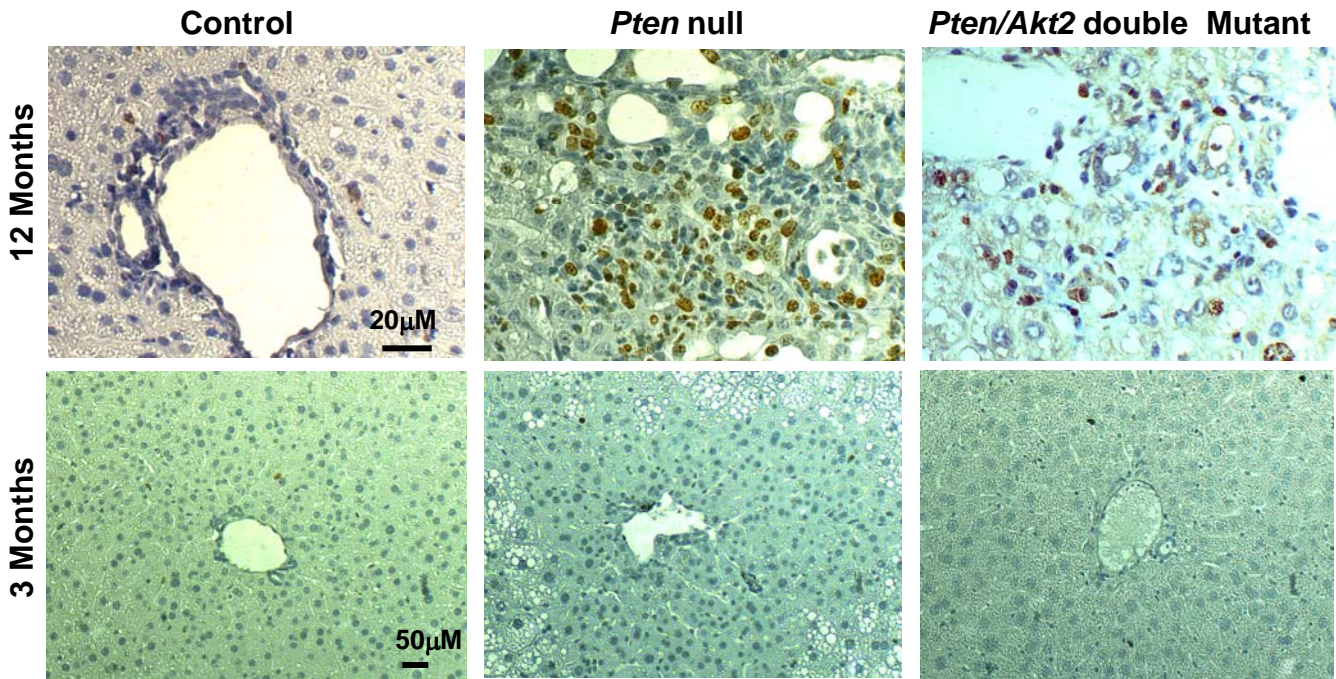
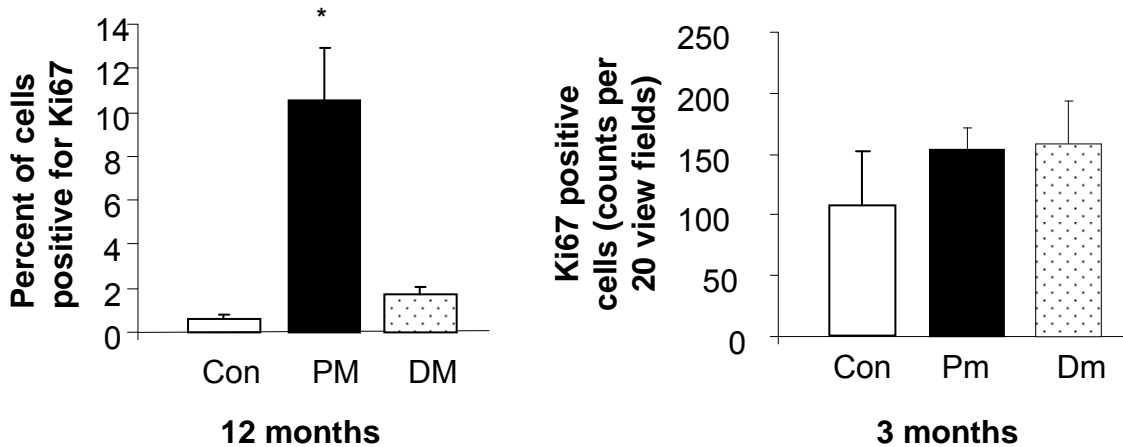


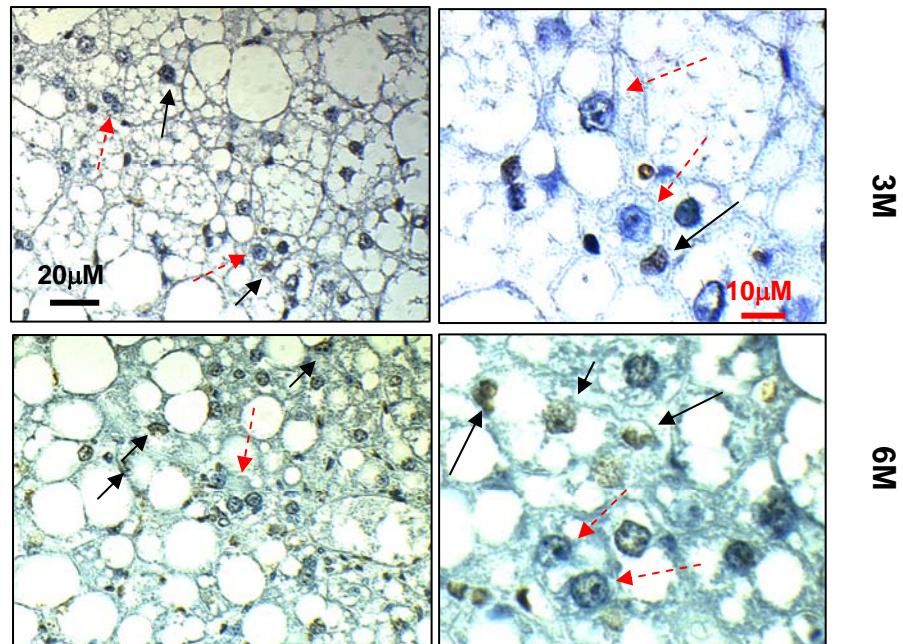
A.



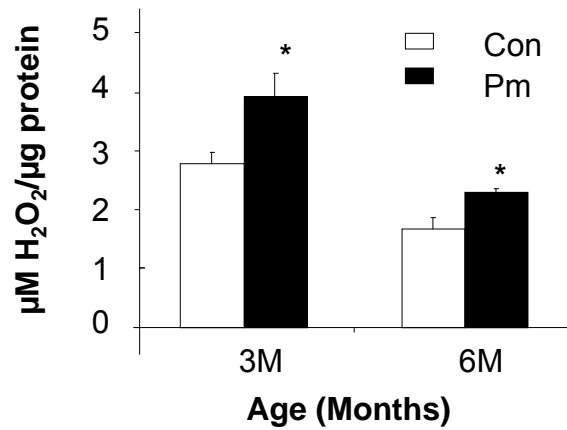
B.



Galicia et al Supplemental Figure 1. Proliferation rate is increased in *Pten* null mice and inhibited by deletion of *Akt2*. A. Representative images of Ki67 stained (brown nuclei) liver sections. Sections are counterstained with hematoxylin. B. Ki67 stained nuclei and total nuclei are counted from 20 randomly selected view fields in the 12 month (left) and 3 month (right) old mice. Data are collected from 5 mice in each group. Data expressed as Mean \pm SEM. * indicate statistical differences from the control (Con) group at $p < 0.05$. Pm, *Pten* null; Dm, *Pten/Akt2* double mutant.

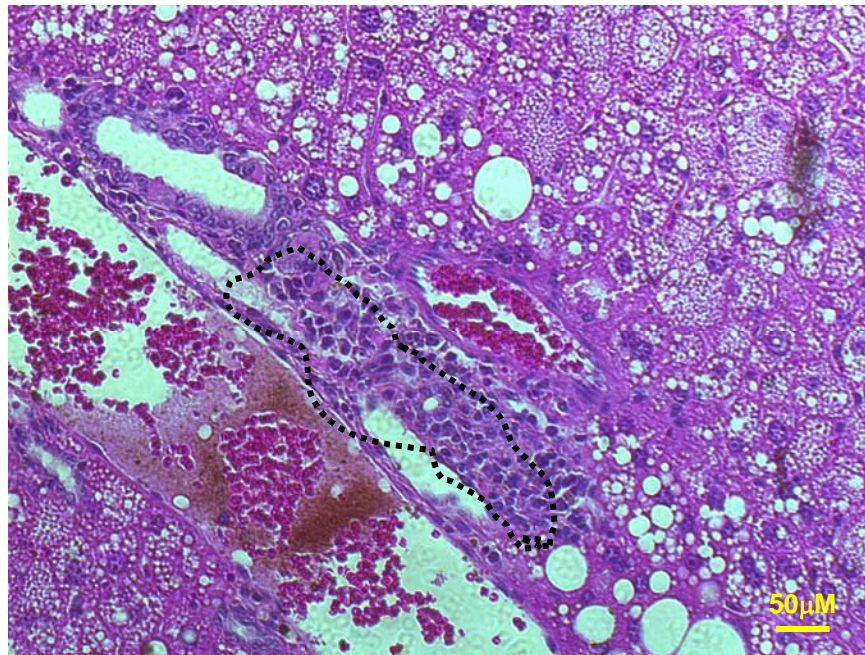


Galicia et al Supplemental Figure 2. Progressive increase of hepatocyte cell death in the *Pten* mutant liver with age. TUNEL analysis revealed more positively stained hepatocytes (brown stained nucleus) in 6 month mutant mice than in 3 month *Pten* mutant mice. Left panels, low magnification images. Right panels, high magnification images. Black solid arrows, TUNEL positive apoptotic cells. Red dashed arrows, TUNEL negative hepatocytes. Hepatocyte apoptosis increases progressively in mutant mice with age.

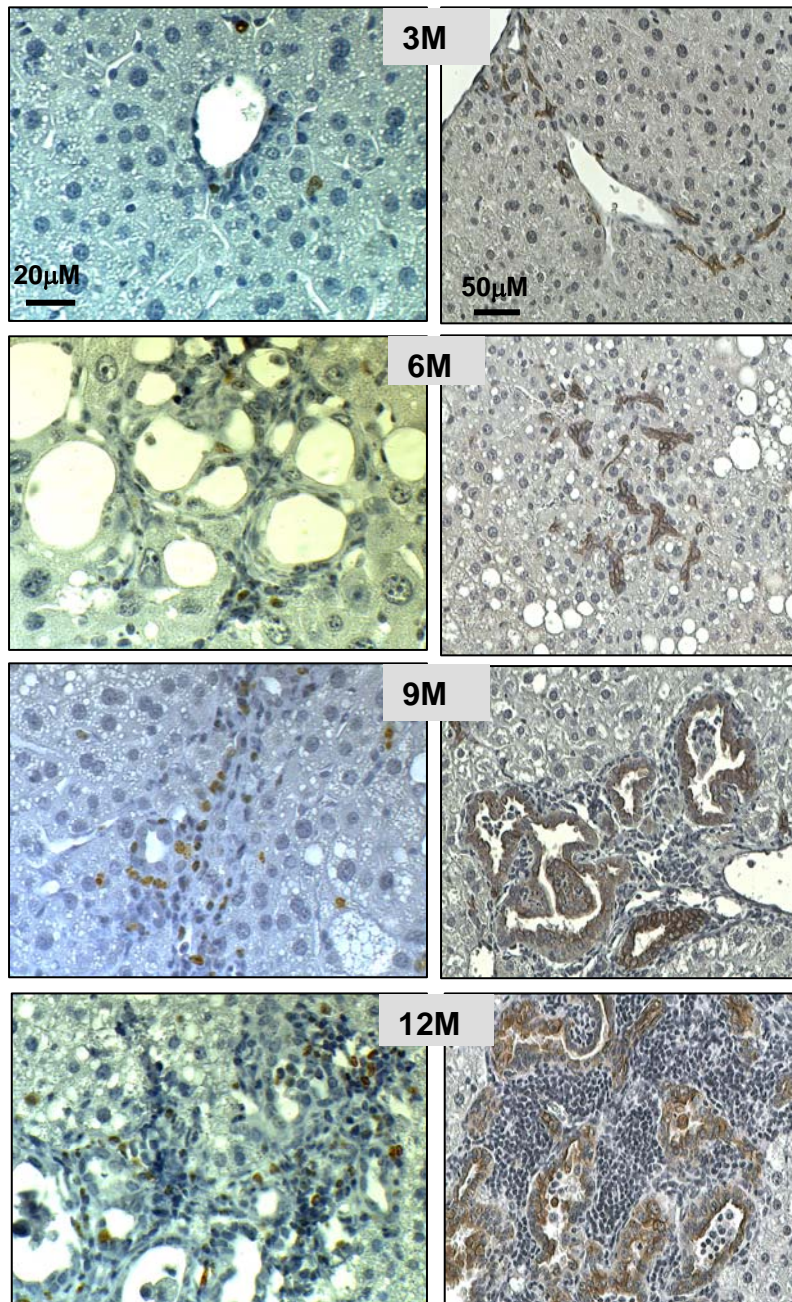


Galicia et al Supplemental Figure 3. Deletion of *Pten* leads to increased levels of hydrogen peroxide in mice. Livers of *Pten* null mice showed higher hydrogen peroxide accumulation at 3 and 6 months of age compared to controls. Values are expressed as the mean \pm SEM. * indicates values significantly different from that of age-matched controls at $p \leq 0.05$. $n=5$ for control and mutant experimental cohorts.

***Pten/Akt2* double mutant: 12 months**

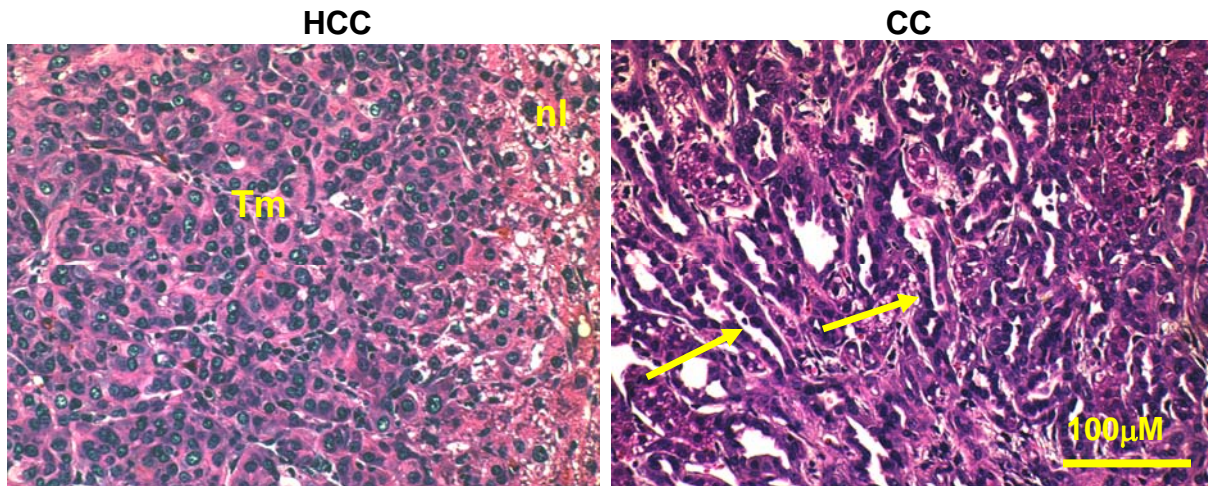


Galicia et al Supplemental Figure 4. *Pten* and *Akt2* double mutant mice develop progenitor cell expansion phenotype after 12 months of age. H&E sections of livers from *Pten* and *Akt2* double mutant mice show expansion of liver progenitors in the peri-ductal region. Dotted area represents progenitor cell populations.



Galicia et al Supplemental Figure 5. Concomitant expansion of cholangiocytes and progenitor cells. Left panels, proliferation of progenitor cells. Brown nuclei staining indicates Ki67 positive cells. The Majority of the Ki67 positive cells are confined to the progenitor cell population. Proliferation of hepatocytes is rarely observed. Right, Liver sections were stained with pan-CK to visualize ductal cells. Expansion of ductal cell populations is observed in Pm mice 9 months and older together and is associated with increasing progenitor cell populations.

A.



Galicia et al Supplemental Figure 6. Morphology of Tumors developed in *Pten* null mice. Left, some tumor areas contain densely growing hepatocytes with eosinophilic cytoplasm (Tm). Right, other tumor areas are composed of cells resembling biliary epithelium organized in tubular formations (arrows).

Table S1. Real time PCR primers used in study

<i>Gene</i>	Primer	Sequence	Amplicon size (bp)
<i>Ck19</i>	Fwd	CCGGACCCTCCCGAGATTA	179
	Rev	CTCCACGCTCAGACGCAAG	
<i>EpCAM</i>	Fwd	AGGGGCGATCCAGAACAACG	223
	Rev	ATGGTCGTAGGGGCTTTCTC	
<i>AFP</i>	Fwd	ATCGACCTCACCGGGAAGAT	143
	Rev	GAGTTCACAGGGCTTGCTTCA	
<i>Wnt10a</i>	Fwd	GACTCCACAACAACCGTGTG	133
	Rev	CCTACTGTGCGGAACTCAGG	
<i>Wnt7a</i>	Fwd	CGACTGTGGCTGCGACAAG	205
	Rev	CTTCATGTTCTCCTCCAGGATCTTC	
<i>GPx</i>	Fwd	ACATTCCCAGTCATTCTACC	151
	Rev	TTCAAGCAGGCAGATACG	
<i>GST</i>	Fwd	TCTGCCTATATGAAGACC	174
	Rev	AGAGAAGTTACTGGAAGC	
PDGFA	Fwd	GTCCAGGTGAGGTTAGAGG	210
	Rev	CACGGAGGAGAACAAGAC	
<i>GAPDH</i>	Fwd	GTCGGTGTGAACGGATTTGG	278
	Rev	GACTCCACGACATACTCAGC	

TableS2. Tumor spectrum in *Pten* and *Pten/Akt2* double mutants

Age	Microscopic tumors ^a		Tumor nodules ^b		Macroscopic tumors ^c	
	<i>Pten</i> null	DM ^d	<i>Pten</i> null	DM	<i>Pten</i> null	DM
8 m	1/5	n/a	0/5	n/a	0/5	n/a
9-12 m	5/9	0/14	4/9	0/14	3/9	0/14
13-15m	10/10	4/16	10/10	2/16	10/10	2/16

^a Tumors identified using microscopy analysis of H&E stained slides.

^b Nodules observed during dissection of the liver that are <1mm in diameter.

^c Mass observed during dissection of the liver that are >1mm in diameter.

^d DM: double mutant for both *Pten* and *Akt2*. *Pten*^{loxP/loxP}; *Akt2*^{-/-}; *Alb-Cre*⁺.