SUPPLEMENTARY FIGURE LEGENDS

Figure S1. PKCε mediates activation of COX-2 pathway in normal prostate epithelial and prostate cancer cells. (A) LNCaP cells were transfected with RNAi duplexes for PKCε (#1 and #2) or NTC, and 48 h later treated with TNFα (10 ng/ml) for 4 h. PGE_2 levels in the culture medium were assessed by enzyme immuno assay. Results were normalized to NTC. (B) RWPE1 cells were infected with either PKCε or control (LacZ) AdVs (MOI = 1 pfu/cell) and 24 h later treated with either LPS (5 μg/ml) or TNFα (10 ng/ml) or vehicle for 4 h. *Left:* PKCε and COX-2 protein levels as determined by Western blot. *Middle and right:* COX-2 mRNA levels determined by Q-PCR. *, $p< 0.05$ and **, $p< 0.01$, n=3.

Figure S2. Effect of different signaling inhibitors on COX-2 mRNA expression in RWPE-1 cells. RWPE-1 cells were incubated with GF109203X (0.5 μM), parthenolide (2.5 μM), wedelolactone (10 μM), BX795 (5 μM) or BKM120 (0.3 μM) for 16 h and then treated with LPS (5 μg/ml, 4 h). COX-2 mRNA levels were determined by Q-PCR, and data normalized to LPS, no inhibitor. Results were expressed as mean ± S.D. of triplicate measurements. *, $p< 0.05$ and **, $p< 0.01$ *vs.* LPS, no inhibitor. Similar results were observed in two additional experiments.

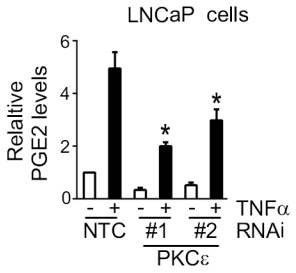
Figure S3. COX-2 inhibition reduced cell viability in RWPE1 cells with PKCε overexpression and Pten loss. RWPE1 stables were treated with different doses of COX-2 inhibitors for different time. (A) Effect of NS398 on the cell viability as determined by MTT assay. (B) Effect of rofecoxib on the cell viability as determined by MTT assay. *, $p< 0.05$ and **, $p< 0.01$, n=3.

Figure S4. COX-2 inhibition impairs PKCε driven tumorigenicity. Male athymic nude mice were treated with NF-kB inhibitor, parthenolide or vehicle control (*i.p.*). Following 7 days of the start of the treatment regimen, CaP8-PKCε cells were injected s.c. into the left flank of the nude mice and tumor growth was monitored. Tumor volume, expressed as mean ± S.D. (n= 8 mice/group).

Figure S5. Rofecoxib could be detected in the plasma of mice fed with the diet. (A) Rofecoxib levels were determined in the plasma collected from the mice fed with control laboratory or rofecoxib diet using mass spectrometry. (B) Mouse urine samples were collected by using metabolic cages over an 8-hour period. Levels of PGIM (l*eft*) and PGEM (*right*) were determined by mass spectrometry. Results were normalized with creatinine.

Figure S1

A


LNCaP cells
Relaltive PGE2 levels
6
4
2
0
*
*
- + - + - +
TNFα
NTC
#1
#2
RNAi
PKCε

B

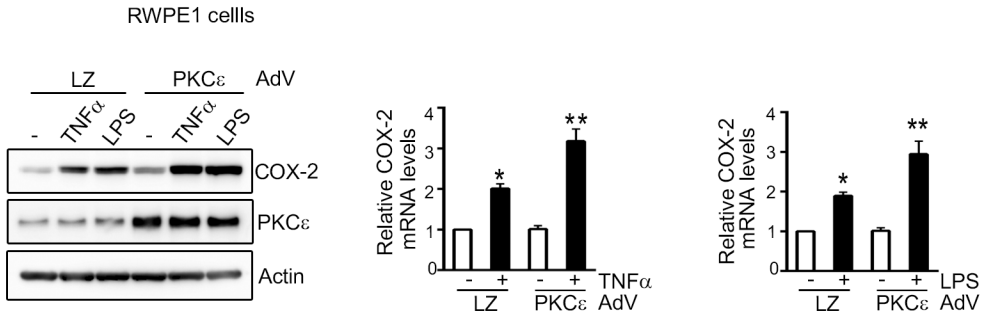

RWPE1 cellls
LZ
PKCε
AdV
- TNFα LPS - TNFα LPS
COX-2
PKCε
Actin
Relative COX-2 mRNA levels
4
3
2
1
0
*
**
- + - +
TNFα
LZ
PKCε
AdV
Relative COX-2 mRNA levels
4
3
2
1
0
*
**
- + - +
LPS
LZ
PKCε
AdV

Figure S2

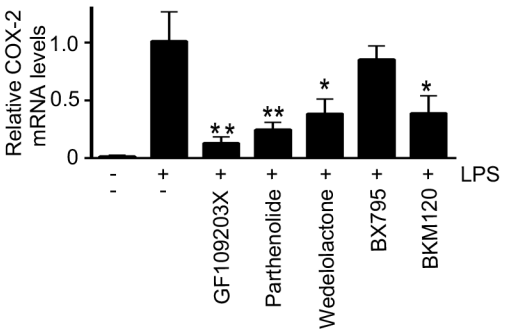

Relative COX-2 mRNA levels
1.0
0.5
0
**
**
*
*
- - + + + + + LPS
GF109203X
Parthenolide
Wedelolactone
BX795
BKM120

Figure S3

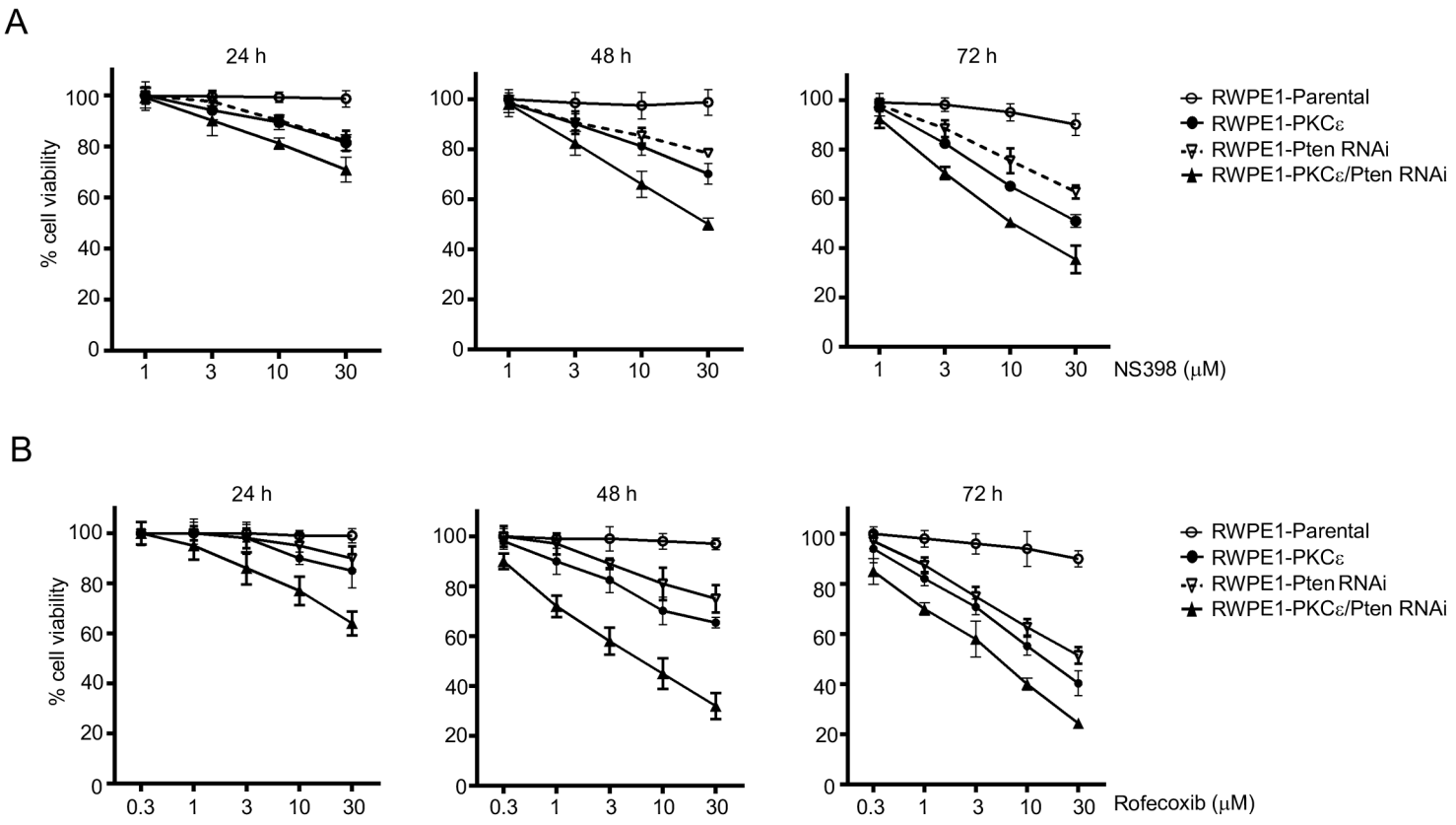

A
24 h
48 h
72 h
% cell viability
100
80
60
40
20
0
1
3
10
30
NS398 (μM)
RWPE1-Parental
RWPE1-PKCε
RWPE1-Pten RNAi
RWPE1-PKCε/Pten RNAi
B
24 h
48 h
72 h
% cell viability
0.3
1
3
10
30
Rofecoxib (μM)
RWPE1-Parental
RWPE1-PKCε
RWPE1-Pten RNAi
RWPE1-PKCε/Pten RNAi

Figure S4

A

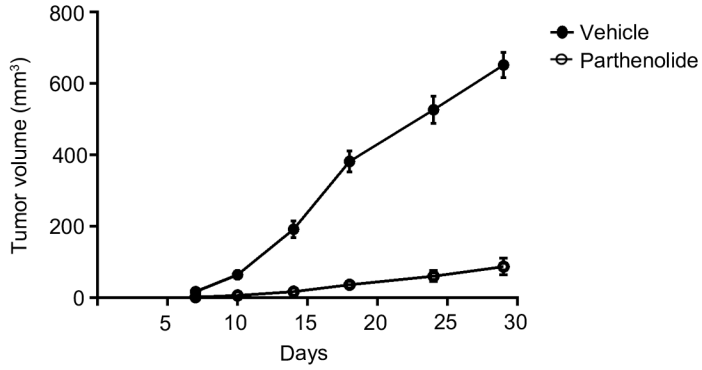

800
600
400
200
0
Tumor volume (mm3)
5
10
15
20
25
30
Days
Vehicle
Parthenolide

Figure S5