Supplementary Information

Hepatic stellate cell stearoyl co-A desaturase activates leukotriene B4 receptor 2-β-catenin cascade to promote liver tumorigenesis

Sonal Sinha, Satoka Aizawa, Yasuhiro Nakano, Alexander Rialdi, Hye Yeon Choi, Rajan Shrestha[1] Stephanie Q Pan, Yibu Chen, Meng Li, Audrey Kapelanski-Lamoureux, Gregory Yochum, Linda Sher, Satdarshan Paul Monga[8] Anthoula Lazaris, Keigo Machida, Michael Karin, Ernesto Guccione, Hidekazu Tsukamoto

Contents

Supplementary Table 1: List of Antibodies Used

Antibody	Vendor	Catalog	Condition	Dilution
Mouse-Beta-actin	Thermo Fisher	sc-47778	IB	1:1000
Rabbit-CTNNB1	Abcam	ab 32572	IB	1:5000
Rabbit-p-ERK1/2	Cell Signaling	9101 s	IB	1:1000
mouse-ERK	Cell Signaling	9107 s	IB	1:1000
Mouse-GAPDH	Thermo Fisher	SC-32233	IB	1:1000
Rabbit-p-(S9) GSK3β	Cell Signaling	5558 s	IB	1:1000
Mouse-GSK3β	Cell Signaling	12456	IB	1:1000
Rabbit-HuR	Novus MBL	En004p	IB	1:1000
Rabbit-LTB4R2	Abcam	ab 8460	IB	1:500
Mouse-Lamin-B2	Cell Signaling	13823 s	IB	1:500
Rabbit-LRP-6	Abcam	ab 66156	IB	1:1000
Rat-NaKATPase	Cell Signaling	3010 s	IB	1:1000
Rabbit-p-(S127)YAP	Cell Signaling	13008 s	IB	1:1000
Rabbit-YAP	Cell Signaling	4912 s	IB, IF	1:1000, 1:100
Rabbit-YAP	Cell Signaling	14074	IF	1:400
Mouse-α-SMA	Sigma-Aldrich	A2547	IF	1:500
Goat-HNF4α	Santa Cruz	Sc-6556	IF	1:200
Mouse-SOX9	Millipore sigma	AB5603	IF	1:500
Rabbit- CYP1B1	Boster Biological Technology	PB9546	IHC	1:1000
Rabbit -LTB4R2	MyBioSource	MBS243185	IHC	1:200
Mouse-AFP	Invitrogen	PA5-21004	IF	1:200
Goat-GFP	Rockland	P/A-600-101-215	IF	1:400
Rabbit-TAZ	Cell Signaling	70148s	IB	1:1000

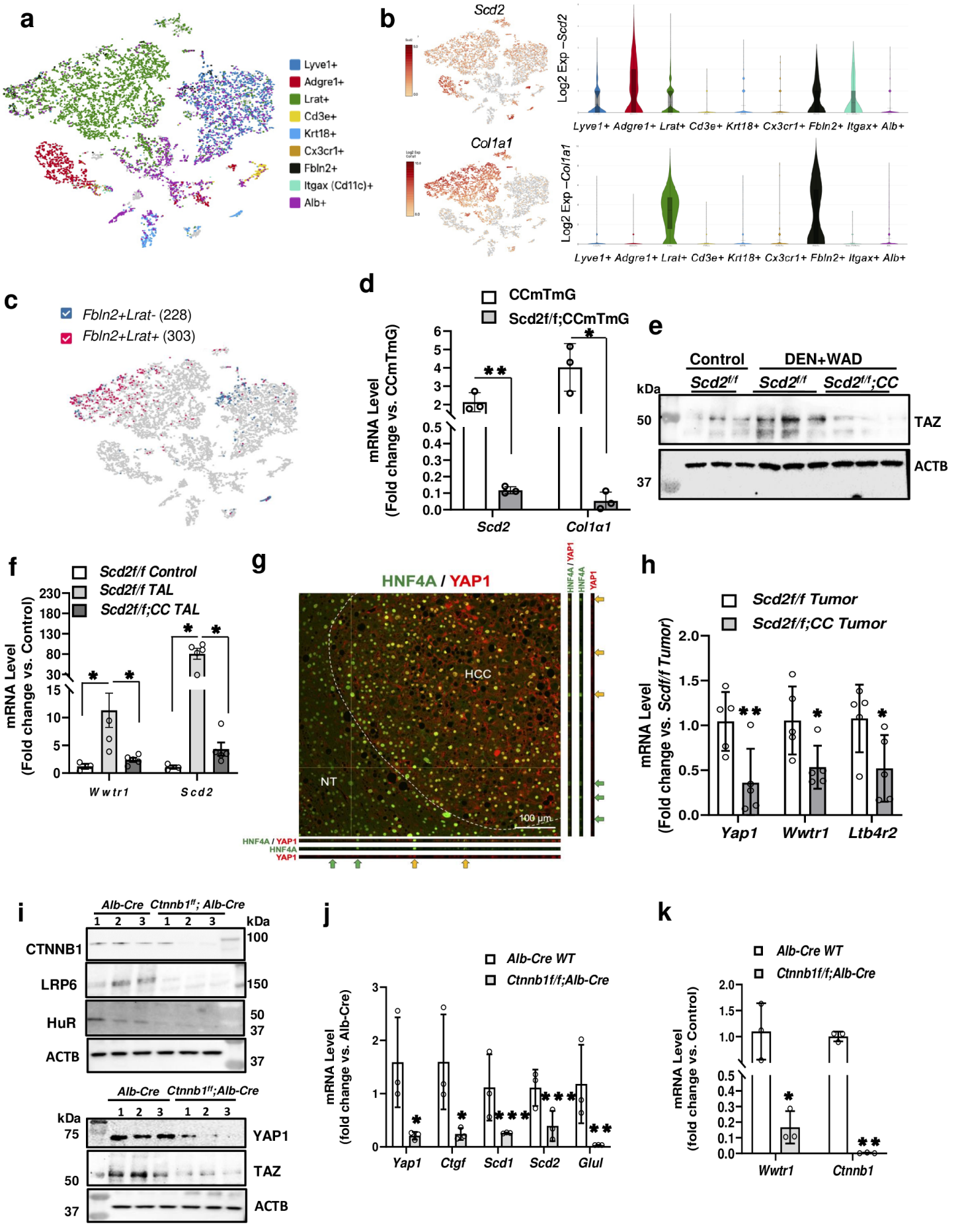
Supplementary Table 2: List of qPCR Primers and gRNAs:

Gene	Sequence	
h-YAP	FW: CCTTCTTCAAGCCGCCGGAG;	REV: CAGTGTCCCAGGAGAAACAGC
h-SCD1	FW: GCAGGACGATATCTCTAGCT	REV: GTCTCCACCTTATCTCCTCCATT
h-LTB4R2	FW: TACCACGCAGTCAACCTTCTG	REV: GCGGTGAAGACGTAGAGCAC
h-CTGF	FW: CAGCATGGACGTTCGTCTG	REV: AACCACGGTTTGGTCCTTGG
h-CYR61	FW: CTCGCTTAGTCGTCACCC	REV: CGCCGAAGTTGCATTCCAG
h-LRG5	FW: GAGTTACGTCTTGCGGGAAAC	REV: TGGGTACGTGTCTTAGCTGATTA
h-PTGS1	FW: TTGACCGCTACCAGTGTGAC	REV: GGAAGTGGGTGAAAGAGGGG
h-PTGS2	FW: CCCCAGGGCTCAAACATGAT	REV: ACCGTAGATGCTCAGGGACT
h-TBXAS1	FW: CCGAGACGAACTGAATGGCT	REV: GGCATCCAGGACCATTTGGA
h-CYP1A1	FW: TCGGCCACGGAGTTTCTTC	REV: GGTCAGCATGTGCCCAATCA
h-CYP1B1	FW: TGAGTGCCGTGTGTTTCGG	REV: GTTGCTGAAGTTGCGGTTGAG
h-ALOX15	FW: CAGCGTGGAAAACAGTG	REV: TCTAGGGAGGGTGGGACATG
h-ALOX12	FW: CTTCTCCGGGTCGTACAACC	REV: CCAAGTCCTCTGCAACGTCA
h-BRIC	FW: AGCATTCGTCCGGTTGCGCT	REV: TCGATGGCACGGCGACTTT
h-CCND1	FW: GGTGGCCGCAGTGCAA	REV: GAAGCGTGTGAGGCGGTAGTA
h-BCL2L1	FW: TGGACAATGGACTGGTTGAG	REV: GGGAAAGCTTGTAGGAGAGAAA
h-36B4	FW: TCTTGGGACAAATTGGACATGG	REV: GTGAGCCACTTGGTGTTGGA
m-36B4	FW: AGATTCGGGATATGCTGTTGGC	REV: TCGGGTCCTAGACCAGTGTTC
m-Cnnd1	FW: TGCAAATGGAACTGCTTCTG	REV: TGGAAAGAAAGTGCGTTGTG
m-Yap	FW: ATGACAACCAATAGTTCCGATCC	REV: CAGGGTGCTTTGGCTGATG
m-Ctgf	FW: AGAACTGTGTACGGAGCGTG	REV: GTGCACCATCTTTGGCAGTG
m-Cyr61	FW: AGAGGCTTCCTGTCTCTTTGGC	REV: CCAAGACGTGGTCTGAACGA
m-Bric5	FW: TGCAAAGGAGACCAACAACA	REV: GGCATGTCACTCAGGTCCAA
m-Ltb4r2	FW: ACAGCCTTGGCTTTCTTCAG	REV: TGCCCCATTACTTTCAGCTT
m-Scd1	FW: TACACTCTGGTGCTCAACGC	REV: AGGATATTCTCCCGGGATTG
m-Scd2	FW: GCTCTCGGGAGAACATCTTG	REV: CAGCCCTGGACACTCTCTCTTC
m-Glul	FW: TGAACAAAGGCATCAAGCAAATG	REV: CAGTCCAGGGTACGGGTCTT
m-Col1α1	FW: CACCCTCAAGAGCCTGAGTC	REV: GTTCGGGCTGATGTACCAGT
m-Wwtr1	FW: CATGGCGGAAAAAGATCCTCC	REV:GTCGGTCACGTCATAGGACTG
h-CYP1B1 gRNA 1	gRNA TGCGCCCGAACTCTTCGTTG	
h-CYP1B1 gRNA 2	gRNA ACTGATCGGAAACGCGGCGG	
h-CYP1B1 gRNA control	gRNA AAAGTCGCCCTCCGCGCTCG	

Supplementary Table 3: Summary of HCC patients whose tissues were analyzed.

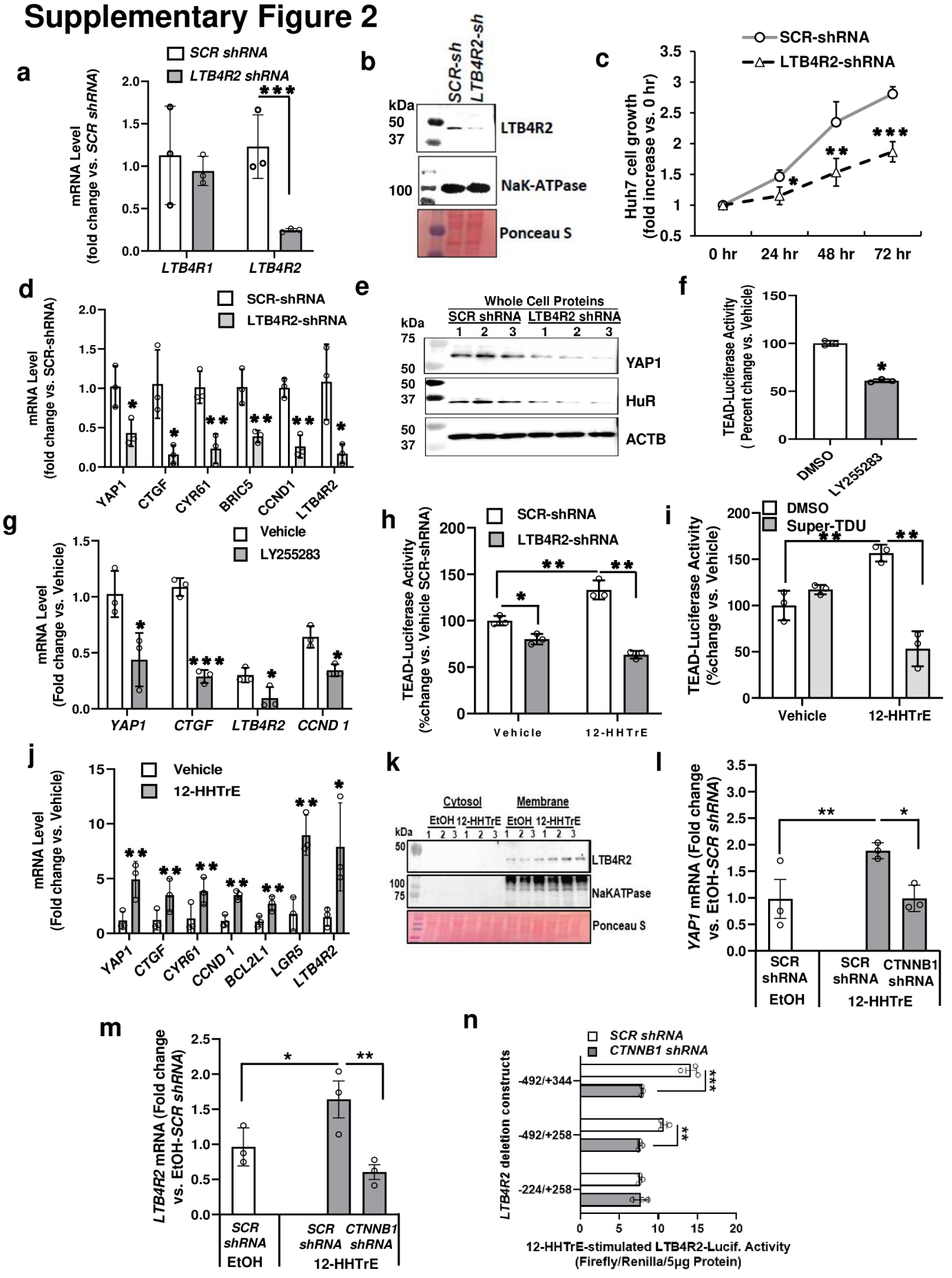
Tissue Type	Diagnosis	Etiology	Sex	Age	Race	Ethnicity
Snap-frozen tissues	HCC/cirrhosis	3 ALD 1 NASH 2 HCV	4 males 2 females	64±8.3	White	1 Hispanic 5 non-Hispanic
Paraffin sections	HCC/cirrhosis	1 ALD 1 NASH 1 HCV 1 HBV	3 males 1 female	72±7.5	Asian White	1 Asian 3 non-Asian

Supplementary Figure 1



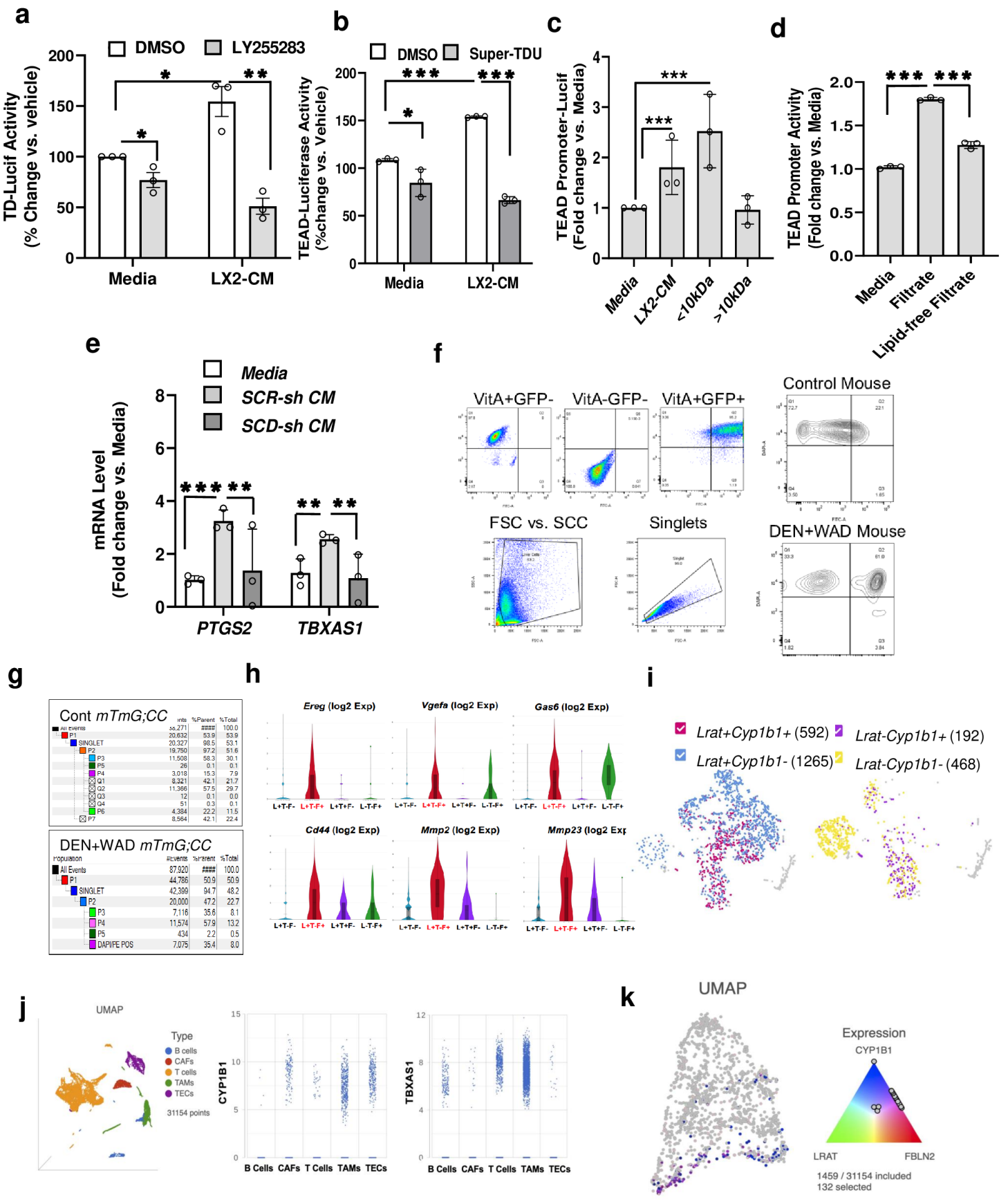
Supplementary Figure 1. Global YAP1/TAZ repression by SCD2 deficiency in aHSC. a A t-SNE plot depicting TME cell type clusters in the DEN+WAD mouse liver identified by scRNA-seq using the specific cell type marker genes. **b** *Scd2* and *Col1a1* expressions by the cell type clusters as shown by t-SNE plot and violin plots (Violin plot parameters and cell numbers for different cell types analyzed are provided in Supplementary Data 3 in Supplementary File). **c** A t-SNE plot showing the distribution of *Fbln2+Lrat-* vs. *Fbln2+Lrat+* cells. Numbers in the parenthesis are cell numbers. **d** Effective *Scd2* and *Col1a1* mRNA reductions in HSC from *Scd2$^{f/f}$;CC;mTmG* vs. *CC;mTmG* mice. *p<0.05, **p<0.01 by two-sided t-test. Data presented are means±SEM (n=3 pairs). **e** TAZ IB analysis showing its upregulation in *Scd2$^{f/f}$ TAL* is blocked in *Scd2$^{f/f}$;CC* (n=3 mouse samples each). **f** *Wwtr1* and *Scd2 mRNA* in *Scd2$^{f/f}$;CC* vs. *Scd2$^{f/f}$* TAL vs. normal *Scd2$^{f/f}$* liver (Control). *p<0.05 by two-sided t-test. Data presented are means±SEM (n=3-5). **g** Representative 3D confocal imaging of HN4A and YAP1 IF staining of *Scd2$^{f/f}$* TAL section from 4 samples analyzed. **h** *Yap1*, *Wwtr1*, and *Ltb4r2* mRNA levels in *Scd2$^{f/f}$;CC* vs. *Scd2$^{f/f}$* tumors. *p<0.05. Data presented are means±SEM (n=5). *p<0.05, **p<0.01 by two-sided t-test. **i** IB analysis of CTNNB1, HuR, LRP6, YAP1, and TAZ along with the house keeping ACTB in *AlbCre* vs. *Ctnnb1f/f;AlbCre* mouse livers. (n=3 each). **j** CTNNB1 and YAP1-target gene mRNA levels in *AlbCre* vs. *Ctnnb1f/f;AlbCre* mouse livers. *p<0.05, **p<0.01, ***p<0.005 vs. Alb-Cre by two-sided t-test. Data presented are means±SEM (n=3 each). **k** *Wwtr1* and *Ctnnb1* mRNA levels in *AlbCre* vs. *Ctnnb1f/f;AlbCre* mouse livers. *p<0.05, **p<0.01 vs. Alb-Cre by two-sided t-test. Data presented are means±SEM (n=3 each). All relevant figures, source data and exact p values are provided in the Source Data file.

Supplementary Figure 2



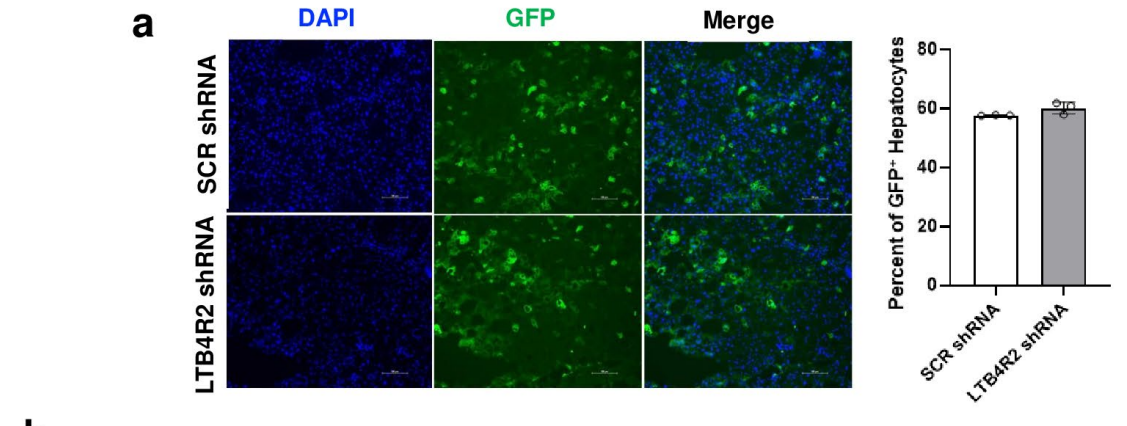
Supplementary Figure 2. 12-HHTRE-LTB4R2 tumor promoter pathway. a *LTB4R1* and *LTB4R2* qPCR data for Huh7 cells with adenoviral expression of *LTB4R2-shRNA* vs. *SCR-shRNA* at MOI=15 after 72 hr post-infection. ***p<0.005 vs. *SCD-shRNA* by two-sided t-test. Data presented are means±SEM (n=3 experiments). **b** IB analysis of LTB4R2 in Huh7 cells with *LTB4R2-shRNA* vs. *SCR-shRNA*. (a represented image from 3 experiments). **c** Growth curve for Huh7 cells with *LTB4R2-shRNA* vs. *SCR-shRNA*. *p<0.05, **p<0.01, ***p<0.005 vs. *SCR-shRNA* by two-sided t-test. Data presented are means±SEM (n=3 experiments). **d** mRNA expression of *YAP1* and its target genes (*CTGF, CYR61* and *BRIC5*), CTNNB1-target gene *CCND1*, and *LTB4R2* in *LTB4R2-shRNA* vs. *SCR-shRNA* Huh7 cells. *p<0.05, **p<0.01 vs. *SCR-shRNA* by two-sided t-test. Data shown are means±SEM (n=3 experiments). **e** IB analysis for YAP1 and HuR of whole cell proteins from Huh7 cells with *LTB4R2-shRNA* vs. *SCR-shRNA*. (n=3 replicates). **f** TEAD-luciferase activity in Huh7 cells treated for 24 hr with the receptor antagonist LY255283 (10µM) vs. DMSO (vehicle). *p<0.05 by two-sided t-test. Data presented are means±SEM (n=3 experiments). **g** mRNA levels of *YAP1, CTGF, LTB4R2,* and *CCND1* in Huh7 cells treated with LY255283 (10µM) vs. DMSO. *p<0.05, ***p<0.005 vs. vehicle by two-sided t-test. Data presented are means±SEM (n=3 experiments)**. h** TEAD-luciferase activity in Huh7 cells with *LTB4R2-shRNA* vs. *SCR-shRNA* which were treated for 24 hr with 12-HHTrE (50nM) or vehicle (ethanol). *p<0.05, **p<0.01 by two-sided t-test. Data presented are means±SEM (n=3 experiments). **i** TEAD-luciferase activity in Huh7 cells treated with 12-HHTrE (50nM) or vehicle (EtOH) in the presence of Super-TDU (300µM) or DMSO. **p<0.01 by two-sided t-test. Data presented are means±SEM (n=3 experiments)**. j** mRNA expression of *YAP1* and its target genes (*CTGF, CYR61*), CTNNB1-target genes (*CCDN1, LRG5*), *BCL2L1* regulated by both YAP1 and CTNNB1 and *LTB4R2* in Huh7 cells treated with 12HHTrE vs. vehicle (EtOH). *p<0.05, **p<0.01 vs. vehicle by two-sided t-test. Data shown are means±SEM (n=3 experiments). **k** IB analysis of LTB4R2 with membrane proteins from Huh7 cells treated with vehicle (-) or 50nM 12-HHTrE (+). (n= 3 experiments). **l** *YAP1* qPCR data in Huh7 cells infected with adenovirus expressing *CTNNB1-shRNA* vs. *SCR-shRNA* (MOI=10) which were subsequently treated with 12-HHTrE vs. EtOH. **p<0.01, *p<0.05 by two-sided t-test. Data presented are means±SEM (n=3 experiments). **m** *LTB4R2* mRNA in the same cell preparations as above. *p<0.05, **p<0.01 by two-sided t-test. Data presented are means±SEM. **n** 12-HHTrE-stimulated LTB4R2-Luciferase activity in Huh7 cells transduced with *SCR-shRNA* vs. *CTNNB1-shRNA*. **p<0.01, ***p<0.005 by two-sided t-test. Data are means±SEM (n=3 experiments). All relevant figures, source data and exact p values are provided in the Source Data file.

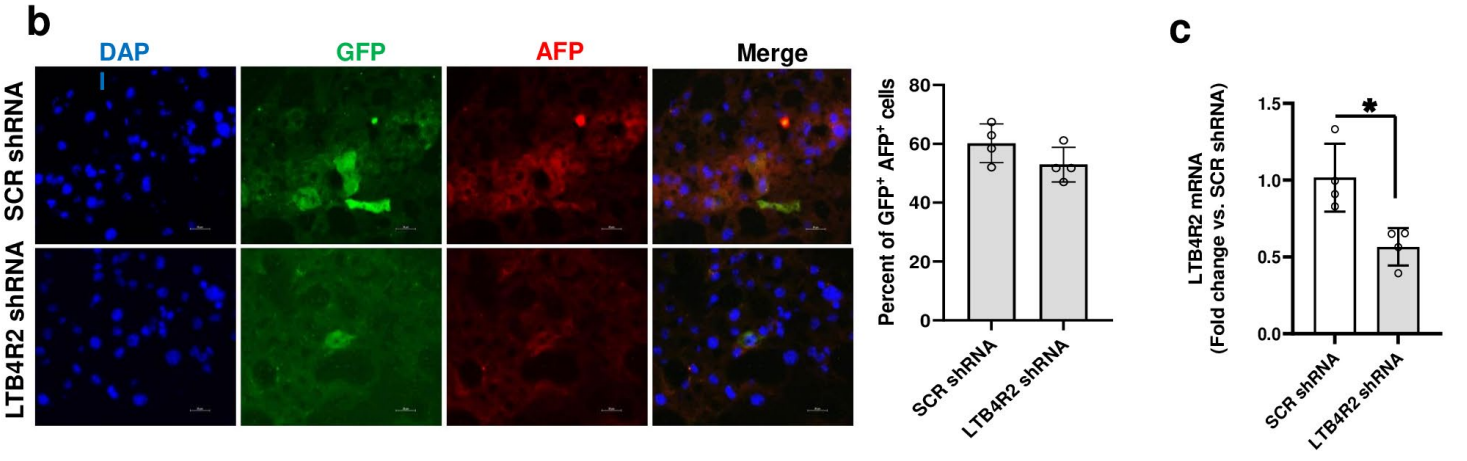
Supplementary Figure 3

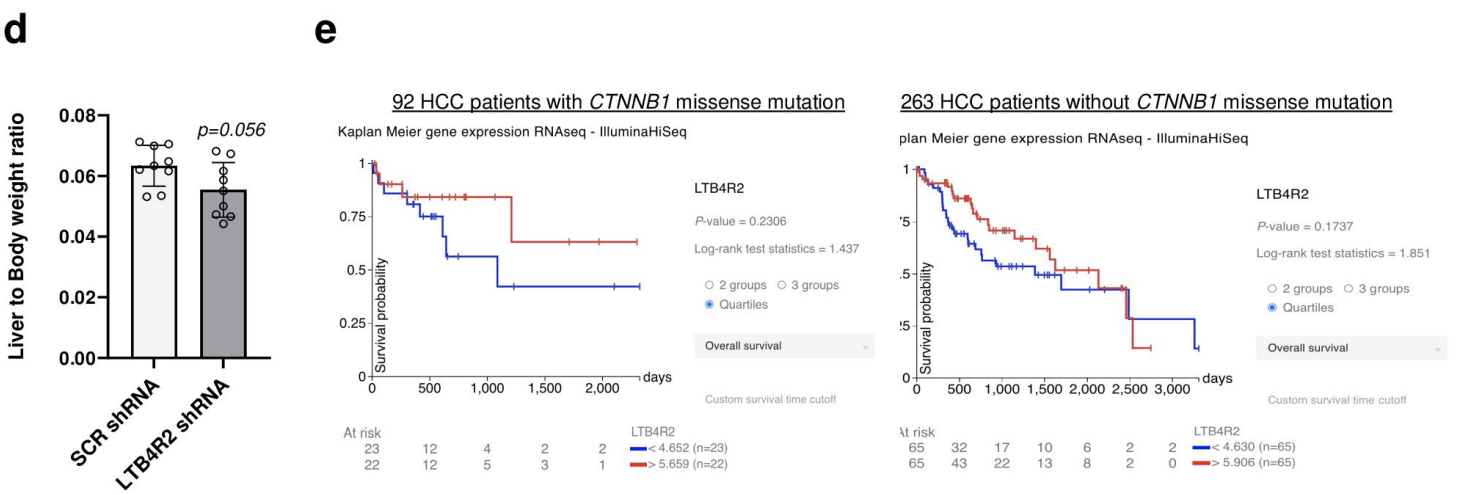


Supplementary Figure 3. Paracrine aHSC activation of LTB4R2 in HCC cells. **a** TEAD-luciferase activity in Huh7 cells treated with LX2-CM in the presence of the LTB4R2 receptor antagonist LY255283 (10μM) vs. DMSO. *p<0.05, ***p<0.005 by two-sided t-test. Data presented are means±SEM (n=3 experiments). **b** TEAD-luciferase activity in Huh7 cells treated with LX2-CM in the presence of Super-TDU (300μM) vs. DMSO. *p<0.05, **p<0.01 by two-sided t-test. Data presented are means±SEM (n=3 experiments). **c** TEAD-luciferase activity in Huh7 cells treated with media (no cell), LX2-CM with molecular cut-off of <10 kDa (filtrate) and LX2-CM with molecular cut-off of >10 kDa (retentate). **p<0.01, ***p<0.005 by one-way posthoc ANOVA test. Data presented are means±SEM (n=3 separate experiments). **d** Lipid removal reduces LX2-CM induction of TEA D-luciferase activity. **p<0.05, ***p<0.01 by one-way posthoc ANOVA test. Data presented are means±SEM (n=3 separate experiments). **e** qPCR data for *PTGS2* and *TBXAS1* in Huh7 cells treated with CM collected from LX2 cells with *SCR-shRNA* (SCR-sh CM) vs. *SCD-shRNA* (SCD-sh CM) compared to media control (Media). *p<0.05, **p<0.01 by one-way posthoc ANOVA test. Data presented are means±SEM (n=3 separate experiments). **f** FACS gating strategy. Freshly isolated C57Bl/6j mouse primary HSCs were used as the positive control for VitA+ (DAPI) and negative control for Col1a1GFP (FITC) (VitA+GFP-); the rat myofibroblast cell line BSC cells used as the negative control for both VitA and GFP (VitA-GFP-); and culture-activated HSCs from Col1a1-GFP mice used as the VitA+GFP+ control. Non-parenchymal liver cells were first subjected to forward vs. side scatter (FSC vs. SCC) gating to collect cells of interest which were then subjected to singlet gating for isolation of single cells. The cells were then gated for VitA (DAPI) and GFP (FITC) as set above for isolation of VitA+Col1a1GFP+ activated HSCs (aHSCs) and VitA-Col1a1GFP+ cells from mTmG;CC mice subjected to regular chow feeding without DEN injection (Control) vs. the DEN+WAD tumorigenesis regimen (DEN+WAD). **g** Numbers of cells separated by FACS for VitA+GFP- (P3), VitA+GFP+ (P4), and VitA-GFP+ (P5) populations from mTmG;CC control vs. DEN+WAD mice. **h** Violin plots of tumor promoter genes among VitA-GFP+ subpopulations based on *Lrat*, *Thy1* and *Fbln2* expression (Cell numbers and violin plot parameters of subpopulations are provided in Supplementary Data 3 in Supplementary File). **i** t-SNE plots of VitA-GFP+ cells clustered based on *Lrat* and *Cyp1b1* expression. **j** UMAP of human HCC TME cells from NCI's Single-cell Atlas in Liver Cancer Data (scATlasLC) (left) and CYP1B1 and TBXA1S expression by different cell types (right). **k** CAF co-expressing *CYP1B1* and *FBLN2* via re-analysis of scATlasLC data. All relevant figures, source data and exact p values are provided in the Source Data file.

Supplementary Figure 4







Supplementary Figure 4. AAV8-based LTB4R2 targeting in mice and LTB4R2 translational relevance. **a** IF microscopy images for GFP transduced by *SCR-shRNA* or *LTB4R2-shRNA* AAV8 vector in DEN+WAD mice and morphometric data for the percent of GFP+ hepatocytes to total hepatocytes. Data presented are means±SEM (n=3 liver sections). No statistical difference by two-sided t-test. **b** IF microscopy images of dual staining for GFP and AFP depicting GFP+AFP+ cells. A bar graph shows morphometric data for the percentage of GFP+AFP+ cells in the two groups. Data presented are means±SEM (n=4 liver sections). Two-sided t-test was performed. **c** *LTB4R2* mRNA in tumors of *SCR-shRNA* vs. *LTB4R2-shRNA* DEN+WAD mice. *p<0.05 vs. SCR-shRNA mice by two-sided t-test. Data presented are means±SEM (n=4 mouse samples each). An exact p value is provided in the Source data file. **d** Liver weight/body weight ratio of *SCR-shRNA* vs. *LTB4R2-shRNA* DEN+WAD mice. Data presented are means±SEM (n=9). Two-sided t-test was performed. **e** Kaplan-Meier survival curves of HCC patients with (left) or without (right) CTNNB1 missense mutation comparing between those with LTB4R2 high (red) vs. low (blue) expression the TCGA-LIHC (the Cancer Genome Atlas Liver Hepatocellular Carcinoma) cohort data. All relevant figures, source data and exact p values are provided in the Source Data file.