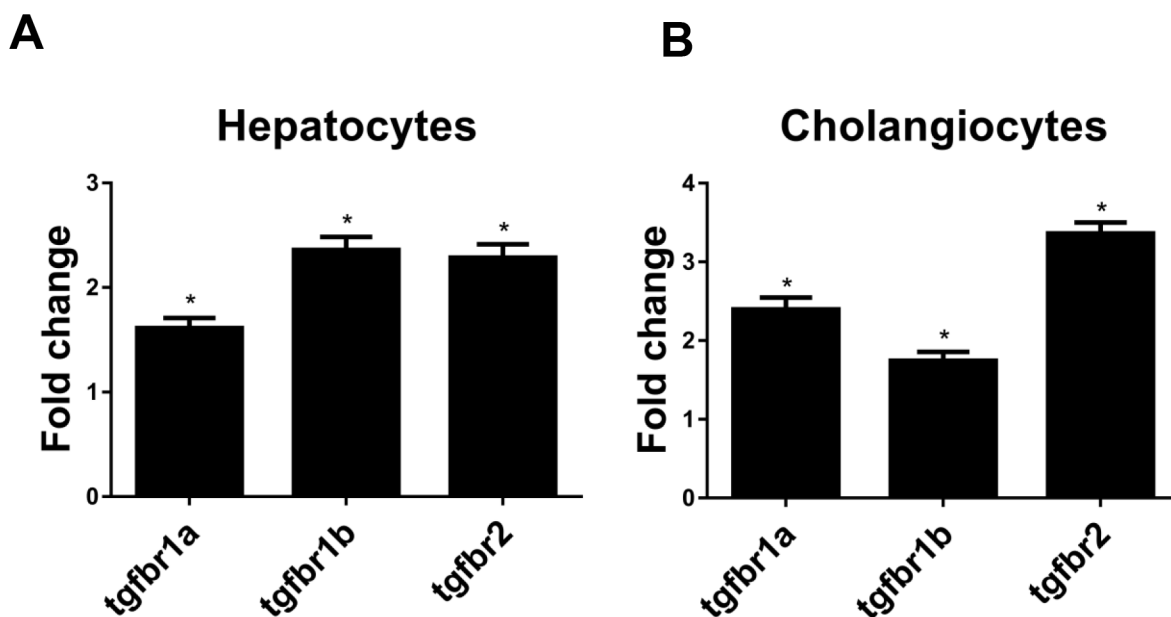
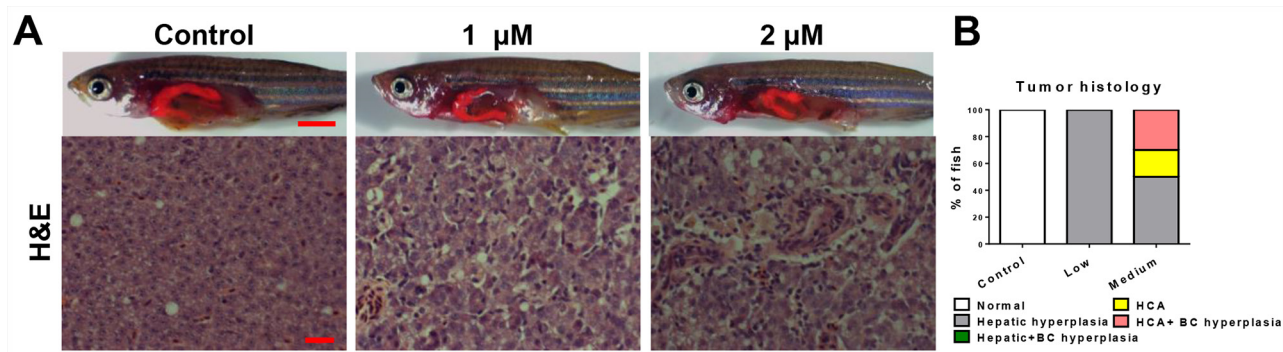


Chronically high level of *tgfb1a* induction causes both hepatocellular carcinoma and cholangiocarcinoma via a dominant Erk pathway in zebrafish

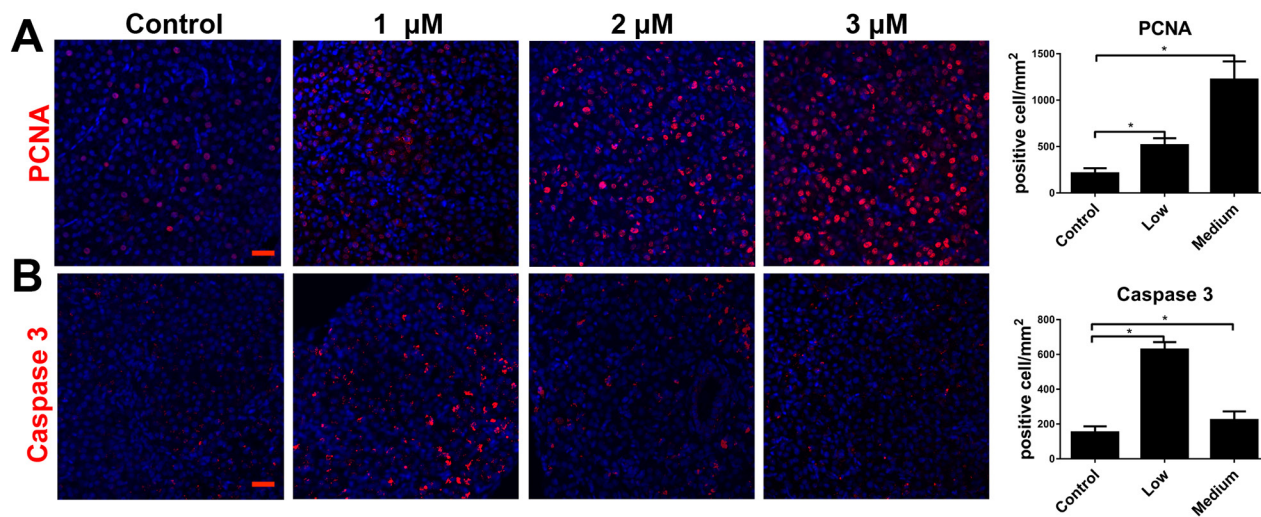
SUPPLEMENTARY MATERIALS



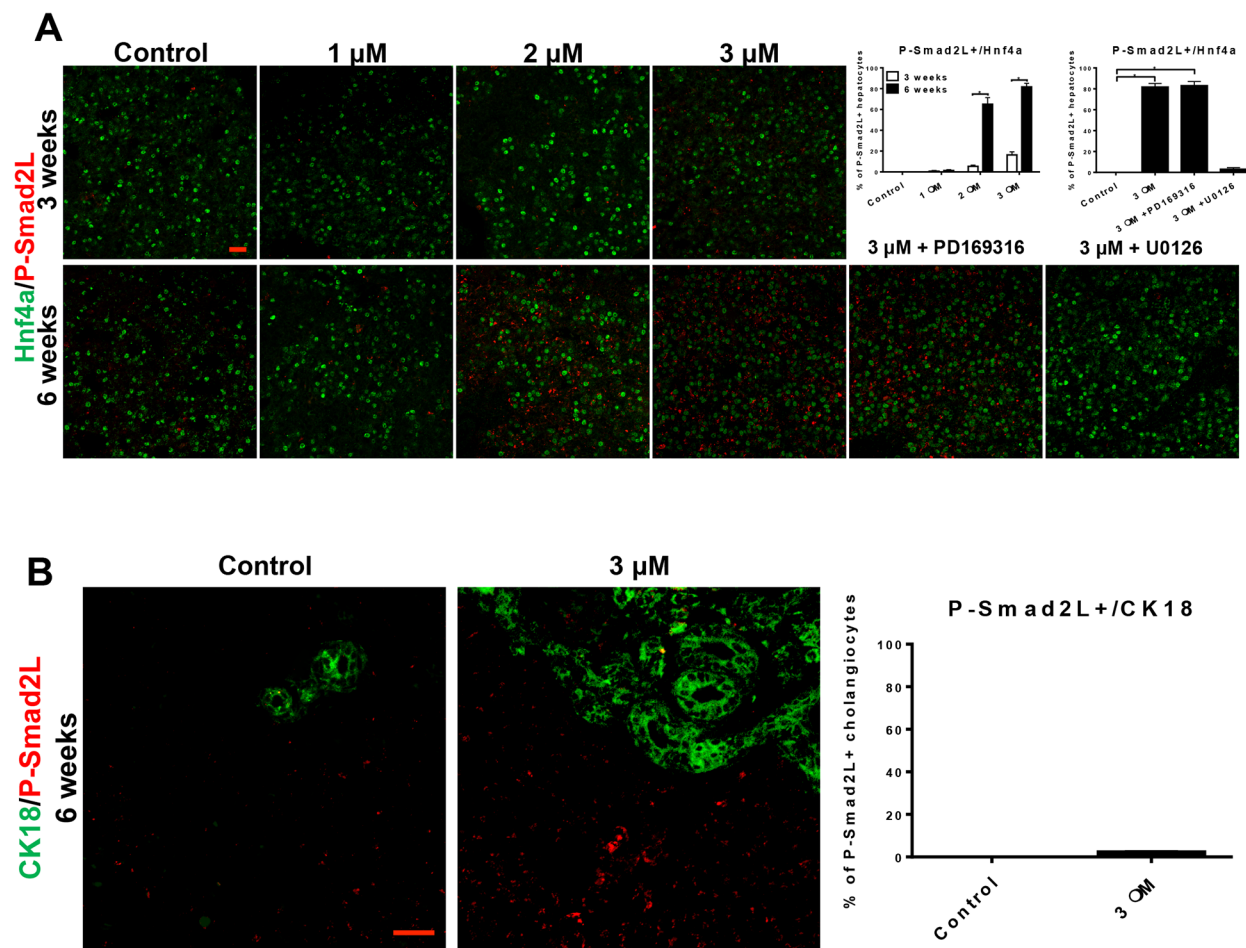
Supplementary Figure 1: Analysis of inflammation gene expressions in hepatocytes and cholangiocytes in *tgfb1a*+ zebrafish. 3-month-old *tgfb1a*+ zebrafish were treated with either 0 μ M (control) or 3 μ M (high) mifepristone for 6 weeks. Liver were dissociated and FACS sorted for hepatocytes and cholangiocytes and inflammation gene expression was analysed by RT-qPCR. (A) Expression of *tgfb1a*, *tgfb1b* and *tgfb2* in hepatocytes of *tgfb1a*+ zebrafish. (B) Expression of *tgfb1a*, *tgfb1b* and *tgfb2* in cholangiocytes of *tgfb1a*+ zebrafish. * $p < 0.05$.



Supplementary Figure 2: Gross morphology and histology of *tgfb1a*+ zebrafish induced for 9 weeks by 1 μ M or 2 μ M of mifepristone. (A) Gross morphology (top row) and histology as stained by H&E (bottom row). Scale bar, 100 μ m for the top row and 20 μ m for the bottom row. (B) Quantification of tumor phenotypes in these *tgfb1a*+ zebrafish (n=20/group).



Supplementary Figure 3: Proliferation and apoptosis of liver cells in *tgfb1a*+ zebrafish induced for 6 weeks by 1 μ M, 2 μ M and 3 μ M mifepristone. (A) PCNA staining (left) and quantification (right) for cell proliferation (n=20/group). (B) Caspase3 staining (left) and quantification (right) for cell apoptosis (n=20/group). Scale bars, 20 μ m. Statistical significance, *p<0.05.



Supplementary Figure 4: Expression of P-Smad2L in hepatocyte and cholangiocytes respectively. 3-month-old *tgfb1a*+ transgenic zebrafish were treated with 1 μ M (low), 2 μ M (medium) and 3 μ M (high) mifepristone for 6 weeks or induced with 3 μ M of mifepristone for 3 weeks, then co-incubated with 3 μ M of mifepristone + 1 μ M of either PD169316 (Smad2 inhibitor) or U0126 (MEK inhibitor) for another 3 weeks. (A) Immunofluorescent co-staining of Hnf4a/P-Smad2L. (B) Immunofluorescent co-staining of CK18/P-Smad2L. n=20/group. Scale bars, 20 μ m. Statistical significance, *p<0.05.

Supplementary Table 1 : List of PCR primers

Gene	Forward	Reverse
<i>b actin</i>	CTCTGGGTCACCGCTTCTTT	CAGATGCTCACGAAACCCCT
<i>Cebpa</i>	TGAAGATTGGCGATCGAGGG	ATTTTCGCCTTGTCCCGACT
<i>illb</i>	GCCTGTGTGTTTGGGAATCT	TGATAAACCAACCGGGACAT
<i>mCherry</i>	GAACGGCCACGAGTTCGAGA	CTTGGAGCCGTACATGAAC
<i>nfkB2</i>	AACAAGACGCAAGGAGCCCA	TGAACCTCCACACGAGCATT
<i>Pparg</i>	CACTCTCCGCTGATATGGTGG	GTAGATGGGCTCGTGTGTCC
<i>srebpl</i>	CTCTGGGTCACCGCTTCTTT	CAGATGCTCACGAAACCCCT
<i>Tnfa</i>	CGTCTGCTTCACGCTCCATA	GATGTGCAAAGACACCTGGC

Supplementary Table 2 : List of antibodies

Primary antibody against	Host species	Dilution	Supplier
Alcam	Mouse	1:5	zn-8, ZIRC
Alexa Fluor 488 donkey anti-goat	Goat	1:500	A11055, ThermoFisher
Alexa Fluor 546 goat anti-rabbit	Rabbit	1:500	A11071, ThermoFisher
Alexa Fluor 488 goat anti-rabbit	Rabbit	1:500	A11070, ThermoFisher
Alexa Fluor 546 goat anti-mouse	Mouse	1:500	A11018, ThermoFisher
Alexa Fluor 488 goat anti-mouse	Mouse	1:500	A11017, ThermoFisher
aSma	Rabbit	1:200	ab15734, Abcam
Bcatenin	Rabbit	1:100	ab6302, Abcam
Caspase 3(Active)	Rabbit	1:200	C92-065, BD biosciences
CD4	Rabbit	1:100	AS-55712, Anaspec
Collagen I	Rabbit	1:100	ab23730 Abcam
Csflr	Rabbit	1:100	AS-55618, Anaspec
Cytokeratin 18	Mouse	1:100	LS-C84878, LifeSpan bioscience
Ecadherin	Mouse	1:100	ab11512, Abcam
HNF4a	Goat	1:50	SC-6556, Santa Cruz
Laminin	Rabbit	1:100	L9393, Sigma
Leptin	Rabbit	1:100	Ab1673, Merck
mCherry	Rabbit	1:100	ab167453, Abcam
Nfkb2	Rabbit	1:100	AS-55484, Anaspec
PCNA	Rabbit	1:200	FL-261, Santa Cruz
P-Erk	Mouse	1:100	M9692, Sigma
P-Erk	Rabbit	1:200	SC-13073, Santa Cruz
P-Smad2 (Ser467)	Mouse	1:100	ab53100, Abcam
P-Smad2 (Ser465/467)	Rabbit	1:100	3101, Cell Signaling
P-Smad2L (Ser245/250/255)	Rabbit	1:100	3104, Cell Signaling
Tgfb1	Rabbit	1:200	AS55450, Anaspec
TGFB1	Rabbit	1:100	ab92486, Abcam
Vimentin	Mouse	1:100	ab8979, Abcam