

**PTEN DIRECTS PROSTAGLANDIN E₂-MEDIATED FIBROBLAST RESPONSES VIA
REGULATION OF E PROSTANOID 2 RECEPTOR EXPRESSION**

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Running head: PTEN regulates EP2 expression

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SUPPLEMENTAL DATA

Table Legend

Table S1. Primer and Probe sequences for quantitative real-time PCR.

Figure Legends

Figure S1. Short hairpin RNA interference of PTEN by stable retroviral transfection in IMR-90 cells. Cells were infected with retrovirus encoding PTEN shRNA and stable transfectants were selected with puromycin. Compared to uninfected cells, a representative blot of shRNA-transfected cells demonstrated complete knockdown of PTEN with a corresponding increase in Akt activity (pAkt).

Figure S2. PGE₂ inhibits TGF- β -induced α -SMA in normal murine embryonic fibroblasts. Results are representative of 3 separate experiments. The graph shows the densitometric analysis of protein expression. Data are pooled from 3 separate blots.

Figure S3. Quantitative PCR of embryonic C57Bl/6 WT murine fibroblasts for α -SMA and collagen I. *P<0.0001 compared to the control condition. **P=0.0005 compared to the control condition. Results are representative of 3 separate experiments. Data are expressed as percent change in gene expression (\pm SD) compared to the serum-free condition.

Figure S4. PTEN reconstitution into *pten*^{-/-} cells inhibits α -SMA (*left panel*) and collagen I (*right panel*) gene expression. The inset blot shows a representative Western blot of *pten*^{-/-} cells transfected with empty vector (-) or PTEN-expressing vector (PTEN). The membrane was stripped and re-probed for GAPDH as a loading control. Results are representative of 2 separate experiments.

Figure S5. PTEN inhibition in normal lung fibroblasts reduces EP2 expression. IMR-90 cells were untreated or were treated with the PTEN inhibitor bpV(pic) and EP2 expression was measured by quantitative PCR. Results are representative of triplicate studies. *P=0.007.

Figure S1.

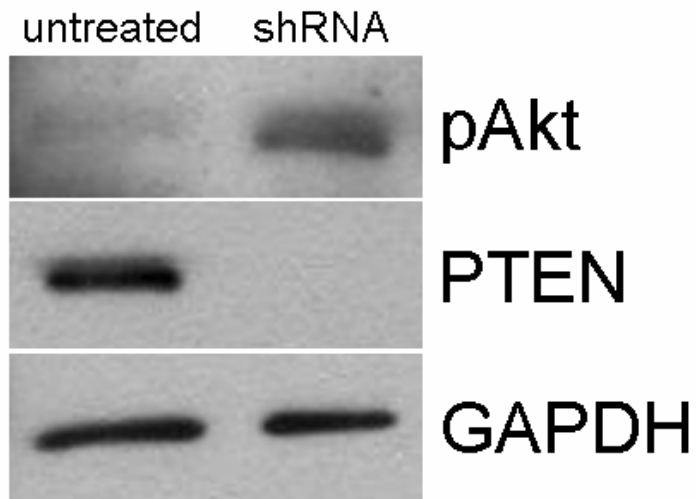


Figure S2.

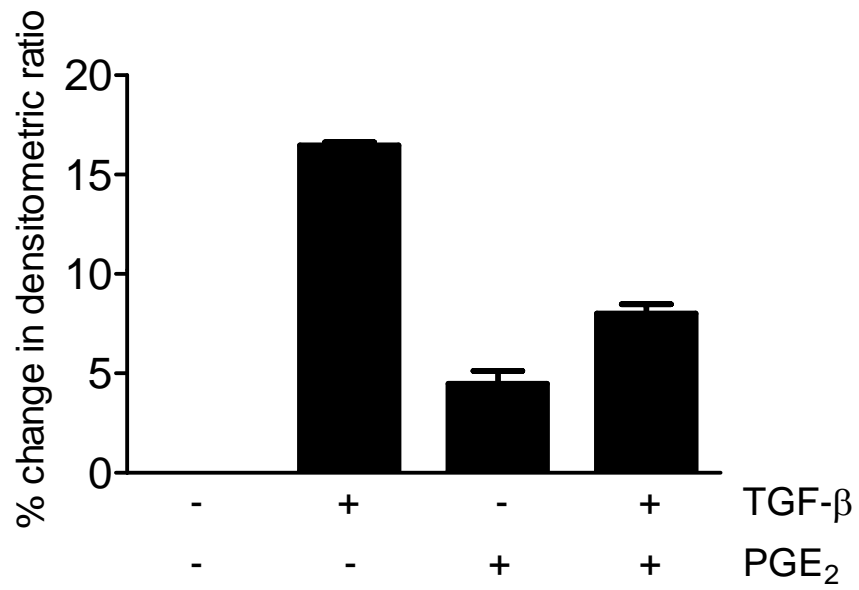
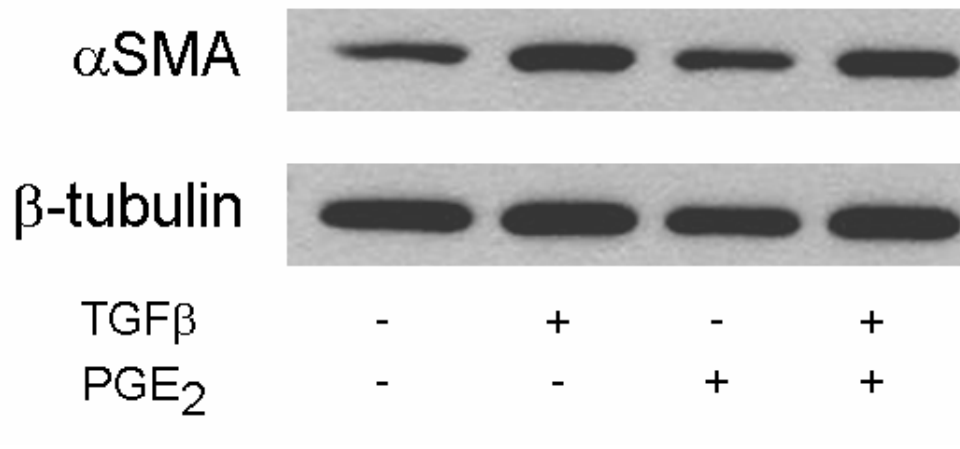


Figure S3.

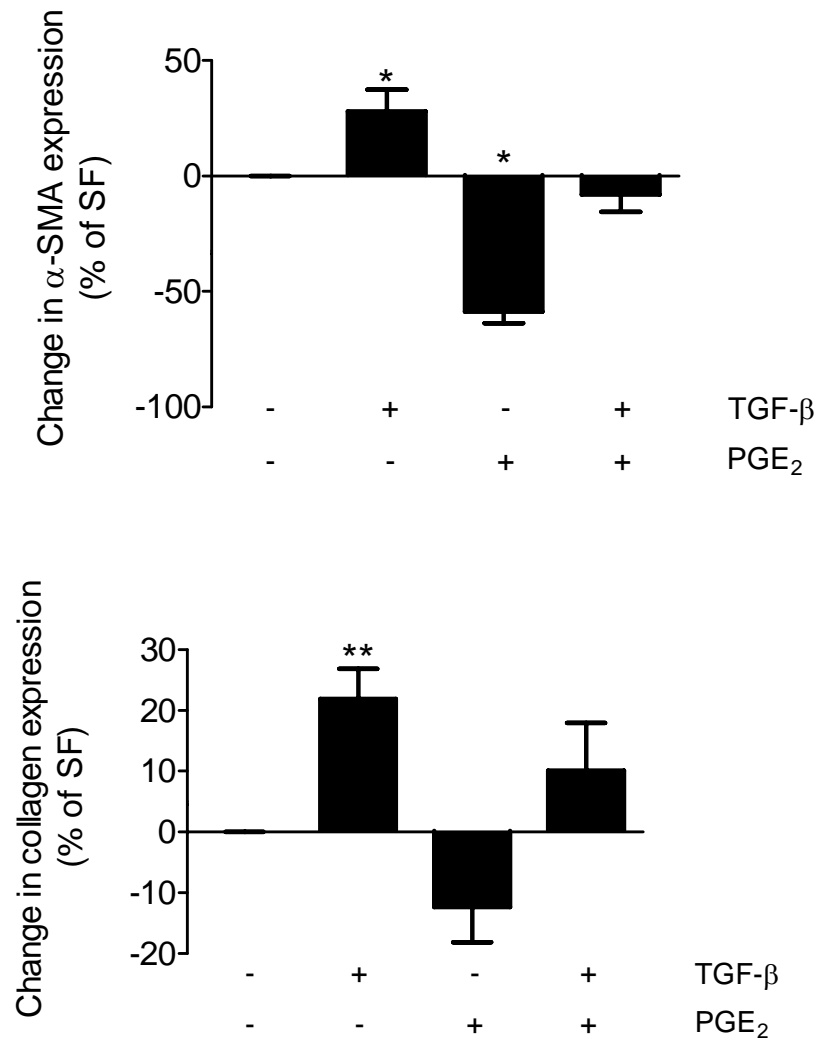


Figure S4.

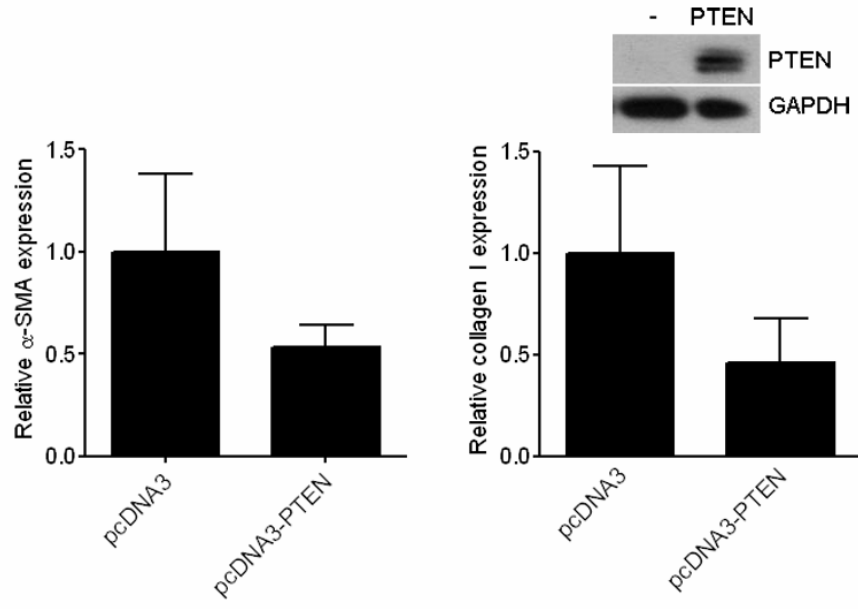


Figure S5.

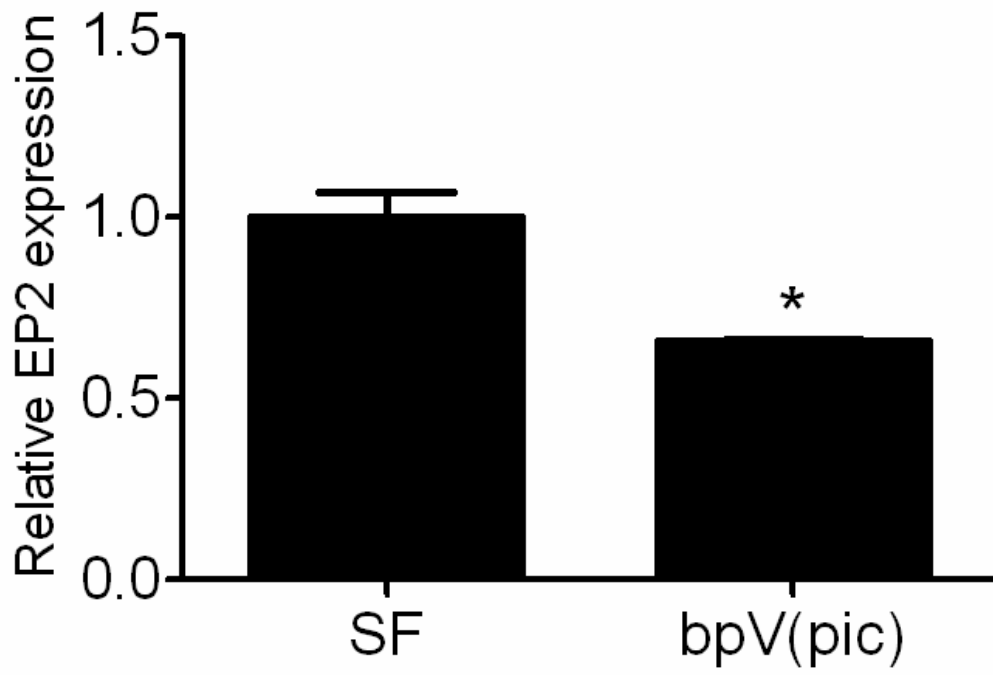


Table S1.

GENE	FORWARD	REVERSE	PROBE
α-SMA	TCCCTGGAGAAGAGCTACGAACT	AAGCGTTCGTTTCCAATGGT	6FAM-CTGACGGGCAGGTGA
Collagen I	GACTGGAAGAGCGGAGAGTACTG	CAGGTCTGACCTGTCTCCATGTT	6FAM-CTGCAACCTGGACGCCATCAAGG
EP1	GTGCCAAGGGTGGTCCAA	AACCACTGTGCCGGGAACTA	6FAM-CCTAACCAAGAGTGCCTGGGAAGCCA
EP2	TGCGCTCAGTCCTCTGTTGT	TGGCACTGGACTGGGTAGAAC	6FAM-CACTGAGAACAACAAGAAGCTCAGCAAACAT
EP3	TCAGATGTCGGTTGAGCAATG	CAGGCGAACTGCAATTAGAA	SYBR Green
EP4	ACGTCCCAGACCCTCCTGTA	CGAACCTGGAAGCAAATTCC	SYBR Green
GAPDH	CTGGTGCTGAGTATGTCGTG	CAGTCTTCTGAGTGGCAGTG	SYBR Green