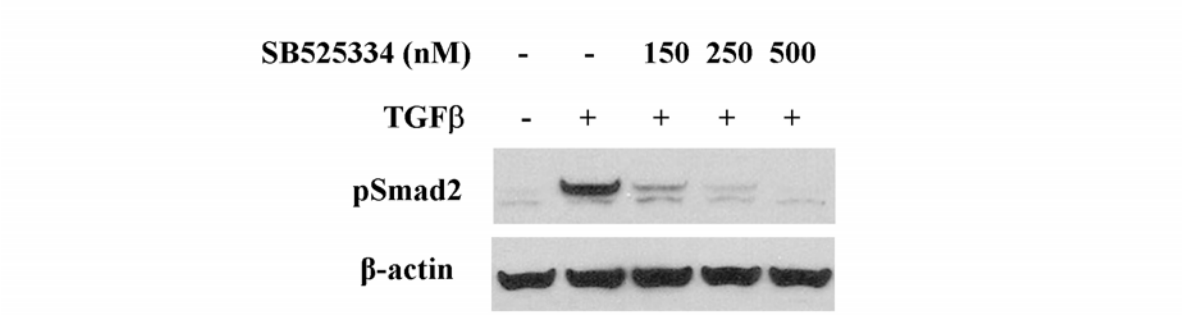

Fig. S1 TGFβ RI inhibitor SB525334 effectively blocks phosphorylation of Smad2 induced by TGFβ. FET cells were treated with TGFβ in the presence of different concentrations of SB525334. Cells were lysed and western blot analyses were performed with an anti-pSmad2 antibody. β-actin was used as a loading control.

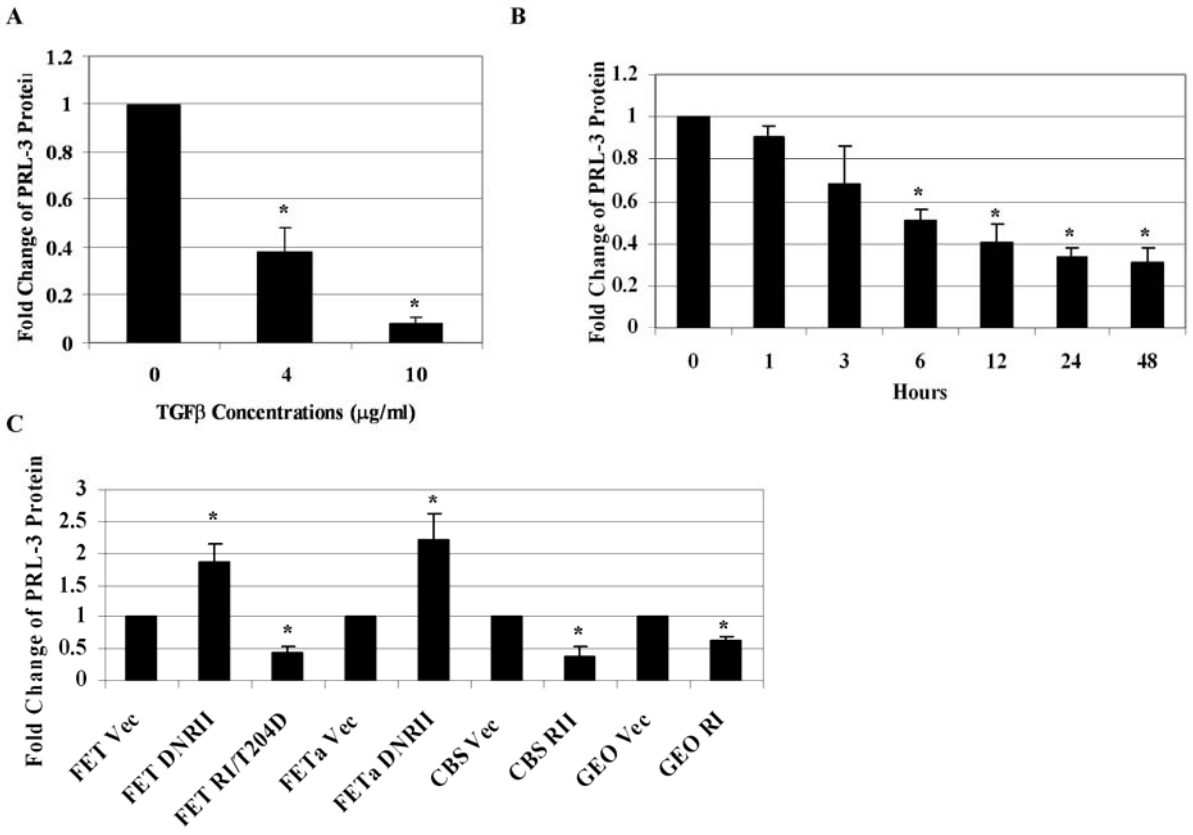

Fig. S2 Quantitative analyses of PRL-3 protein expression. FET cells were treated with TGFβ1 for 48 hrs (A) or 4 ng/ml TGFβ1 for the indicated time periods (B). FET cells were transfected with a DNRII or a constitutively active RI (RI/T204D) and FETα, CBS and GEO cells were transfected with DNRII, RII or RI respectively (C). Cells were harvested and western blot analysis was performed with an anti-PRL-3 antibody. Actin was used as a loading control. PRL-3 expression were calculated by densitometry of the PRL-3 band normalized by that of the actin band and expressed as a ratio of PRL-3 expression in the presence and absence of TGFβ (A, $*P < 0.01$; B, $*P < 0.005$) or a ratio of PRL-3 expression in DNRII, RII or RI-transfected cells and vector-transfected control cells (C, $*P < 0.02$). Values are means ± S.D. from triplicate experiments. *P* values were calculated using Student's *t*-test.

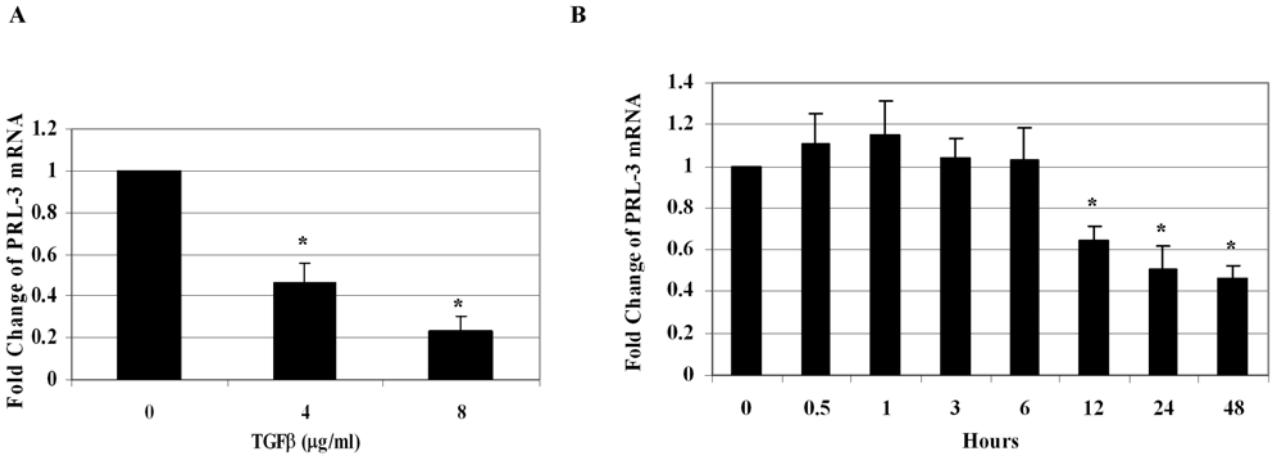

Fig. S3 Quantitative analyses of PRL-3 mRNA expression. FET cells were treated with TGFβ for 24 hrs (A) or 4 ng/ml TGFβ1 for the indicated time periods (B). Cells were harvested and RT-PCR analysis was performed. GAPDH was used as a loading control. PRL-3 mRNA expression were calculated by densitometry of the PRL-3 band normalized by that of the GAPDH band and expressed as a ratio of PRL-3 mRNA levels in the presence and absence of TGFβ. Values are means ± S.D. from triplicate experiments (A, **P* < 0.005; B, **P* < 0.01). *P* values were calculated using Student's *t*-test.

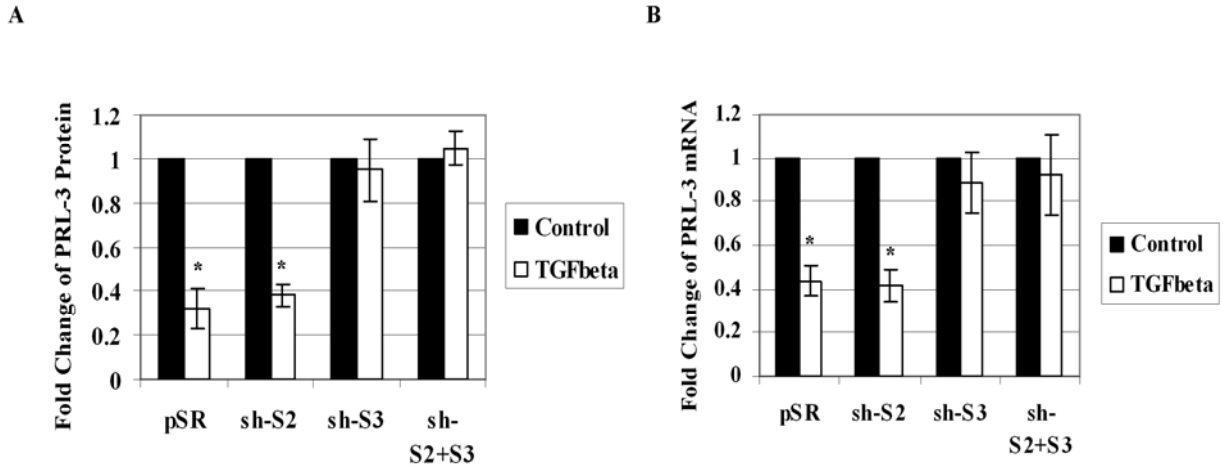

Fig. S4 Quantitative analyses of PRL-3 mRNA and Protein expression. FET cells were transfected with empty vector (pSR), Smad2 shRNA (sh-S2), Smad3 shRNA (sh-S3) or both shRNAs (sh-S2+S3). Cells were treated with TGFβ for 48 hrs (A) or 24 hrs (B). Cells were harvested and western blot (A) or RT-PCR (B) analyses were performed. PRL-3 protein or mRNA expression were calculated by densitometry of the PRL-3 band normalized by that of the actin or GAPDH band respectively and expressed as a ratio of PRL-3 levels in the presence and absence of TGFβ. Values are means ± S.D. from triplicate experiments (A, $*P < 0.004$; B, $*P < 0.003$). *P* values were calculated using Student's *t*-test.

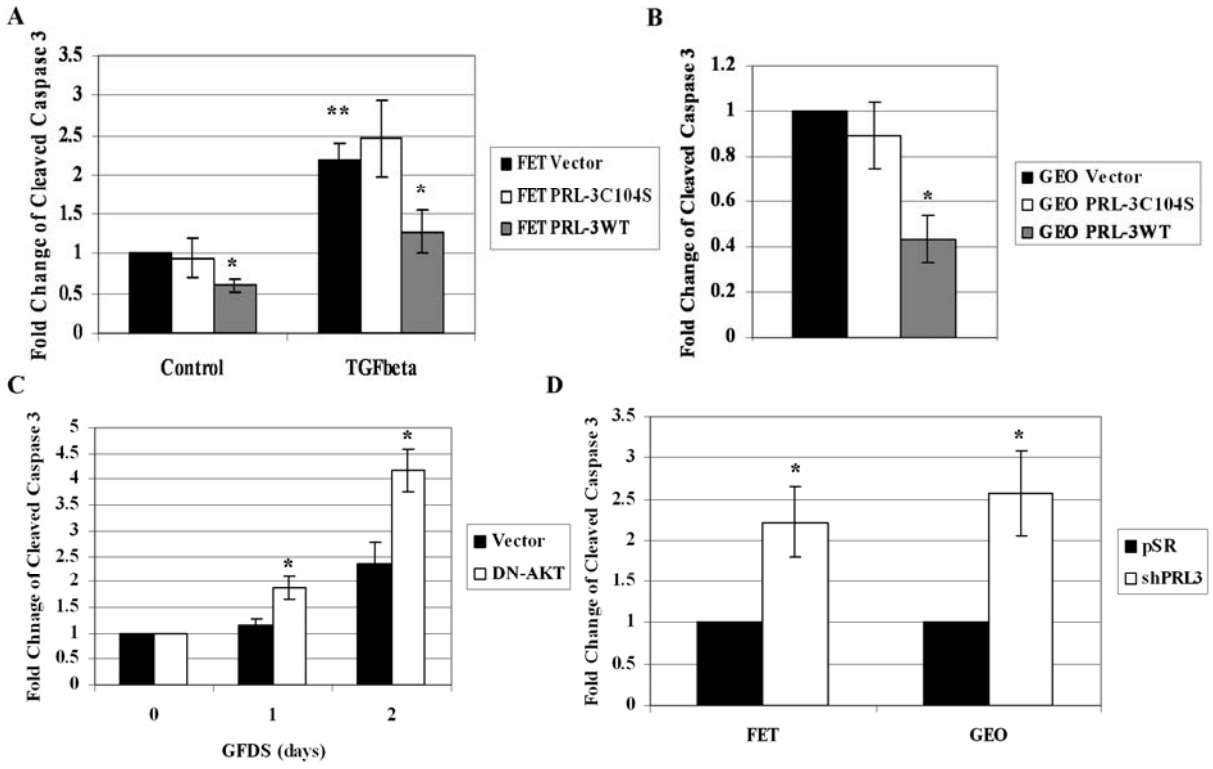

Fig. S5 Quantitative analyses of Caspase 3 Cleavage. A & B, Wild type or catalytic inactive mutant of PRL-3 was stably transduced into FET and GEO cells. Cleaved caspase 3 in FET cells under GFDS in the presence or absence of TGFβ1 for 2 days (A) and in GEO cells under GFDS for 3 days (B) was detected by western blot analyses. Levels of cleaved caspase 3 were calculated by densitometry of the cleaved caspase 3 band normalized by that of the actin band and expressed as fold change of cleaved caspase 3 levels as compared to vector-transfected control cells in the absence of TGFβ. Values are means ± S.D. from triplicate experiments (A, $*P < 0.01$ vs. vector control; $**P < 0.006$ vs. untreated control; B, $*P < 0.006$ vs. vector control). C, Levels of cleaved caspase 3 were determined by western blot analyses in FET/PRL-3 cells transfected with an empty vector or a DN-AKT construct under GFDS for 0, 1 or 2 days. Levels of cleaved caspase 3 were calculated by densitometry of the cleaved caspase 3 band normalized by that of the actin band and expressed as fold change of cleaved caspase 3 levels under GFDS as compared to non-GFDS conditions. Values are means ± S.D. from triplicate experiments. $*P < 0.007$ vs. vector control. D, FET and GEO cells expressing vector control (pSR) or PRL-3 shRNA (shPRL3) were under GFDS for 2 or 3 days respectively. Caspase 3 cleavage was detected by western blot analyses. Levels of cleaved caspase 3 were calculated by densitometry of the cleaved caspase 3 band normalized by that of the actin band and expressed as fold change of cleaved caspase 3 levels as compared to pSR-transfected control. Values are means ± S.D. from triplicate experiments. $*P < 0.01$. *P* values were calculated using Student's *t*-test.

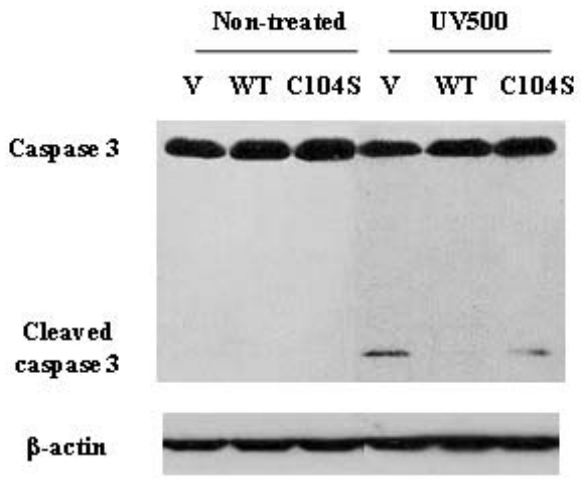

Fig. S6 PRL-3 protects FET cells from UV induced apoptosis. Exponentially growing wild type or mutant PRL-3-expressing FET cells were exposed to UVC (254 nm) at a dose of 50 J/m^2 with an Ultra Lum UVC-508 crosslinker (Ultra Lum, Paramount, CA) or were mock-treated. Twenty-four hours later, cells were lysed and western blot analyses were performed with an anti-PRL-3 antibody. β-actin was used as a loading control.

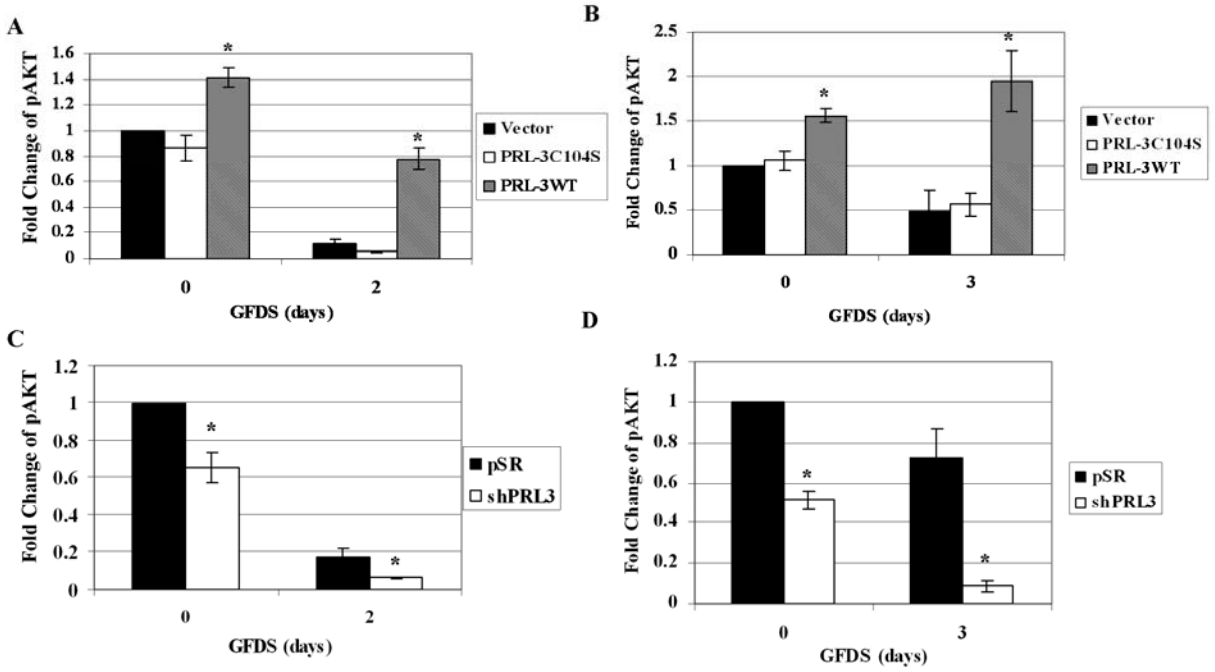

Fig. S7 Quantitative analyses of AKT phosphorylation. A & B, Empty vector, wild type (WT) or catalytic inactive mutant (C104S) of PRL-3 was stably transduced into FET and GEO cells. C & D, Vector control (pSR) or PRL-3 shRNA (shPRL3) was stably transduced into FET and GEO cells. AKT phosphorylation at Ser 473 in FET cells under GFDS for 2 days (A & C) and in GEO cells under GFDS for 3 days (B & D) was detected by western blot analyses. Levels of AKT phosphorylation were calculated by densitometry of the pAKT band normalized by that of the total AKT band and expressed as fold change of pAKT as compared to vector-transfected control cells under non-GFDS conditions. Values are means ± S.D. from triplicate experiments (A, $*P < 0.005$; B, $*P < 0.003$; C, $*P < 0.03$; D, $*P < 0.005$). *P* values were calculated using Student's *t*-test.

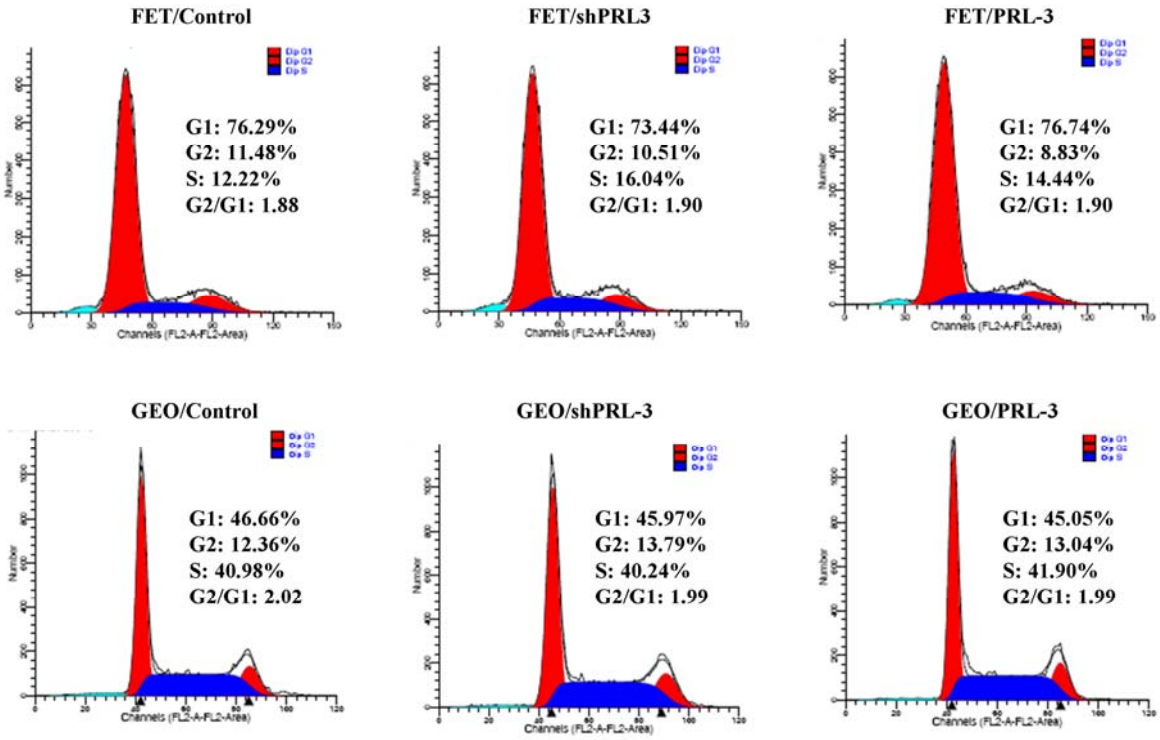

Fig. S8 PRL-3 has little effect on cell cycle profiles in FET and GEO cells. FET and GEO cells with PRL-3 overexpression or knockdown were compared with control cells for cell cycle profile.1x10^6 cells were fixed with 70% ethanol and stained with propidium iodide. Cell cycle was then analyzed by flow cytometry. Percentages of cells in G1, G2, or S phase are shown.

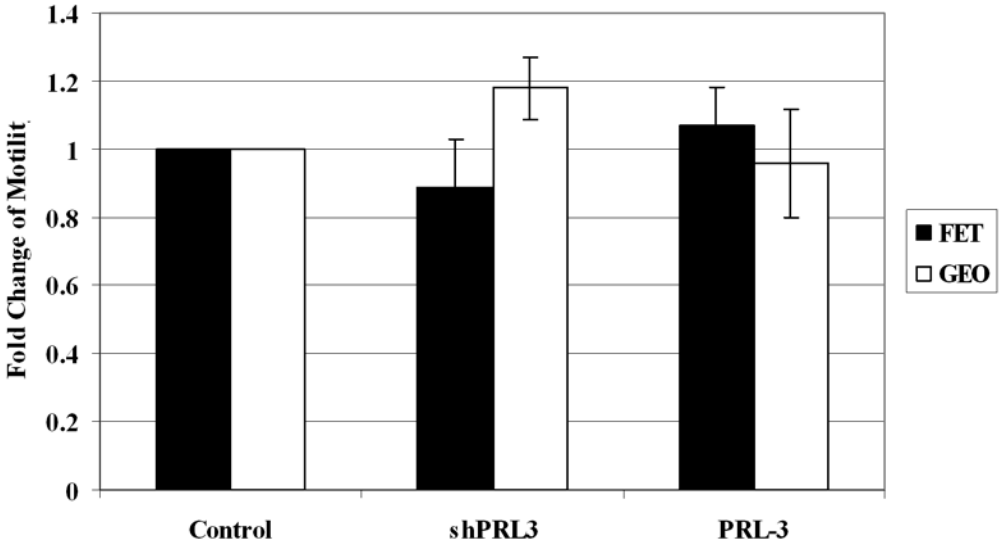

Fig. S9 PRL-3 has little effect on motility of FET and GEO cells. FET and GEO cells with PRL-3 overexpression or knockdown were compared with control cells for cell motility. Transwell motility assays were performed utilizing 8-μm pore, 6.5 mm polycarbonate transwell filters (Corning Costar Corp.). Cells were seeded onto the upper surface of the filters in medium in the absence of serum and growth factors and allowed to migrate towards medium containing 10% FBS. After 18 hr incubation, MTT was added to the medium. The cells on the upper surface of the filter were removed with a cotton swab, and the cells that had migrated to the underside of the filter were stained with MTT. The dye was solublized in DMSO and quantified at 570 nm. Values are means ± S.D. from triplicate experiments.

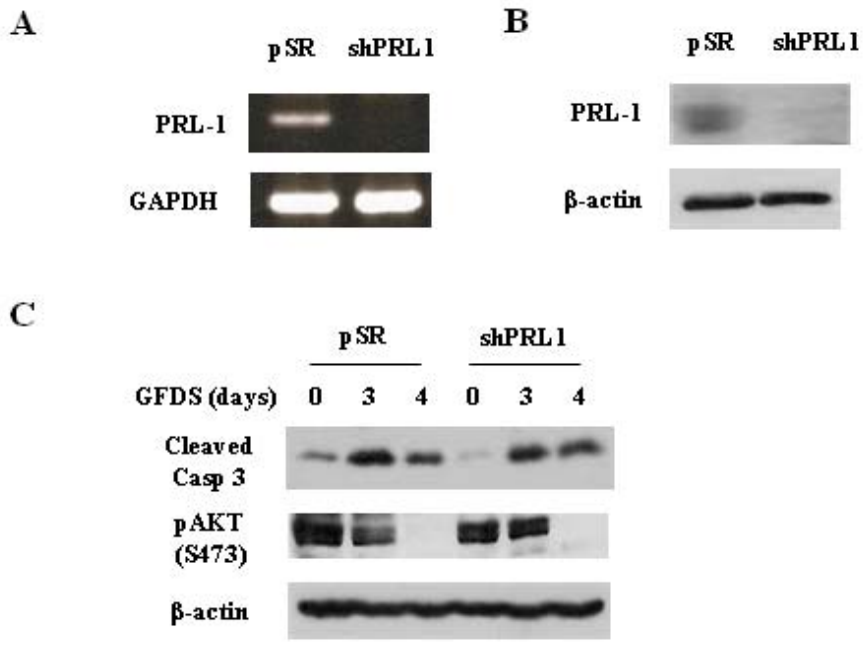

Fig. S10 Knockdown of PRL-1 expression has no effect on GFDS-induced apoptosis. Empty vector (pSR) or PRL-1 shRNA-expressing vector (shPRL1) was stably transduced into GEO cells. A, RNA was isolated and RT-PCR was performed. B, Cells were lysed and western blot analyses were performed with an anti-PRL-1 antibody. β-actin was used as a loading control. C, GEO control cells (pSR) and cells with PRL-1 knockdown (shPRL1) were deprived of growth factors for 3 or 4 days. Cells were lysed and western blot analyses were performed with an anti-caspase 3 or anti-phosphor-AKT (S473) antibody. β-actin was used as a loading control.

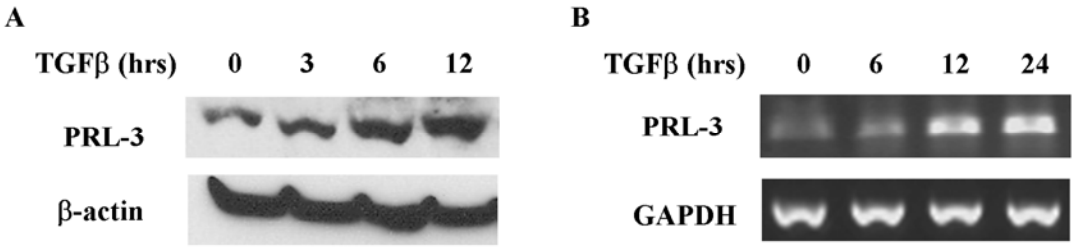

Fig. S11 TGFβ increases PRL-3 expression in SW480 cells. SW480 cells were treated with TGFβ for the indicated time periods. A, Western blot analyses were performed with an anti-PRL-3 antibody. β-actin was used as a loading control. B, RT-PCR analyses were performed to detect PRL-3 mRNA expression. GAPDH was used as a loading control.

Supplemental Materials:
E1 probe:

5'-GGGCTTGGGTTGAGTCTAGGGGGCTGGAGAGTCTGCTAACAGCTG-3'**;**

Mutant E1 probe:

5'- GGGCTTGGGTTGACATAAGGGGGCTGGAGACATAGCTAACAGCTG-3';

E4 probe:

5'-ACCTCTGAGTGAAGGGGGCTGTCTGCCCATCCACCAATGT-3';

Mutant E4 probe:

5'- ACCTCTGAGTGAAGGGGGCTCATAGCCCATCCACCAATGT-3'.

The following primers were used to amplify E1 and E4 respectively: 5'-GTAGCAGGCAGTAGTGACAGG-3' (Forward) and 5'-GTGACACAAGTGACCCAAGCTG-3' (Reverse); 5'-AGTTCAAGTTCATTCTTCCTCTGG-3' (Forward) and 5'-TCTCAGAGCTGGATAGTCATAAAG-3' (Reverse).

The negative control PCR reactions generated a 252-bp product from a distal region without Smad-binding sites, using the following primers: 5'-GGAGTGAGAGCTGTGTTACACG-3' (Forward) and 5'-ACAGGCAGAAGTTTCAGCCCAC-3' (Reverse).

Western blot analysis Cells were lysed in NP40 lysis buffer [50 mmol/L Tris-HCl (pH 7.5), 120 mmol/L NaCl, 0.5% NP40, 1 mmol/L EDTA, 50mmol/L NaF, 1 mmol/L NaVO3, 10mmol/L sodium 2-glycerolphosphate, 1 mmol/L phenylmethylsulfonyl fluoride, protease inhibitor cocktail (Sigma, St. Louis, MO)] at 4°C. The supernatants were cleared by centrifugation. Protein (30μg to 100μg) was fractionated on an 8-15% acrylamide denaturing gel and transferred onto a nitrocellulose membrane (Life Science,

Amersham) by electroblotting. The membrane was blocked with 5% non-fat dry milk in TBST (50mM Tris pH7.5, 150mM NaCl, 0.05% Tween 20) for 1 hour at room temperature or overnight at 4^{o}C and washed in TBST. The membrane was then incubated with primary antibodies for 1 hour at room temperature or overnight at 4^{o}C. After washing with TBST for 15 minutes, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Life Science, Amersham) for an hour at room temperature. After further washing in TBST for 15 minutes, the proteins were detected by the enhanced chemiluminescence system (Amersham).

RT-PCR analysis Total cellular RNA from FET cells treated with or without TGFβ was isolated using the RNeasy™ Mini Kit (Qiagen, Valencia, CA). Two and a half μg of RNA was reverse-transcribed with M-MLV reverse transcriptase (Promega, Madison, WI) using a random primer. Two microliters of this cDNA product was used to amplify human PRL-3. Primer sequences for PRL-3 were 5'-GGGACTTCTCAGGTCGTGTC-3' (forward) and 5'-AGCCCCGTACTTCTTCAGGT-3' (reverse). Conditions for amplification were: one cycle of 94°C for 2 min followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec, 72 °C for 30 sec. The GAPDH gene was used as an internal control.

DNA Fragmentation ELISA assays Cells were seeded in 96-well plates and then deprived of growth factors by changing to SM medium for the indicated time periods (24 - 72 h) or treated with 4ng/ml TGFβ or 25 μmol/L LY294002 for 24 - 48 hrs. DNA fragmentation ELISA assays were performed according to the manufacturer's protocol (Roche Applied Science, Indianapolis, IN). Statistical analyses were done using Student's *t*-test.

Plasmids and retroviral infections cDNAs encoding human wild type PRL-3 and its catalytically inactive mutant (C104S) were subcloned into a pBabe based retroviral expression vector. shRNAs directed against human PRL-3 (5'-CAGCAAGCAGCTCACCTAC-3') (Basak et al., 2008), PRL-1 (5'-GGAGAAGTATCGTCCTAAA-3'), Smad2 (5'-GCACTTGCTCTGAAATTTG-3') (Yang et al., 2008a) and Smad3 (5'- GGATTGAGCTGCACCTGAATG-3') (Jazag et al., 2005) were cloned into the pSuper-Retro vector (Oligoengine, Seattle, WA). 293GP packaging cells (Clonetech) were co-transfected with a vesicular stomatitis virus-G (VSV-G)-expressing vector and retroviral expression constructs using Effectene (Qiagene, Valencia, CA). The viruses were harvested 48 h later and used to infect desired target cells.