Supplement Table 1. Mitotic index detected by immunohistochemical staining with an antibody to histone H3.

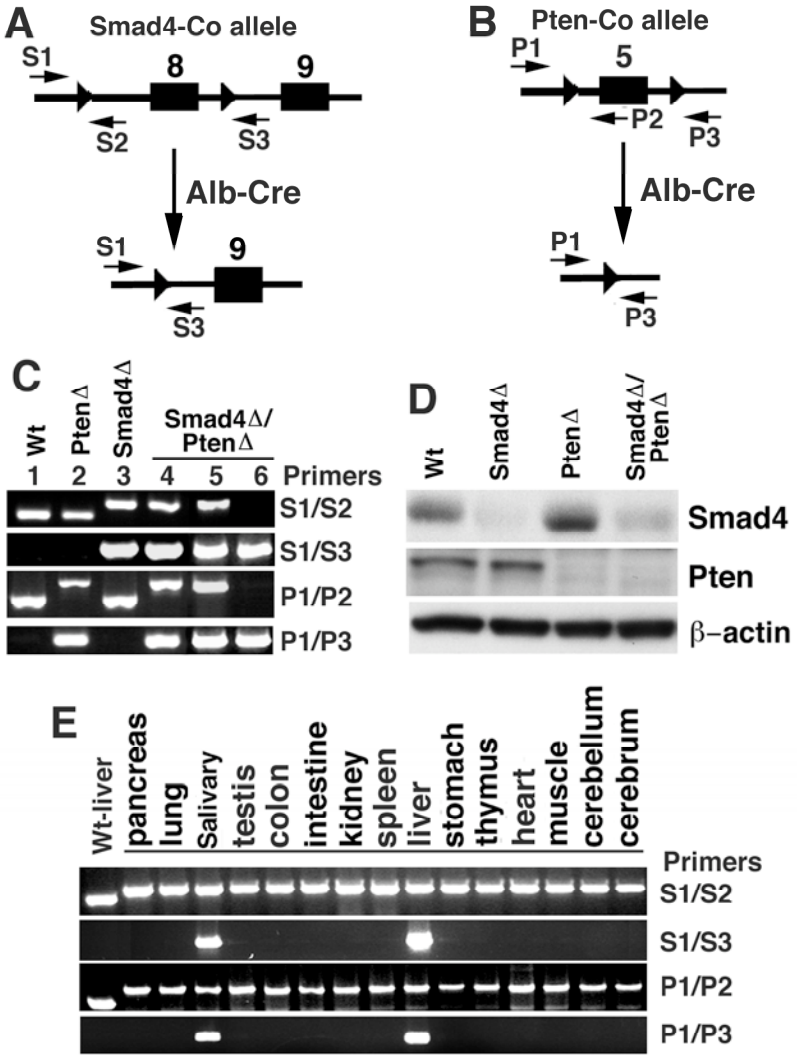
	Wt	PtenΔ	Smad4Δ	Hyperplasia	Dysplasia	Carcinoma in situ	Fully developed tumor
H3-positive cells	0	4	2	46	42	53	86
Total cells counted	50	128	142	438	618	669	985
Percent	0	3.1%	1.5	10.5	6.8	7.9	8.7

Supplement Fig. 1. Detection of Cre-loxP mediated recombination and expression of SMAD4 and PTEN. (A,B) Structures of *Smad4* conditional allele (A) and *Pten* conditional allele (B) before and after *Albumin-Cre* mediated recombination. (C) PCR products in wild type (Wt), *Pten*$^{Co/Co}$*;Alb-Cre* (PtenΔ), *Smad4*$^{Co/Co}$*;Alb-Cre* (Smad4Δ), and *Smad4*$^{Co/Co}$;*Pten*$^{Co/Co}$*;Alb-Cre* (Smad4Δ/PtenΔ) mice are as indicated. Lanes 1-4 are samples from livers and lane 5 is from a liver tumor and lane 6 is from an establish cell line from a liver tumor. *Smad4* wild type and conditional alleles are amplified using primers S1: 5-GAC CCA AAC GTC ACC TTC AG-3 and S2: 5-AAG AGC CAC AGG TCA AGC AG-3. The PCR product of the wild type allele is about 390 bp and the conditional allele is 450 bp, respectively. The recombination allele of Smad4 is amplified using primers S1: 5-GAC CCA AAC GTC ACC TTC AG-3 and S3: 5-GGG CAG CGT AGC ATA TAA GA-3. A fragment of about 500 bp is produced.
Pten wild type and conditional alleles are amplified using primers P1: 5-ACT CAA GGC AGG GAT GAG C-3, and P2: 5-AAT CTA GGG CCT CTT GTG CC-3'. The PCR product of the wild type allele is about 900 bp and the conditional allele is 1000 bp, respectively. The recombination allele of Pten is amplified using primers P1: 5-ACT CAA GGC AGG GAT GAG C-3, and P3: 5-GCT TGA TAT CGA ATT CCT GCA GC-3. A fragment of about 300 bp is produced. (D) Western blot analysis of SMAD4 and PTEN protein levels in livers of various genotypes as indicated. (E) Albumin-Cre promotes deletion of Smad4 and Pten is only detected in salivary gland and liver among 15 tissues/organs analyzed by PCR. The first lane is the liver of wild type mice and all remaining are from *Smad4*$^{Co/Co}$;*Pten*$^{Co/Co}$*;Alb-Cre* mice.

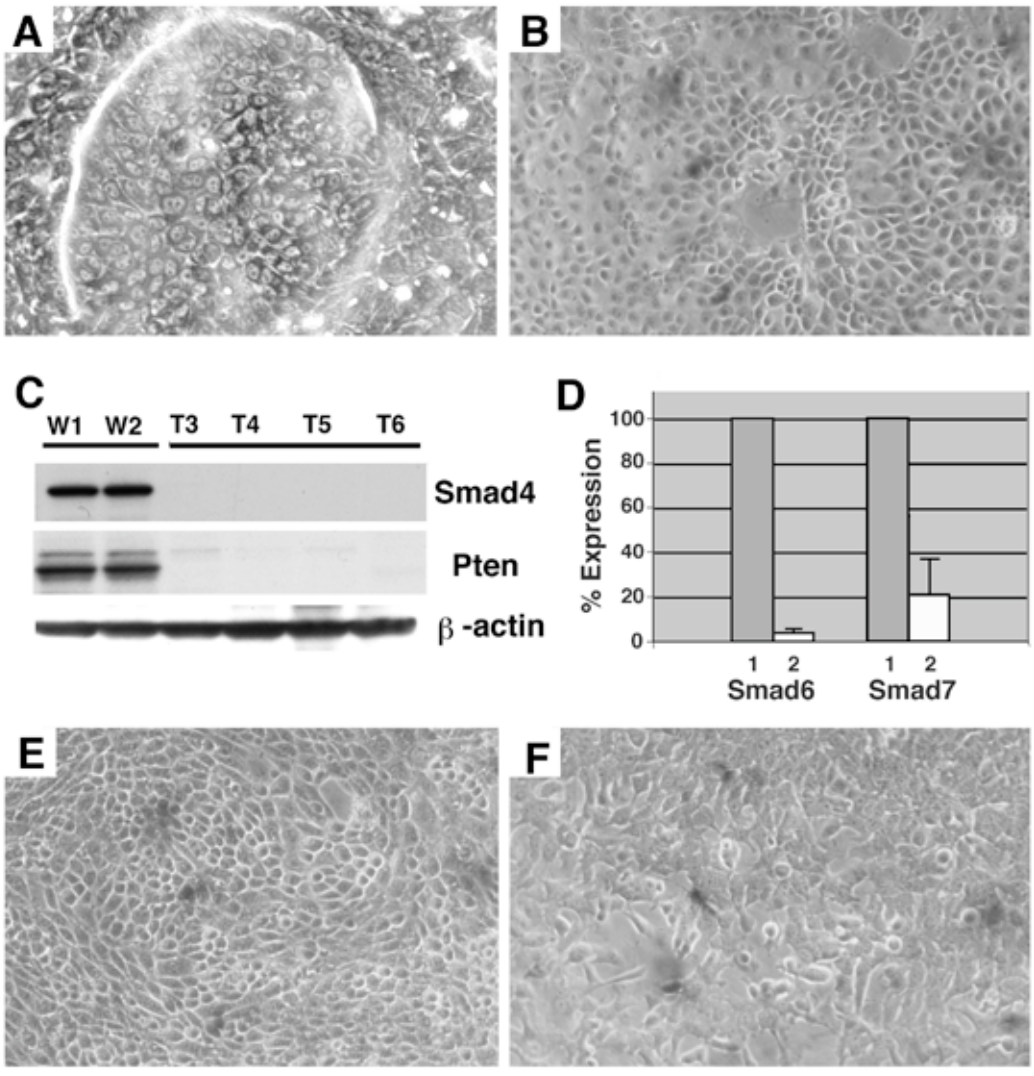
Supplement Fig. 2. Characterizaton of cell lines. (A) An area of epithelial-like cells found in the culture from a tumor of a 8 month-old *Smad4*$^{Co/Co}$;*Pten*$^{Co/Co}$;*Alb-Cre* mouse. (B) Morphology of one of the established cell lines (858) from CC. (C) Expression of PTEN and SMAD4 in cultured wild type and tumor cell lines by western blot analysis. (D) Analysis of *Smad6* and *Smad7* expression in cell lines developed from wild type liver and tumors by Real-time PCR. Results shown are averaged from 3 samples in each group. Expression levels of wild type are set at 100%. (E) Morphology of one cell line (644) that was derived from a one month-old *Smad4*$^{Co/Co}$;*Pten*$^{Co/Co}$ mice. (F) Morphology of Hepa1-6 cells that was derived from a HCC.

Supplement Fig. 3. Analysis of gene expression in human and mouse CC.
(A,B) Percentage of SMAD4, pPTEN and pFOXO1 in 21 pAKT positive human CC. (C,D). Immunohistochemistry using an antibody against total FOXO1 protein in mouse CC at different stages. Magnification: x700.

Supplement Fig. 1



Supplement Fig. 2



Supplement figure 3

A

	Positive	Negative	Total
SMAD4	11 (52%)	10 (48%)	21
pPTEN	15 (71%)	6 (29%)	21
pFOXO1	16 (76%)	5 (24%)	21