the total body and prostate weights (A) and histology of prostate tissues (dorsolateral and ventral prostates) (B) with the control that were treated with vehicle alone (2.5% DMSO, 5% ethanol).

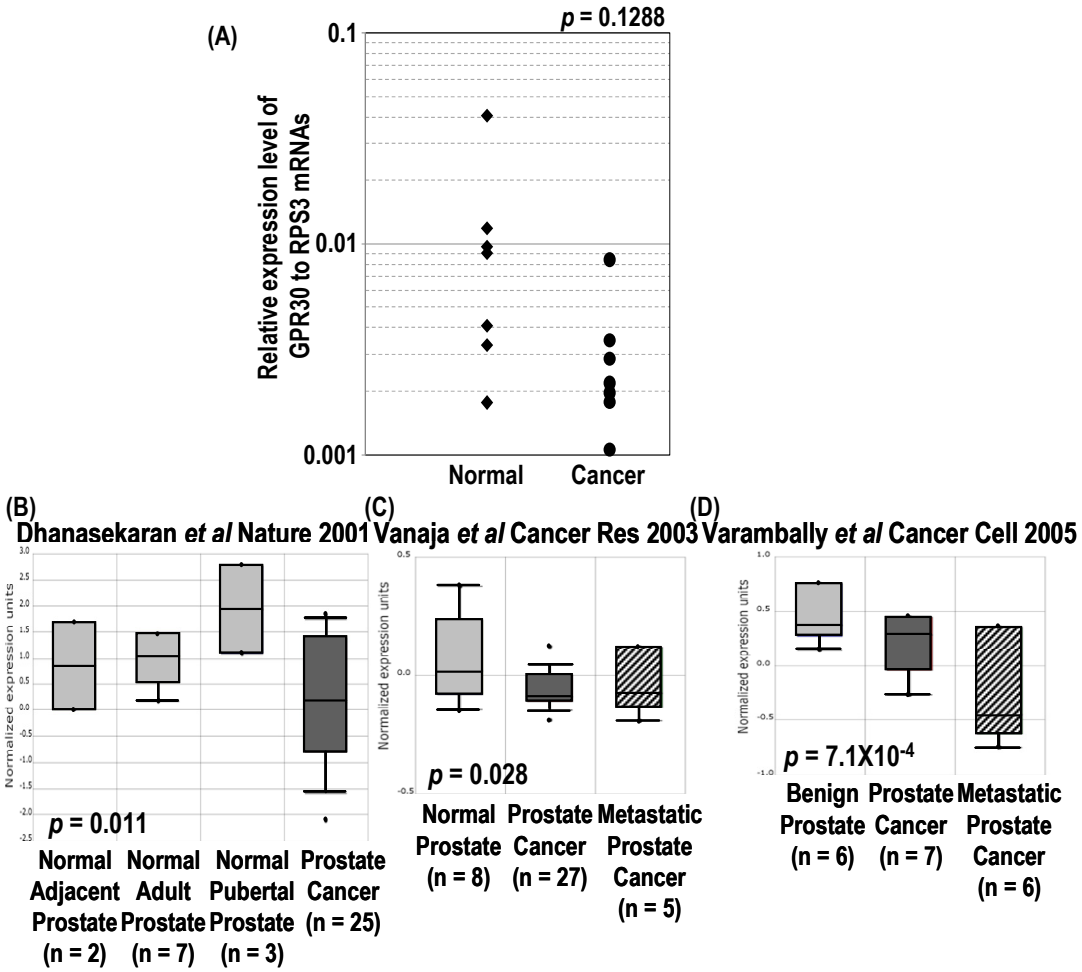


Figure S4. Higher GPR30 mRNA expression in benign or adjacent normal prostate tissues as compared with expression levels in PCa tissues. (A) Results of quantitative RT-PCR analysis of GPR30 for 7 pairs of PCa samples and their adjacent normal tissues. Data were analyzed with t-test and *p*-value is presented. (B-D) GPR30 mRNA expression data for normal/benign prostate tissues, normal adjacent prostate tissues, localized prostate cancers, and metastases were obtained from the Affymetrix GeneChip data in the Oncomine database. The normalized data were analyzed statistically using Student's *t*-test for comparison between normal tissues and cancers in (B) and using Pearson correlation analysis for determination of the correlation of GPR30 expression with different groups of samples (C and D). Box plots and *p*-values of the statistical analyses are presented.

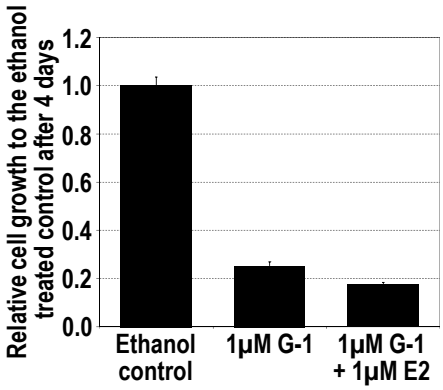


Figure S5. No effect of 17β-estradiol (E2) on G-1–induced inhibition of cell growth in PC-3. The cells were treated with 1 μM G1 for 4 days with or without E2, and control cells were treated with ethanol. Cell growth of the control after treatment for 4 days was set at 1, and relative cell growth of the G-1–treated PC-3 cells with or without E2 to the control is presented. Columns, means; bars, standard deviations; n=3.

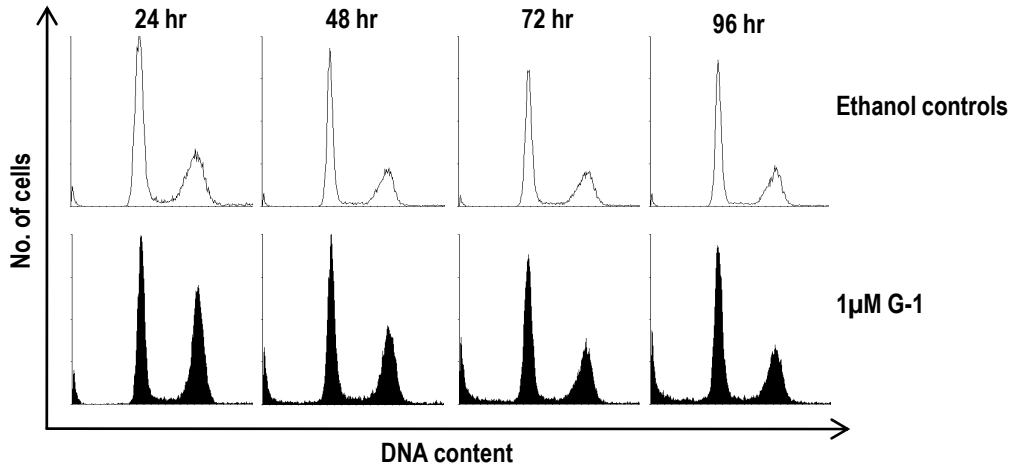
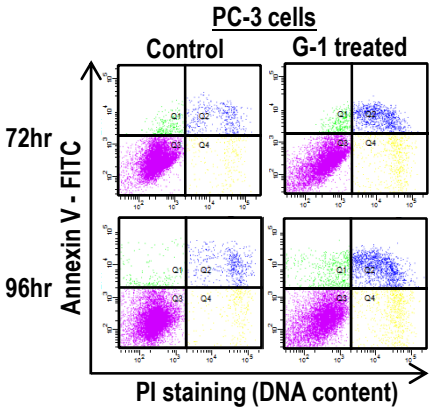


Figure S6. Histograms of propidium iodide (PI)–stained PC-3 cells with or without G-1. PC-3 cells were treated with 1 μM G-1 for 4 days, and control cells were treated with ethanol. The treated cells were harvested at each time point as indicated and stained with PI, and the stained cells were harvested at each time point as indicated and stained with PI; the stained cells were analyzed by flow cytometry for cell-cycle phase profiling. The experiments were repeated three times, and one representative set is presented.



	Control	G-1 treated
72hr	Q1: 2.53%	Q1: 2.80%
	Q2: 3.95%	Q2: 8.64%
	Q1+Q2: 6.48%	**Q1+Q2: 11.44%**
96hr	Q1: 2.50%	Q1: 5.47%
	Q2: 4.89%	Q2: 12.12%
	Q1+Q2: 7.39%	**Q1+Q2: 17.59%**

Q1 = Early apoptotic cells
Q2 = Late apoptotic cells

Figure S7. Induction of apoptosis by G-1 in PC-3 in the prolonged treatments. PC-3 cells were treated with 1 μM G-1 for 3 or 4 days, and control cells were treated with ethanol. The treated cells were harvested at each time point as indicated and stained with AnnexinV conjugated with FITC and PI using the BD AnnexinV apoptosis kit. The stained cells were analyzed by flow cytometry. The experiments were repeated three times, and one representative set of dot plots (left) is presented (right). Percentages of FITC-positive and PI-negative cells (Q1) and both FITC- and PI- positive (Q2) were tabulated. Sum of Q1 and Q2 represents the total percentage of apoptotic cells.

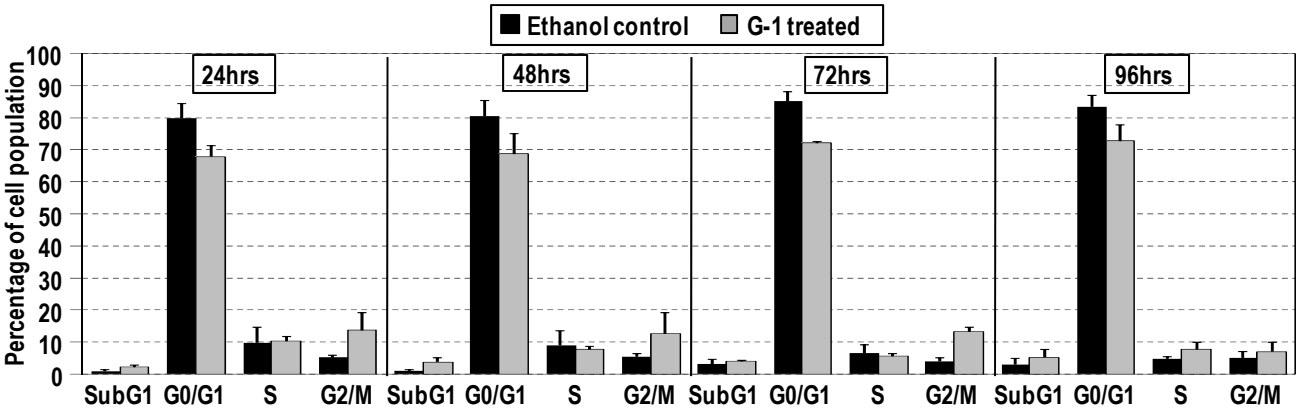


Figure S8. Induction of G2/M arrest by G-1 in LNCaP. The cells were treated with 1 μM G-1 for 4 days, and control cells were treated with ethanol. The treated cells were stained with PI at each time point as indicated. The stained cells were analyzed by flow cytometry for cell-cycle phase profiling. The experiments were repeated three times. Percentages of cells at subG1, G0/G1, S, and G2/M phases are presented. Columns, means; bars, standard deviations; n=3.

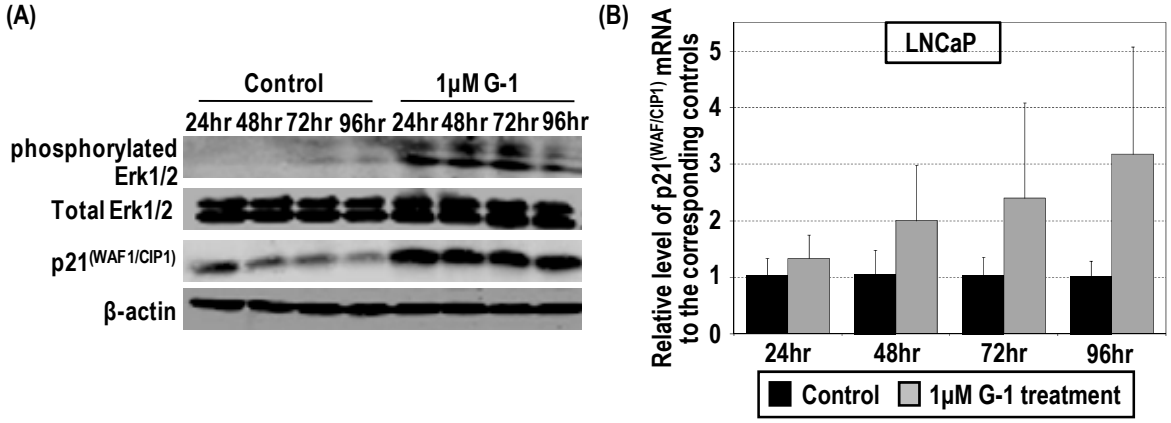


Figure S9. Phosphorylation of Erk1/2 and upregulation of p21 by G-1 in LNCaP. The cells were treated with 1 μM G-1 for 4 days (24-96hr), and control cells were treated with ethanol. (A) Protein levels of phosphorylated Erk1/2, total Erk1/2, p21, and β-actin in the cells were determined by Western blot analysis. (B) Total RNA was extracted from the treated cells at each time point as indicated and subjected to quantitative RT-PCR analysis of p21 and RPS3 to determine the levels of p21 mRNA in the cells. Columns, means; bars, standard deviations; n=3.