Supplemental Materials:

I. Supplementary Methods

Cell Lines, Treatments and Reporter Assays

HepG2 cells and HC-AFW1 cell lines with known exon-3 deletions in *CTNNB1* gene leading to deletion of 116 and 49 amino acids, respectively, were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and used for the experiments.[1,2] For knockdown studies *in vitro,* HepG2 and HC-AFW1 cells were transfected with small interfering RNA (siRNA) against Yap1 (Catalogue # 4392420; ID # S20366) and/or validated siRNA directed against exon-4/5 of β-catenin gene (Catalogue # AM51331; ID # 42816) from Life Technologies (Grand Island, NY), according to the manufacturer's recommendations, and incubated for 24 and 48 hours.

For the treatment with chemical inhibitors, the two cell lines were plated at 2.0 X 10^3/well in 96-well plate and grown for 12 hours. After 24-hour serum deprivation, PNU74654 (β-catenin inhibitor; Santa Cruz Biotechnology, Santa Cruz, CA) IWR1-endo (β-catenin inhibitor; Santa Cruz Biotechnology), and/or Verteporfin (Yap-Tead inhibitor; VWR International GmbH, Darmstadt, Germany) were added to the medium at 200 μM, 20 μM, and 15 μM final concentration, respectively, and cells incubated for 24 and 48 hours. To assess cell proliferation, HB cells were plated at the concentration of 2.0 x 10^3/well in 96-well plates, allowed to attach and adjust for the next 12 hours (which corresponds to the 0 hour time point in our graphs), and grown for additional 24 and 48 hours.

The proliferation was assessed at these three time points - 0, 24, and 48 hours with the BrdU Cell Proliferation Assay Kit (Cell Signaling Technology, Danvers, MA) by measuring the absorbance at 450 nm following the manufacturer's protocol. To measure apoptosis, HCC cell lines were plated at the concentration of 2.0 x 10^3/well in 96-well plates, incubated for 12 hours, and then subjected to 24-hour serum deprivation, which corresponds to the 0 hour time point in the apoptosis graphs. HB cells continue to growth in serum-free medium for additional 24 and 48 hours. Apoptosis was assessed at these three time points - 0, 24, and 48 hours with the Cell Death Detection Elisa Plus Kit (Roche Molecular Biochemicals, Indianapolis, IN) by measuring the absorbance at 405 nm, following the manufacturer's instructions.

For assessment of β-catenin transcriptional activity, the TopFlash (TCF reporter plasmid: 21-170; Millipore, Billerica, MA) assay was used following the manufacturer's protocol. Briefly, HepG2 or HC-AFW1 cells grown on 6-well plates were transfected in triplicates with 0.8 μg of TOPflash construct or its mutated control reporter (FopFlash) along with 0.2 μg of internal control reporter *Renilla reniformis* luciferase (pRL-TK; Promega) and siRNAs. The cells were harvested 48 hours post transfection using Dual Luciferase Assay System kit according to manufacturer's protocol (Promega, E1910).

For TEAD transcriptional activity assays, HepG2 and Hep3B cells were plated on 6-well dishes and cotransfected with 800 ng of a 20:1 mixture [8xGTIIC-luciferase (Plasmid 34615; Addgene) and pRL-TK] and siRNAs using Lipofectamine 2000 (Invitrogen). After 48 hours, luciferase activity was assayed

using the dual luciferase reporter assay system. TOPflash, FOPflash and TEAD luminescent signal were normalized to *Renilla* luminescent signal and t-test used to determine the p-value for statistical significance.

Protein Extraction and Western Blotting

HB cell lines were homogenized in lysis buffer [30 mM Tris (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS, 10% glycerol, and 2 mM EDTA] containing the Complete Protease Inhibitor Cocktail (Roche Molecular Biochemicals) and sonicated. Protein concentrations were determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) using bovine serum albumin as standard. Aliquots of 40 µg were denatured by boiling in Tris-Glycine SDS Sample Buffer (Life Technologies, Carlsbad, CA), separated by SDS PAGE, and transferred onto nitrocellulose membranes (Life Technologies) by electroblotting. Membranes were blocked in 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and probed with rabbit polyclonal anti-Yap (Cell Signaling Technology, Danvers, MA; 1:500), mouse monoclonal anti- β-catenin (BD Biosciences, San Jose, CA; 1:1000) and mouse monoclonal anti- β-Actin (Sigma-Aldrich, St. Louis, MO; 1:20000) antibodies. Each primary antibody was followed by incubation with horseradish peroxidase-secondary antibody diluted 1:5000 for 1 h and then revealed with the Super Signal West Pico (Pierce Chemical Co., New York, NY).

Quantitative Reverse Transcription Real-time Polymerase Chain Reaction (QRT-PCR).

Primers for human *Yap, β-catenin, Cyr61, CTGF, Survivin, Axin2, DKK1, Jag1, Cyclin D1*, *c-Myc*, *Glypican-3 (Gpc3), Epcam, α-fetoprotein (Afp)* and *RNR-18* genes were designed or purchased predesigned from Applied Biosystems (Foster City, CA) and the information is listed in Online Supplementary Table 4. PCR reactions were performed with 100 ng of cDNA from HB cell lines, using an ABI Prism 7000 Sequence Detection System and TaqMan Universal PCR Master Mix (Applied Biosystems). Cycling conditions were: 10 min of denaturation at 95℃ and 40 cycles at 95℃ for 15 s and at 60℃ for 1 min. Quantitative values were calculated by using the PE Biosystems Analysis software (Applied Biosystems) and expressed as N target (NT). NT = $2^{-\text{ÄCt}}$, wherein ÄCt value of each sample was calculated by subtracting the average Ct value of the target gene from the average Ct value of the *RNR-18* gene.

Coprecipitation studies

HepG2 (HB), Hep3B (HCC) and PLC/PRF/5 (HCC) cells (ATCC) were grown to confluence and whole cell lysate was prepared in radioimmunoprecipitation assay (RIPA) buffer. Five hundred μg of lysate in a 1 ml volume (in the presence of protease and phosphatase inhibitors) were precleared using appropriate control IgG (normal goat) together with 20 μl of protein A/G agarose for 30 minutes to an hour at 4℃ (Santa Cruz Biotechnology). The supernatant obtained after centrifugation (1000 X *g*) at 4℃ was incubated with 10 μl (20 μg) of agarose-conjugated, goat anti-β-catenin antibody (Santa Cruz Biotechnology) for 1 hour or overnight at 4℃. Reverse immunoprecipita tion was also performed by incubating the supernatant with 10 μl of anti-Yap antibody (Cell Signaling) for 1

hour at 4℃ using end-over-end rotation, followed by addition of 20 µl of protein A/G agarose for an hour. The beads were collected by centrifugation (1000 X *g*), washed four times for 5 minutes in cold RIPA buffer. Beads were resuspended in an equal volume of standard electrophoresis loading buffer with SDS and fresh β-mercaptoethanol and boiled for 5 minutes. Thirty µl of samples were resolved on ready gels and transferred as described earlier. β-Catenin immunoprecipitate was probed for Yap and vice versa using antibodies described elsewhere in this report. The blots were stripped and reprobed with antibody to detect protein pulled down for stoichiometry.

Constructs and Reagents

Constitutive activated Yap (YapS127A) was kindly provided by Dr. Kunliang Guan (University of California, San Diego) and cloned into a PT3EF5α vector. S94A mutation was introduced into YapS127A construct using the QuickChange Site-Directed Mutagenesis kit (Stratagene, Santa Clara, CA). N-terminal deleted and activated β-catenin (ΔN90-β-catenin) and pCMV-SB constructs have been previously described[3].

Hydrodynamic Tail Vein Injection

Six- to eight-week-old wild-type FVB/N mice (Charles River, Wilmington, MA) were used. For hydrodynamic tail vein injection, YapS127A or YapS94AS127A (10µg), ΔN90-β-catenin (10µg), and pCMV/SB (0.8µg) were diluted into 2ml 0.9% NaCl solution, sterile filtered with 0.2µm filters, and then injected into mouse lateral tail vein within 5–7 seconds as described[3]. Mice were killed at the time of morbidity that ranged from 1 day to 11.7 weeks after Yap/β-catenin injection. A

list of all mice used in the study along with constructs utilized, sex and time of sacrifice are noted in Online Supplementary Table 5. Parts of livers were used for histopathological analysis and parts were flash frozen for RNA isolation.

For lineage tracing experiment, *AlbCreERT2* mice[4] were crossed with *R26R-EYFP* mice[5] to obtain *AlbCreERT2;R26R-EYFP* double transgenic mice. Mice were treated with Tamoxifen at 6 to 7 weeks old. One week post Tamoxifen treatment, mice were hydrodynamically injected with YapS127A (10μg), ΔN90-β-catenin (10μg), and pCMV/SB (0.8μg) plasmids. Mice were euthanized and liver tissues harvested at 11 weeks post injection. This strategy allows to label and trace cells that are derived from albumin and EYFP-positive mature hepatocytes.

All mice were housed, fed, and monitored in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco.

Immunohistochemistry, Immunofluorescence, Electron Microscopy and Analysis for Proliferation and Apoptosis

Parts of livers were fixed in 10% buffered formalin and paraffin embedded. Four μm sections were stained with Hematoxylin and Eosin (H&E). All tumors were diagnosed by a pediatric pathologist (S.R.) and two liver pathologists (M.E and F.D.). Immunohistochemistry was performed on liver sections for β-catenin (Santa Cruz Biotechnology; SC-7199; 1:150), Myc-tag (SC-788; 1:100), endogenous c-Myc (SC-764; 1:50), Cyclin D1 (Neomarker; 9041-P; 1:50), Yap (Cell Signaling Technology; 1:50), Flag-tag (Cell Signaling Technology; 1:50), Dlk1 (10636-1-AP; Proteintech, Chicago, IL; 1:50), PCNA (SC-56; 1:4000), and

Ki67 (Bethyl Laboratories, Montgmomery, TX; 1:1000), as described elsewhere.[6,7] Briefly, slides were passed through xylene, graded alcohol, and rinsed in PBS. Endogenous peroxide was inactivated using 3% hydrogen peroxide. Slides were microwaved in zinc sulfate for PCNA or citrate buffer for the other antibodies, immersed in Ultra V Block (Lab Vision Products, Fremont, CA) followed by one hour incubation at room temperature with the primary antibody. After washes, the sections were incubated in the appropriate biotin-conjugated secondary antibody (Chemicon, Temecula, CA), for 30 minutes at room temperature. Signal was detected using the Vectastain ABC Elite kit (Vector Laboratories, Inc., Burlingame, CA) and developed using DAB (Vector Laboratories). Sections were counterstained with Shandon hematoxylin solution (Thermo Fisher Scientific, Pittsburgh, PA) and passed through the dehydration process and covered. For negative control, the sections were incubated with secondary antibodies only.

For patients, if 10% or greater cells in a tumor showed nuclear immunoreactivity for β-catenin or Yap, these were labelled as positive tumors. Tumors were called simultaneously positive for nuclear β-catenin and Yap only if serial sections displayed nuclear positivity in the same areas for both proteins examined.

For mice, proliferation and apoptotic indices were determined in wild-type livers and Yap/β-catenin animals by counting Ki67-positive cells and apoptotic figures stained with the ApoTag Peroxidase In Situ Apoptosis Kit (Millipore, Billerica, MA), respectively, on at least 3000 hepatocytes.

For electron microscopy, specimens of 1-2mm³ from representative liver tissue were cut with a razor blade, fixed in 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO) in phosphate buffered saline, post-fixed with osmium tetroxide (Carl Roth, Karlsruhe, Germany), embedded in glycidether 100 (Carl Roth), and cut with with a diamond knife with a Leica Ultracut ultramicrotome (Leica Microsystems, Wetzlar, Germany) to 500 and 750 nm thick semi-thin slides and stained according to Richardson. Ultrathin sections of 70-90 nm were stained with uranyl acetate and lead citrate (Sigma-Aldrich) and examined with a Zeiss Libra 120 electron microscope (Carl Zeiss Microscopy GmbH, Goettingen, Germany).

For immunoflourescence staining, liver tissue was freshly isolated from euthanized animals, embedded in O.C.T. compound and frozen in cold 2-Methylbutane. Frozen sections were fixed in acetone, blocked with 5% serum of species in which secondary antibody was raised, and incubated with primary antibodies: EpCAM, Clone G8.8, 1:200 (eBioscience Inc., San Diego, CA); beta-catenin, 1:200 (BD Biosciences, San Diego, CA); and EYFP, 1:200 (Abcam, Cambridge, MA), overnight at 4℃ followed by incubation for corresponding secondary antibody Alexa Fluor IgG (Invitrogen, Carlsbad, CA) at 1:500 dilution for 30 min at room temperature. The immunofluorescence signal was visualized using an immunofluorescence microscope after the sections were mounted with VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Inc).

References

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II. Supplemental Tables

A. **Supplemental Table 1:** Summary of nuclear localization of Yap and β-catenin in 94 human hepatoblastoma cases.

B. **Supplemental Table 2:** Summary of nuclear localization of Yap and β-catenin in 103 human hepatocellular carcinoma cases.

C. **Supplemental Table 3:** Summary of nuclear localization of Yap and β-catenin in 62 human intrahepatic cholangiocarcinoma cases.

D. **Supplementary Table 4:** Information on primers used for QRT-PCR analysis in the current study.

E. **Supplementary Table 5:** Summary of all mice used in the current study for hydrodynamic injections.

Supplemental Table 1: Summary of nuclear localization of Yap and β-catenin in 94 human hepatoblastoma cases.

HB Case number	Age	Sex	Morphology	Nuclear β-catenin	Nuclear YAP
1	30m	M	Mixed	Y	N
2	18m	F	Embryonal	Y	Y
3	36m	M	Fetal	Y	Y
4	12m	F	Fetal	Y	Y
5	6m	M	Fetal	Y	Y
6	8m	M	Mixed	N	N
7	12m	M	Fetal/Embryonal	Y	Y
8	24m	M	Fetal	Y	Y
9	28m	M	Fetal	Y	Y
10	52m	M	Fetal	Y	Y
11	13m	M	Embryonal/mesenchymal	N	N
12	11m	M	Fetal	Y	Y
13	15m	F	Embryonal	Y	Y
14	27m	F	Fetal	Y	Y
15	108m	M	Feta	Y	Y
16	62m	M	Fetal	Y	Y
17	19m	M	Fetal	Y	Y
18	22m	F	Embryonal/Mesenchymal	N	Y
19	10m	F	Mixed	Y	N
20	26m	F	Fetal	Y	Y
21	14m	M	Embryonal	Y	Y
22	18m	M	Embryonal	Y	Y

23	9m	F	Fetal/Embryonal	Y	Y
24	12m	M	Fetal/Embryonal	Y	Y
25	21m	F	Embyonal	Y	Y
26	58m	F	Embryonal	Y	Y
27	26m	M	Mesenchymal	N	N
28	58m	F	Embryonal/Mesenchymal	N	N
29	20m	F	Fetal	Y	Y
30	34m	M	Mixed	Y	N
31	42m	F	Fetal	Y	Y
32	58m	M	Fetal	Y	Y
33	13m	M	Mixed	Y	Y
34	48m	M	Embryonal	Y	Y
35	60m	M	Mixed	Y	Y
36	11m	M	Mixed	N	Y
37	50m	M	Fetal	Y	Y
38	18m	M	CF/Embryonal	Y	Y
39	24m	F	CF/Embryonal	Y	Y
40	28m	M	Fetal	Y	Y
41	14m	F	Embryonal	Y	Y
42	46m	M	Fetal	Y	Y
43	60m	F	Mixed	Y	N
44	16m	F	Embryonal	Y	Y
45	34	M	Fetal	Y	Y
46	12m	M	Fetal	Y	Y
47	22m	F	Embryonal	Y	Y
48	40m	M	SCU/Embryonal	Y	Y

49	26m	M	SCU	Y	Y
50	38m	M	Mesenchymal	Y	Y
51	12m	F	Mixed	Y	Y
52	9m	M	Mixed	Y	Y
53	25m	F	Fetal	Y	Y
54	14m	F	Embryonal	Y	Y
55	36m	M	Fetal	Y	Y
56	16m	M	Fetal	Y	Y
57	45m	M	Mesenchymal	Y	N
58	12m	F	SCU	Y	Y
59	NA	NA	Fetal	Y	Y
60	28m	F	Embryonal	Y	Y
61	14m	M	CF/Embryonal	Y	Y
62	NA	NA	Fetal	Y	Y
63	NA	NA	Fetal/Embryonal	Y	Y
64	NA	NA	Fetal	Y	Y
65	NA	NA	Feta	Y	Y
66	NA	NA	Fetal/Embryonal	Y	Y
67	25m	F	Embryonal	Y	Y
68	NA	NA	CF/Embryonal	Y	Y
69	30m	M	SCU	Y	Y
70	12m	F	Fetal	Y	Y
71	42m	M	Embryonal	Y	Y
P1	9m	F	CF/Embryonal	Y	Y
P2	5m	F	CF/Embryonal	Y	Y
P3	51m	F	CF/Embryonal	Y	Y

P4	108m	F	Fetal/CF	Y	Y
P5	24m	M	Embryonal/SCU	Y	Y
P6	24m	M	Embryonal/SCU	Y	Y
P7	11m	F	Embryonal/SCU	Y	Y
P8	36m	M	Embryonal/Teratoid	Y	Y
P9	24m	F	Embryonal/CF	Y	Y
P10	24m	M	Embryonal/SCU	Y	Y
P11	8m	M	CF/Embryonal	Y	Y
P12	12m	M	CF/Well differentiated fetal	Y	Y
P13	12m	F	Embryonal/SCU/mesenchymal	Y	Y
P14	60m	F	Well differentiated Fetal	Y	Y
P15	12m	F	Embryonal/CF/SCU	Y	Y
P16	36m	M	CF/Embryonal	Y	N
P17	22m	M	Embryonal	Y	N
P18	72m	M	CF/Embryonal	Y	N
P19	48m	F	Embryonal/CF	Y	N
P20	48m	F	Embryonal/CF	Y	N
P21	24m	M	Treated CF	N	Y
P22	12m	M	Fetal	N	Y
P23	6m	F	SCU/Embryonal	N	Y
P24	12m	M	Fetal/Embryonal	N	Y

Abbreviations: N-no nuclear staining (shaded orange); Y-positive nuclear staining (shaded yellow); 1-71-patients from Europe; P1-P24-patients from Children's Hospital, University of Pittsburgh; m-months; M-male; F-female; NA-not available; CF-crowded fetal (Fetal HB with mitosis); SCU-small cell undifferentiated

Supplemental Table 2: Summary of nuclear localization of Yap and β-catenin in 103 human hepatocellular carcinoma (HCC) cases.

HCC Case number	Etiology	Gender	Cirrhosis	Survival (months)	Differentiation	Nuclear β-Catenin	Nuclear Yap
1	HCV	F	Y	83.3	Well	N	Y
2	HCV	M	Y	33.4	Poorly	N	Y
3	HBV	M	Y	140	Well	Y	Y
4	HBV	M	N	142	Poorly	N	Y
5	HBV	M	Y	46.9	Well	Y	N
6	HBV	M	N	58.6	Poorly	N	Y
7	HBV	M	N	44	Well*	Y	Y
8	HCV	M	Y	48	Moderately	N	Y
9	HBV	M	Y	70	Well	N	N
10	Ethanol	M	Y	61	Well*	Y	N
11	HBV	M	Y	52	Well	N	Y
12	HCV	M	Y	64.6	Well*	Y	N
13	HCV	M	Y	55.2	Well	N	Y
14	HBV	M	Y	48	Moderately	N	Y
15	Ethanol	M	Y	84	Modertely*	Y	N
16	Ethanol	M	Y	64	Well	Y	N
17	HBV	M	Y	59	Poorly	N	Y
18	HCV	M	N	42	Poorly	N	Y
19	HBV	M	N	66	Well	Y	N
20	Wilson's disease	M	N	55.2	Well	N	Y
21	HBV	M	Y	50	Moderately	N	Y
22	HCV	M	Y	61	Moderately	N	Y

23	HBV	F	N	102	Well	N	Y
24	HCV	F	Y	133	Well	N	Y
25	Ethanol	M	Y	82.4	Poorly	N	Y
26	Wilson disease	M	Y	96.4	Well	N	Y
27	HCV	M	Y	122.4	Moderately	N	Y
28	HBV	M	Y	72	Poorly	N	Y
29	HBV	M	Y	60	Well	N	Y
30	HBV	M	Y	45	Well	N	N
31	HBV	M	Y	88	Well*	Y	N
32	HCV	M	Y	95	Poorly	N	Y
33	HBV	M	Y	86	Poorly	N	Y
34	HBV	M	Y	52.9	Well	Y	N
35	HBV	M	Y	43.1	Moderately	Y	Y
36	HBV	M	Y	62	Well	N	Y
37	HBV	M	Y	58.2	Well	N	Y
38	HBV	M	Y	52.7	Well	N	Y
39	HCV	M	Y	48.6	Well	N	Y
40	HCV	M	N	39	Poorly	N	Y
41	HBV	M	Y	48.3	Well	N	Y
42	HCV	F	Y	80.9	Well	Y	N
43	HBV	M	Y	88.3	Well	N	N
44	HBV	M	N	50.8	Well	Y	N
45	NA	M	Y	49.7	Well	N	Y
46	Ethanol	M	Y	43.9	Well*	Y	N
47	HBV+	M	Y	62	Moderately	N	Y
48	Ethanol	M	Y	38.2	Well	Y	Y

49	Ethanol	M	Y	44.2	Moderately	N	Y
50	NA	M	N	5.9	Well*	N	Y
51	HBV	F	N	0.17	Well	N	Y
52	HBV	M	Y	2	Well	Y	N
53	HBV	M	Y	27.3	Well	N	N
54	HBV	F	Y	32	Poorly	N	Y
55	HBV	M	Y	10.4	Poorly	N	Y
56	HBV	M	Y	2	Well	N	Y
57	HBV	M	N	9.5	Moderately*	Y	N
58	HBV	M	Y	5	Well	N	Y
59	HBV	M	Y	7.7	Well	N	Y
60	HCV	M	Y	15.2	Well	N	Y
61	HCV	M	Y	8	Well	N	Y
62	HCV	M	Y	1.2	Well	Y	N
63	HCV	M	Y	6	Well	N	N
64	HCV	M	Y	1.4	Well	N	Y
65	HCV	M	Y	5	Well	N	N
66	Hereditary Hemochromatosis	M	Y	11.9	Poorly	N	Y
67	HBV	M	Y	20	Well	N	Y
68	HBV	M	Y	24.2	Moderately	Y	N
69	Ethanol	M	Y	6.2	Well	Y	N
70	Ethanol	M	Y	15.8	Well	N	Y
71	Ethanol	M	Y	22.2	Well	Y	N
72	Ethanol	M	Y	12.2	Well	N	Y
73	HBV	F	Y	20.6	Well	N	N

74	HBV	M	N	14	Well	N	Y
75	HBV	M	N	12.8	Well*	Y	N
76	Ethanol	M	Y	28.8	Well	Y	N
77	HCV	M	Y	5.2	Well	N	Y
78	HCV	M	Y	18	Well	N	Y
79	HCV	M	Y	24.4	Poorly	N	Y
80	Ethanol	M	Y	10.2	Moderately	N	Y
81	HCV	M	Y	17.6	Poorly	N	Y
82	HBV	F	Y	22.00	Well	N	Y
83	HBV	M	Y	18.00	Well*	Y	N
84	Wilson disease	M	Y	30.00	Well	Y	N
85	HCV	M	Y	24.4	Well	N	Y
86	HCV	M	Y	12	Poorly	N	N
87	HBV	M	Y	10	Well	N	Y
88	HCV	M	Y	6.2	Well	Y	N
89	HBV	M	Y	32	Well	N	Y
90	HBV	M	Y	20	Well	N	N
91	Ethanol	M	Y	20.5	Moderately*	Y	N
92	Ethanol	M	Y	16.2	Well	N	N
93	HBV	M	Y	28.4	Well	N	Y
94	HBV	M	Y	22	Well	Y	N
95	HBV	M	N	15.4	Poorly	N	Y
96	Ethanol	M	Y	5	Poorly	N	Y
97	HBV	M	N	4.2	Well	N	Y
98	NA	M	N	8	Moderately	Y	N
99	HCV	F	Y	14	Poorly	N	Y

100	HCV	M	Y	8.2	Moderately	N	Y
101	HBV	M	Y	18	Well	N	Y
102	HBV	M	Y	16	Well	N	Y
103	HCV	F	Y	25	Well	N	N

Positive nuclear staining is shaded yellow; Negative nuclear staining is shaded as orange. ***** Indicates HCC with progenitor cell features as indicated by immunoreactivity to EpCAM.

Abbreviations: M-male; F-female; NA- not available; N-no; Y-yes; HCV-hepatitis C virus; HBV-hepatitis B virus.

Supplemental Table 3: Summary of nuclear localization of Yap and β-catenin in 62 human intrahepatic cholangiocarcinoma (ICC) cases.

ICC Case number	Etiology	Gender	Differentiation	Nuclear β-Catenin	Nuclear Yap
1	HCV	M	Well	N	Y
2	HCV	M	Moderately	N	Y
3	None	M	Well	N	Y
4	None	M	Moderately	N	Y
5	PSC	M	Well	N	Y
6	None	M	Well	N	Y
7	PSC	F	Poorly	N	Y
8	HCV	M	Well	Y	Y
9	HCV	M	Well	N	Y
10	HBV	M	Well	N	Y
11	None	M	Poorly	N	Y
12	HCV/Hepatolithiasis	M	Moderately	N	Y
13	None	M	Moderately	N	Y
14	HBV	M	Well	N	Y
15	None	F	Well	N	Y
16	None	F	Poorly	N	Y
17	None	M	Well	N	Y
18	Hepatolithiasis	M	Well	N	Y
19	PSC	M	Poorly	N	Y
20	PSC	M	Moderately	N	Y
21	HCV	M	Well	N	Y
22	None	M	Moderately	N	N
23	HBV/Hepatolithiasis	M	Well	Y	Y

24	None	M	Well	N	Y
25	Hepatolithiasis	M	Poorly	N	Y
26	HCV	M	Well	N	Y
27	None	M	Well	N	Y
28	Hepatolithiasis	M	Moderately	N	Y
29	None	M	Moderately	N	Y
30	Hepatolithiasis	M	Well	N	Y
31	None	F	Moderately	N	Y
32	None	M	Well	N	Y
33	HCV	M	Poorly	N	Y
34	HCV	F	Well	N	Y
35	HBV	M	Well	N	Y
36	HCV	M	Well	N	Y
37	None	M	Moderately	N	Y
38	Hepatolithiasis	M	Poorly	N	Y
39	None	M	Well	N	Y
40	None	M	Well	N	Y
41	None	M	Well	N	Y
42	HCV	F	Well	N	Y
43	None	M	Poorly	N	Y
44	HCV	M	Well	N	Y
45	None	M	Well	N	Y
46	None	F	Moderately	N	Y
47	PSC	M	Moderately	N	Y
48	HBV	M	Poorly	N	Y
49	Hepatolithiasis	F	Poorly	N	Y

50	Hepatolithiasis	F	Well	N	Y
51	HCV	M	Moderately	N	Y
52	None	F	Well	N	Y
53	None	M	Well	N	Y
54	None	F	Well	N	Y
55	Hepatolithiasis	F	Moderately	N	Y
56	HCV	M	Poorly	N	Y
57	None	M	Well	N	Y
58	None	M	Well	N	Y
59	None	M	Well	N	Y
60	HBV/Hepatolithiasis	M	Well	N	Y
61	HCV	M	Moderately	N	Y
62	None	M	Well	N	Y

Abbreviations: N-no nuclear staining (shaded orange); Y-positive nuclear staining (shaded yellow); PSC-primary sclerosing cholangitis; HCV-hepatitis C virus; HBV-hepatitis B virus

Supplementary Table 4: Information on primers used for QRT-PCR analysis.

A. Sequence of primers generated for QRT-PCR analysis.

Gene	Primer	
	Forward primer (5′–3′ sequence	Reverse primer (5′–3′ sequence)
GPC3	TCGACAGCCTCTTTCCAGTCA	GGTCACGTCTTGCTCCTCG
CTGF	GGGCCTCTTCTGCGATTTC	ATCCAGGCAAGTGCATTGGTA
Cyr61	CTGCGCTAAACAACTCAACGA	GCAGATCCCTTTCAGAGCGG
Jag1	CCTCGGGTCAGTTTGAGCTG	CCTTGAGGCACACTTTGAAGTA
AFP	CTTCCCTCATCCTCCTGCTAC	ACAAACTGGGTAAAGGTGATGG
EpCam	GCGGCTCAGAGAGACTGTG	CCAAGCATTTAGACGCCAGTTT
Survivin	GCCACGCATCCCAGCTT	TTTCCAAATACCACTGTCTCCTTCTC

B. Additional primers were also purchased from Life Technologies and the relevant information is as follows:

Gene	Sequence information
Yap	Hs00902712_g1
β-catenin	Hs00355049_m1
Axin2	Hs00610344_m1
DKK1	Hs00183740_m1
Cyclin D1	Hs00765553_m1
c-Myc	Hs00153408_m1
RNR-18	Human 18S rRNA (20x) 4319413E

Supplemental Table 5: Information on all mice used in the study.

Plasmid	Sex	Strain	Harvest time point (Post injection)	Gross images	Body weight(g)	Liver weight(g)	Liver ratio
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	1 day	normal	22.9	1.4	6.11
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	3 days	normal	25.3	1.3	5.14
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	3 days	normal	25.5	1.5	5.88
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	5 days	normal	22.1	1.3	5.88
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	5 days	normal	20.6	1.2	5.83
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	7 days	normal	20.2	1.3	6.44
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	7 days	normal	19.3	1.6	8.29
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	3 Weeks	normal	33.1	2.3	6.95
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	3.5 Weeks	tumor	33.4	3	8.98
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	4 Weeks	normal	35.2	1.8	5.11
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	4.5 Weeks	tumor	31.8	2.8	8.81
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	4.5 Weeks	tumor	29.7	2.6	8.75
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	6.5 Weeks	tumor	34.9	11.7	33.52
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	6.5 Weeks	tumor	32.5	9.7	29.85
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	9 Weeks	tumor	24.3	4.7	19.34
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	9.7 Weeks	tumor	25.1	8	31.87
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	10 Weeks	tumor	30.9	6.6	21.36
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	10 Weeks	tumor	35.1	5.2	14.81
YapS127A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	11.7 Weeks	tumor	26	6.6	25.38
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	10 Weeks	normal	21.6	1.2	5.56
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	10 Weeks	normal	23.2	1.2	5.17
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Male	FVB	10 Weeks	normal	24	1.2	5.00
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	11 Weeks	normal	28.3	1.4	4.95
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	11 Weeks	normal	26.5	1.2	4.53
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	12 Weeks	normal	28.1	1.1	3.91
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	12 Weeks	normal	22.1	1	4.52
YapS127AS94A (10ug)+ΔN90-β-catenin(10ug)+SB(0.8ug)	Female	FVB	12 Weeks	normal	27.9	1.3	4.66

III. Supplementary Figures Legends

Supplementary Figure 1:

A. Western blot using whole cell lysates from four liver tumor cell lines showing β-catenin expression and truncations due to deletions in exon-3. Yap protein expression is also show in the same four cell lines. Protein loading for each cell line is verified by a blot for β-actin.

B-E. Pharmacological inhibition of β-catenin by small molecules PNU74654 and IWR1 endo and Yap-TEAD pathway by small molecule Verteporfin or their combination led to significantly decreased cell proliferation and increased apoptosis in both HepG2 and HC-AFW1 cells when compared to no treatment or treatment of cells with DMSO (solvent for inhibitors) or IWR1 exo (negative control for IWR1 endo). Experiments were conducted at least 3 times in triplicate. Statistical analysis: a- different from Control; b- different from DMSO (solvent); c- different from IWR1 exo; d- different from IWR1 endo; e- different from PNU74654; f- different from Verteporfin; g- different from IWR1 endo + Verteporfin. All p values in the current study were less than 0.0005.

Supplementary Figure 2:

A. Decreased expression of Yap and its targets such as *CTGF, Cyr61, Jag1* and *survivin*, after Yap knockdown in HC-AFW1 cells at 24 and 48 hours after transfection. β-Catenin knockdown led to suppression of *Yap* expression and of its targets *Cyr61* and *survivin,* while no demonstrable effect was evident on *CTGF* or *Jag1*. Combined knockdown of both *Yap*

and *β-catenin*, led to a more pronounced effect on *Cyr61* and *survivin* expression. β-Catenin silencing in HC-AFW1 cells after 24 and 48 hours of transfection causes reduced expression of *β-catenin* and its targets, including *Axin2, c-Myc, Cyclin D1* and *DKK1*. Yap silencing did not impact *β-catenin* expression but affected expression of *c-Myc* and *Cyclin D1*. Dual knockdown of *β-catenin* and *Yap* had a more robust impact on expression of *c-Myc* and *Cyclin D1*. All experiments were conducted at least 3 times and in triplicate. Statistical analysis: a- different from Control; b- different from Scrambled siRNA; c- different from Yap siRNA; d- different from β-catenin siRNA. All p values < 0.001.

B. A significant decrease in β-catenin transactivation was observed in a TopFlash reporter assay 48 hours after silencing of *Yap* in HC-AFW1 cells ($p<0.01$). Transfection of the same cells with FopFlash containing TCF-mutant showed no luciferase activity.

Supplementary Figure 3:

A. H&E staining of a representative liver section showing absence of any microscopic tumor foci at one year after hydrodynamic injection of Δ90β-catenin only.

B. A representative gross specimen and H&E on a liver section show absence of macroscopic or microscopic tumor foci at 22.5 weeks after hydrodynamic injection of Yap127A alone.

C. Gross morphology of livers after co-injection of Yap and β-catenin genes showing multiple tumors at 3, 3.5, 6.5, 9 and 9.7 weeks post injection.

D. IHC for Glutamine Synthetase (GS) shows preneoplastic (PN) lesions to be positive at 3 weeks after Yap/β-catenin injection. In contrast, all HB observed at 3.5, 6.5 and 11.7 weeks after Yap/β-catenin injection were GS-negative as shown in representative liver sections.

Supplementary Figure 4:

A. Representative histology from 3 week old Yap/β-catenin livers showing single or clusters of preneoplastic clear-cell hepatocytes (arrowhead) (200x).

B. Representative histology from additional Yap/β-catenin livers showing HB occurrence. A tumor at 6.5 weeks post injection shows the least maturation, being composed of cells with relatively uniform nuclei that are ovoid to angulated with inconspicuous nucleoli arranged in a vague trabecular and glandular pattern with suggestion of rosettes/canaliculi in an occasional focus. There is an increase in nucleo:cytoplasmic ratio in these cells. These areas resemble those that can be seen in embryonal hepatoblastomas though classic rosettes and clear-cut blastemal cells are not identified. Another photomicrograph showing a tumor from 9 weeks with no significant pleomorphism with tumor cells arranged in a trabecular pattern with one to two cell plate thick trabeculae and sinusoids in between. Mitoses are easily noted. The nuclei are rounded with inconspicuous nucleoli. These areas resemble a crowded fetal pattern of hepatoblastoma. The left tumor from 11.7 weeks is a good example of a matured HB. Tumor cells are bigger with uniform round nuclei with a small

nucleolus, the cytoplasm is either eosinophilic or vacuolated. A trabecular arrangement is noted and mitoses are easily recognized. This tumor corresponds to human fetal subtype of HB. Another tumor also from 11.7 weeks shows a more immature appearance of cells with nucleo:cytoplasmic ratio and angulated to ovoid nuclei arranged in a trabecular pattern, 2-3 cell thick. Again note the lack of pleomorphism.

Supplementary Figure 5:

A-C. Semi-thin sections of liver parenchyma seven days after hydrodynamic injection of Yap and β-Catenin plasmids. Single (A, inset B shows magnified area of A) or groups (C) of transfected/altered hepatocytes (arrows) with irregular cell borders, enlarged nuclei and prominent nucleoli are distributed within the acinus, whereas the portal tract (asterisk) is surrounded by unaltered hepatocytes with round/oval nuclei and regular cell shapes. Richardson staining. Length of the lower edge A- 0.25 mm, B- 83 μm, C- 62 μm.

D-G. Ultrastructural analysis of liver parenchyma. Transfected/altered hepatocytes reveal atypical, irregularly shaped nuclei and several prominent nucleoli, notched nuclear membranes, and nuclear inclusions (arrowheads). Note the loss and irregular distribution of glycogen deposits in the cytoplasm (D, boxed magnified area in E). Hepatocytes are still in close contact to neighboring hepatocytes, forming tight cell junctions and small bile canaliculi (F, boxed magnified area in G).

H-M. Kinetics of HB development after Yap/β-catenin injection in mice. First row: Histological examination of Yap/β-catenin mouse livers one week post injection

show several altered hepatocytes with an enlarged clear cytoplasm and atypical nuclear morphology. These altered hepatocytes expressed the injected genes, as indicated by the positivity to FLAG-tag and MYC-tag staining. Second row: Subsequently, small hepatocellular tumors begin to emerge in the liver parenchyma as early as 3 weeks post injection. Of note, in the transition from preneoplastic lesions to small tumors, altered hepatocytes remodeled towards a smaller size and rounded nuclear shape, especially in the tumor core, whereas enlarged hepatocytes were still present either in the periphery of tumors or in the neighboring parenchyma (arrowheads). Original magnification: x 400 first row; x 200 second row.

N-P. Livers from tamoxifen-injected Alb-CreERT2;R26R EYFP mice administered hydrodynamically Yap127A/ΔN90β-catenin at 11 weeks showing tumor (Tu.) that is strongly positive for β-catenin (green) and EYFP (red) showing co-localization indicating the hepatocyte origin of HB in the current mouse model.

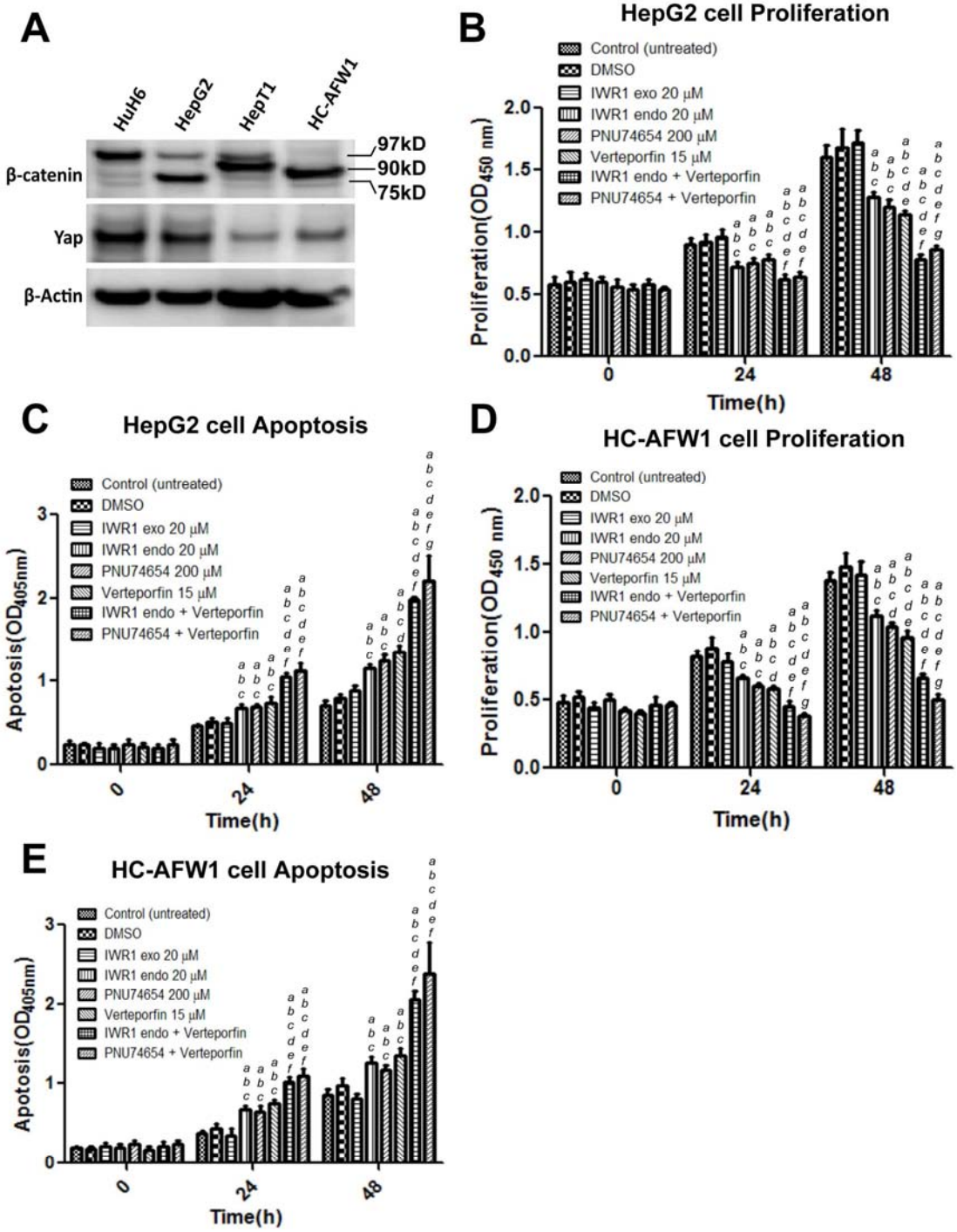
Supplementary Figure 6:

A. Immunohistochemistry using antibody that detects endogenous protein only, identifies c-Myc expression localized to HB, which were observed at various time points after Yap/β-catenin injection (200x).
B. Real-time PCR displays increased expression of several Yap1 targets, including *Jag1, Cyr61, survivin* and *CTGF* and of immature hepatocyte markers such as *Glypican-3 (GPC3), α-fetoprotein (Afp)* and *EpCAM* in Yap/β-catenin livers versus livers of wild-type (WT) mice.

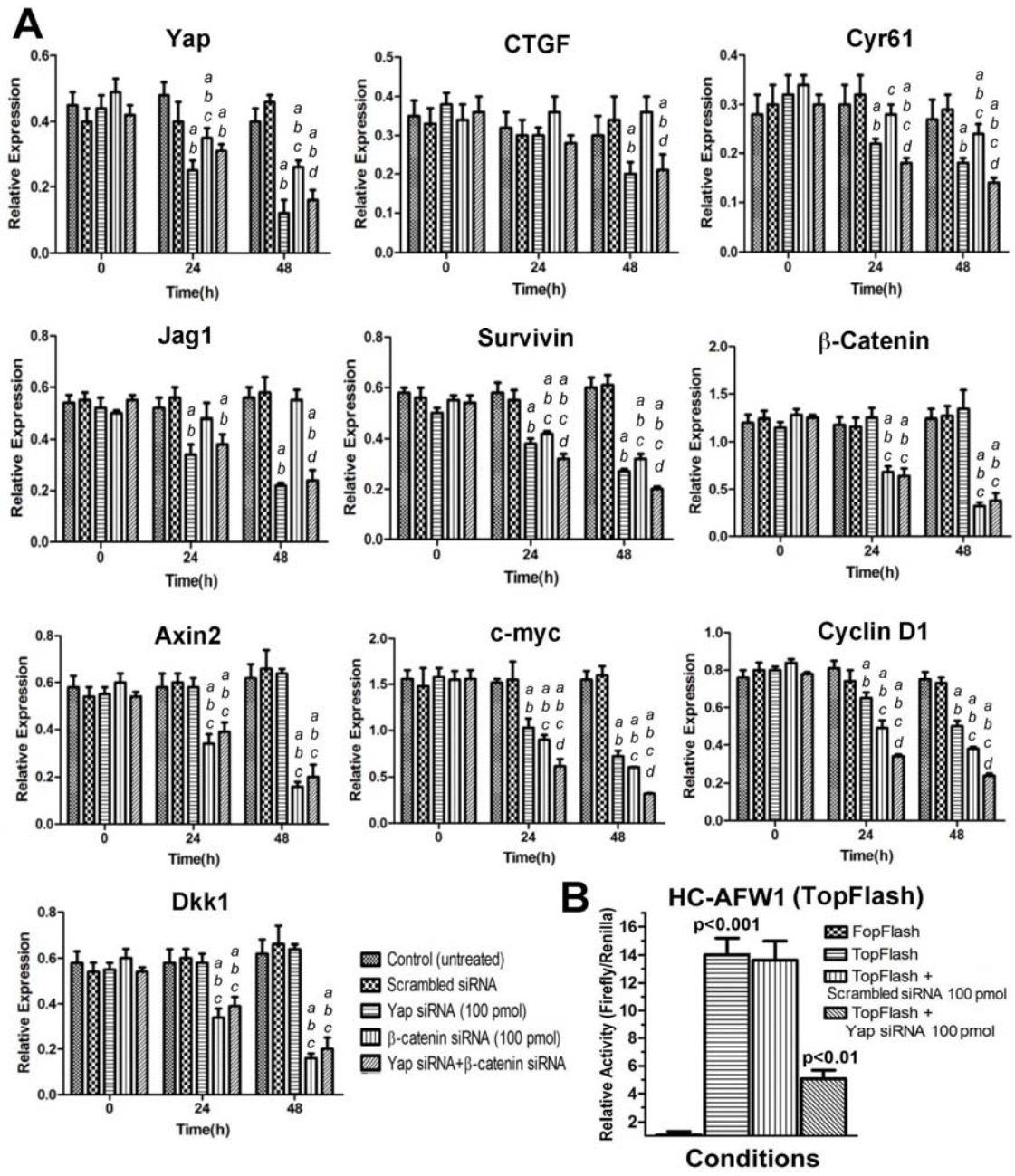
C. Enhanced cell proliferation as indicated by greater numbers of cells in S-phase of cell cycle detected by IHC for Ki-67 was observed in Yap/β-catenin mice as compared to wild-type mice (WT). The differences from WT mice were statistically significant at all times examined (a: $p<0.001$). A significant increase in the number of apoptotic nuclei were also observed in Yap/β-catenin injected livers at different times as compared to WT mice (a: $p<0.001$). 3-5 samples per each group per each time point were analyzed. (200x)

IV. ONLINE SUPPLEMENTARY FIGURES:

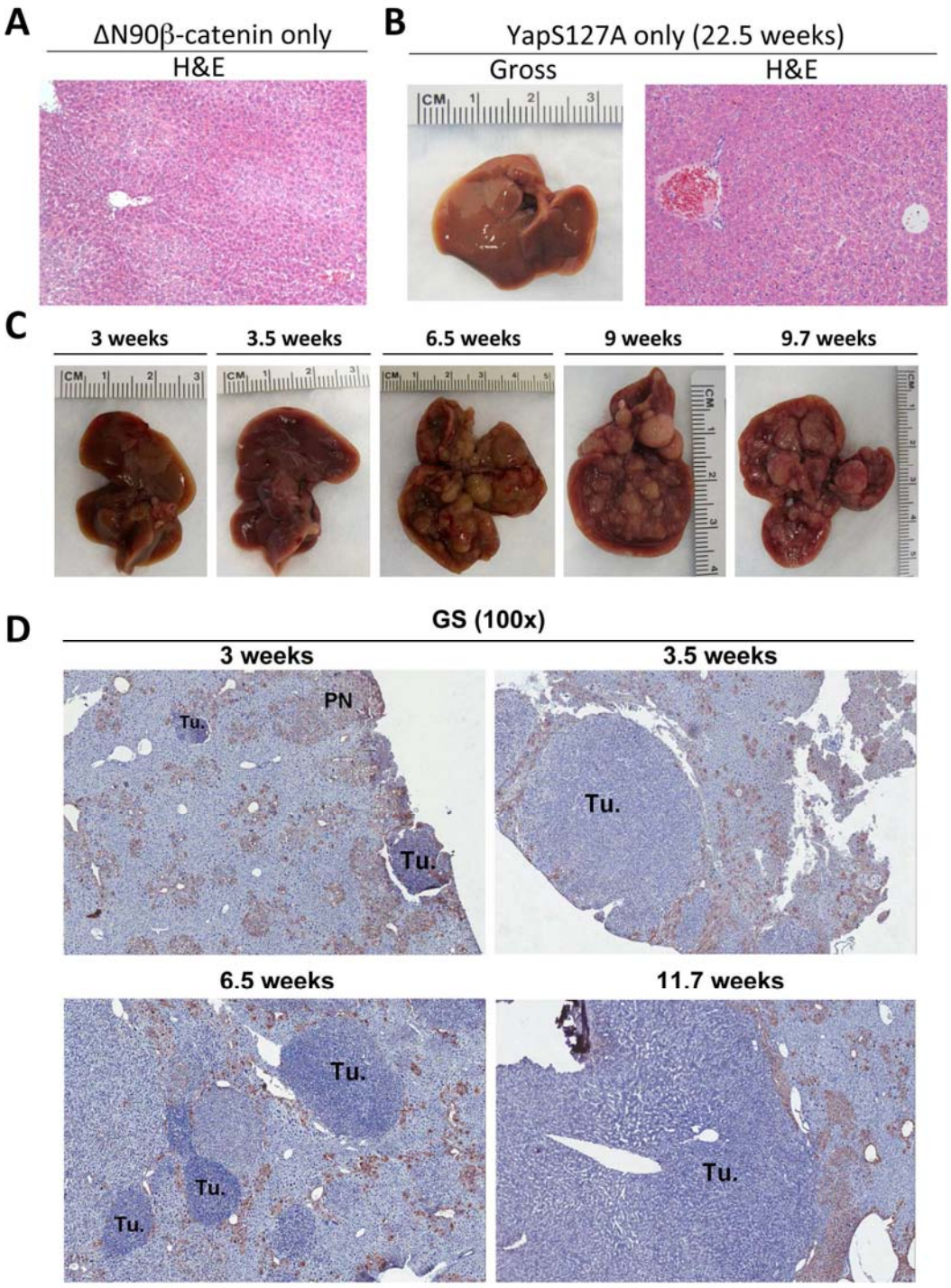
Online Supplementary Figure 1



Online Supplementary Figure 2



Online Supplementary Figure 3



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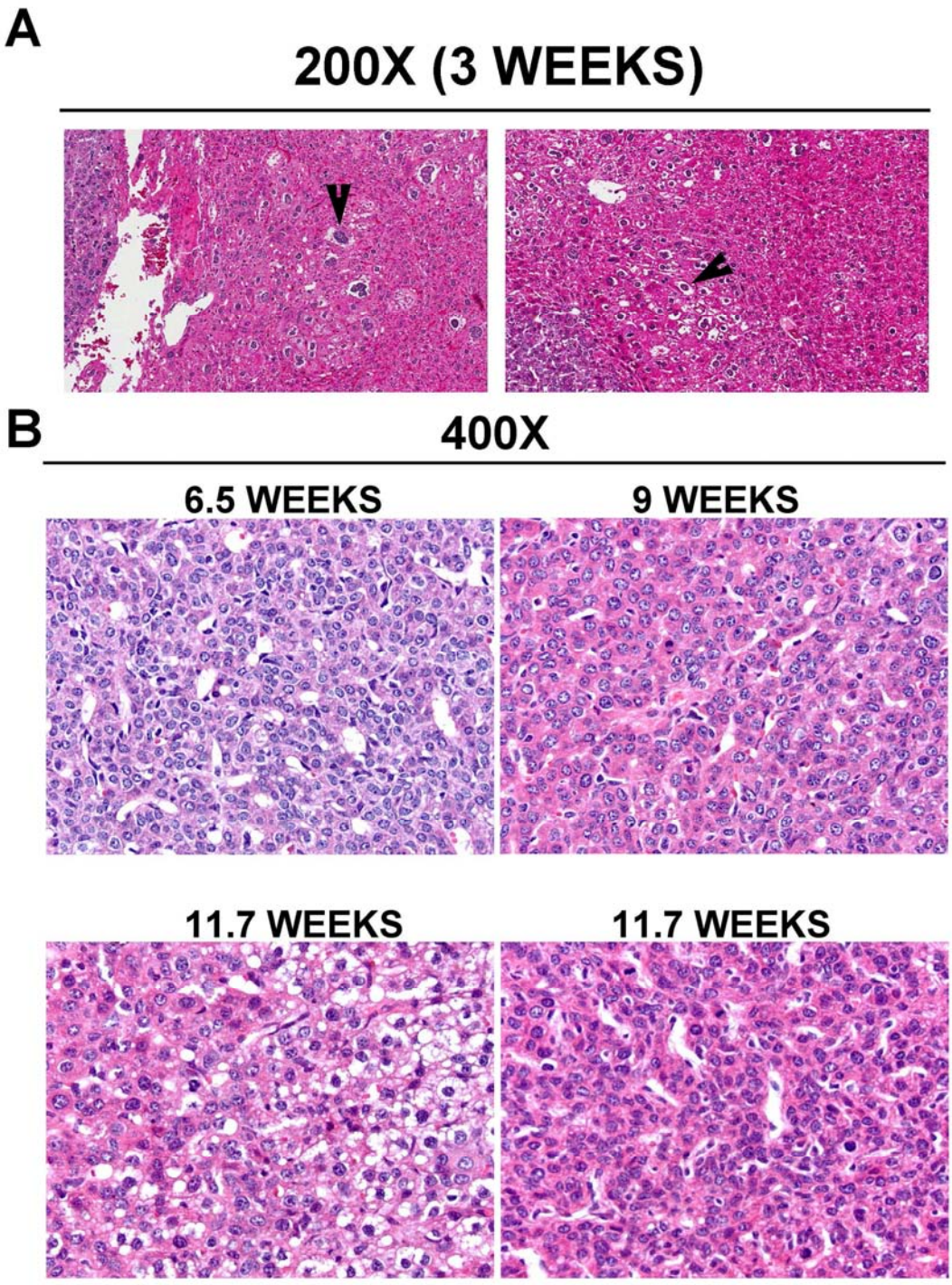
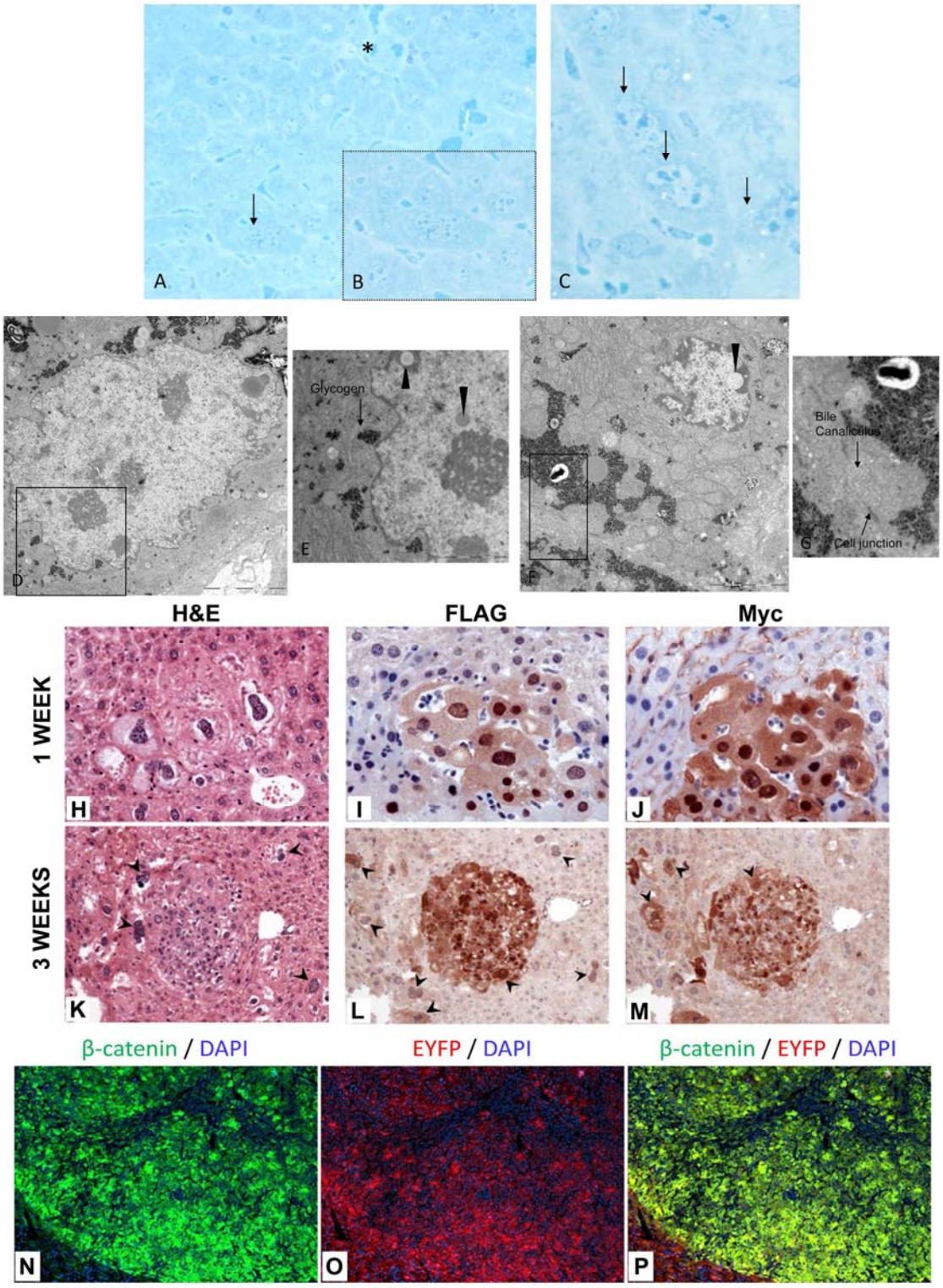


Online Supplementary Figure 5



Online Supplementary Figure 6