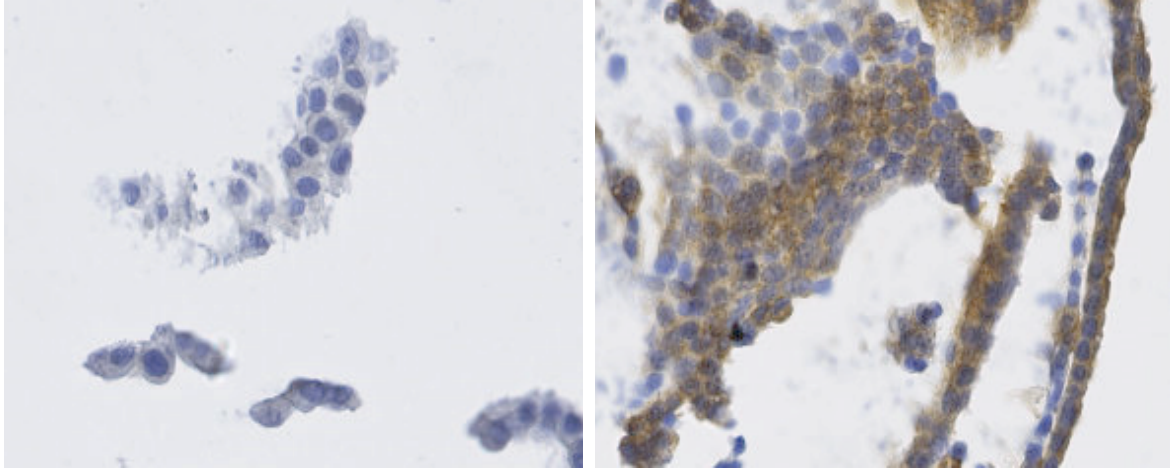


STRAP staining

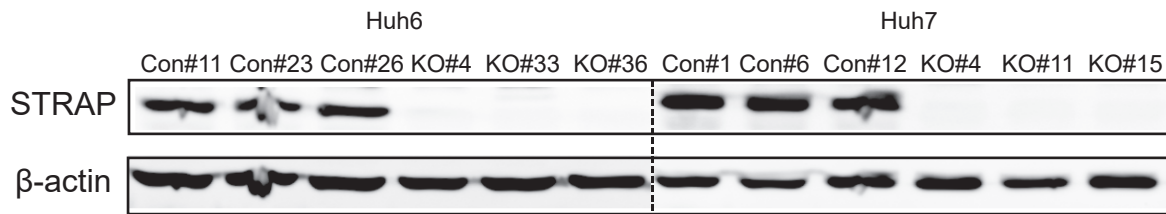


Knock-out

Control

Huh6

Figure S1. Specificity of STRAP antibody tested on a STRAP knock-out Huh6 clone and control thereof. For this purpose, both cell lines were formalin-fixed and embedded in paraffin.



Exons	Clones	AA alteration	Size	Sequences
1	Wild type	-	-	CACGCGACCCGTGGTTGATTGGCCTTCAGTGGCATCACGCCTTATGGGTATTCTT TAATCAGCTGGCCACACGCGACCCGTGGTTGAATT
1	Huh6 KO#33	Insertion	+81	CACGCGACCCGTGGTTGA CCCCGTCTCCCTGGCTTTAGCCACCTCTCCATCCTCTT GCTTTCCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTA TT
2	Wild type	-	-	TGCAAACTGAATAAGGATGCCACCAAAGCAGCTACAG
2	Huh6 KO#4	Deletion	-1	TGCAAACTGA:TAAGGATGCCACCAAAGCAGCTACAG
		Insertion	+1	TGCAAACTGA A TAAGGATGCCACCAAAGCAGCTACAG
		Deletion	-22	TGCAACAC::::::::::::::::::::::::::::AGCTACAG
2	Huh6 KO#36	Insertion	+2	TGCAAACTGA AA TAAGGATGCCACCAAAGCAGCTACAG
		Insertion	+1	TGCAAACTGA A TAAGGATGCCACCAAAGCAGCTACAG
2	Huh7 KO#4	Insertion	+1	TGCAAACTGA A TAAGGATGCCACCAAAGCAGCTACAG
2	Huh7 KO#11	Insertion	+1	TGCAAACTGA A TAAGGATGCCACCAAAGCAGCTACAG
		Deletion	-1	TGCAAACTGA:TAAGGATGCCACCAAAGCAGCTACAG
		Insertion	+~300	from Ecoli and other unknown sources
2	Huh7 KO#15	Insertion	+1	TGCAAACTGA A TAAGGATGCCACCAAAGCAGCTACAG
		Deletion	-1	TGCAAACTGA:TAAGGATGCCACCAAAGCAGCTACAG

Figure S2. Knockout of the STRAP gene by CRISPR/Cas9 technology in HCC cell lines. Huh6 and Huh7 were transfected with STRAP-targeting vectors and single-cell sorted by FACS. The surviving clones were subjected to western blot. Depicted are STRAP protein levels of independent control and knock-out clones selected for further analysis. The lower panel shows the sequence alterations of each STRAP knock-out clone. All control clones showed only the wild-type sequence. The insertion of 81 nucleotides in Huh6 KO#33 results in additional 27 amino acids within the WD40 region, which apparently makes the protein highly unstable as demonstrated by no detectable protein using two antibodies.

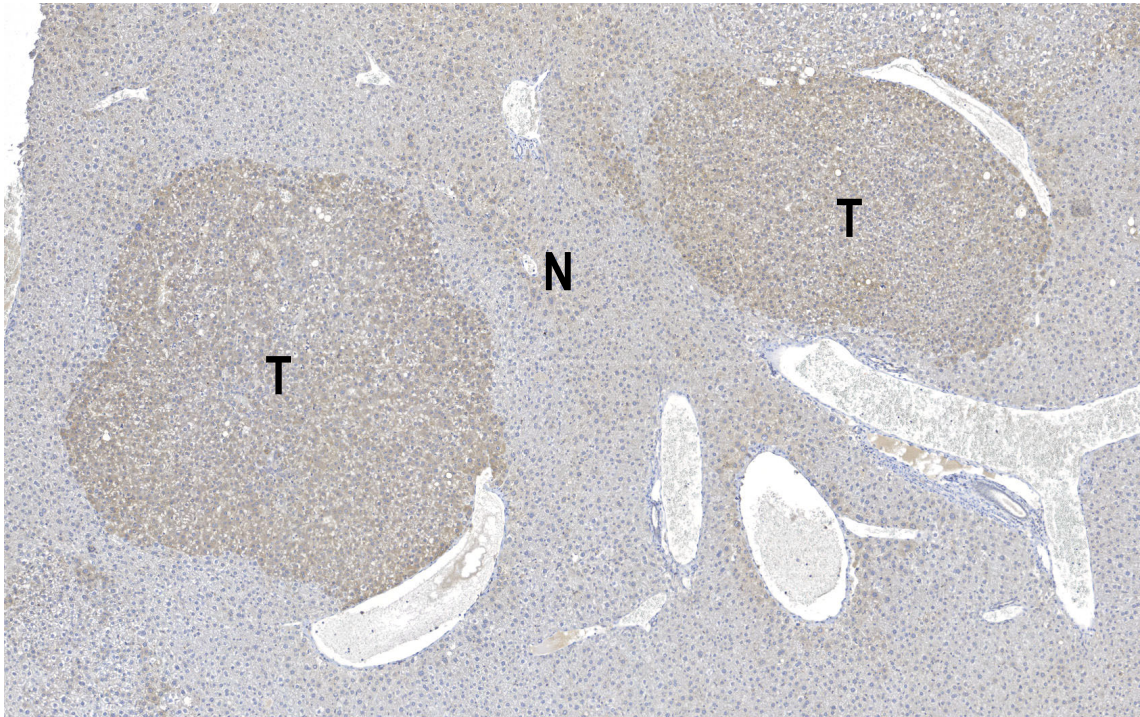


Figure S3. Elevated expression of STRAP in DEN-induced mouse liver tumors (T) compared with flanking normal liver tissue (N).

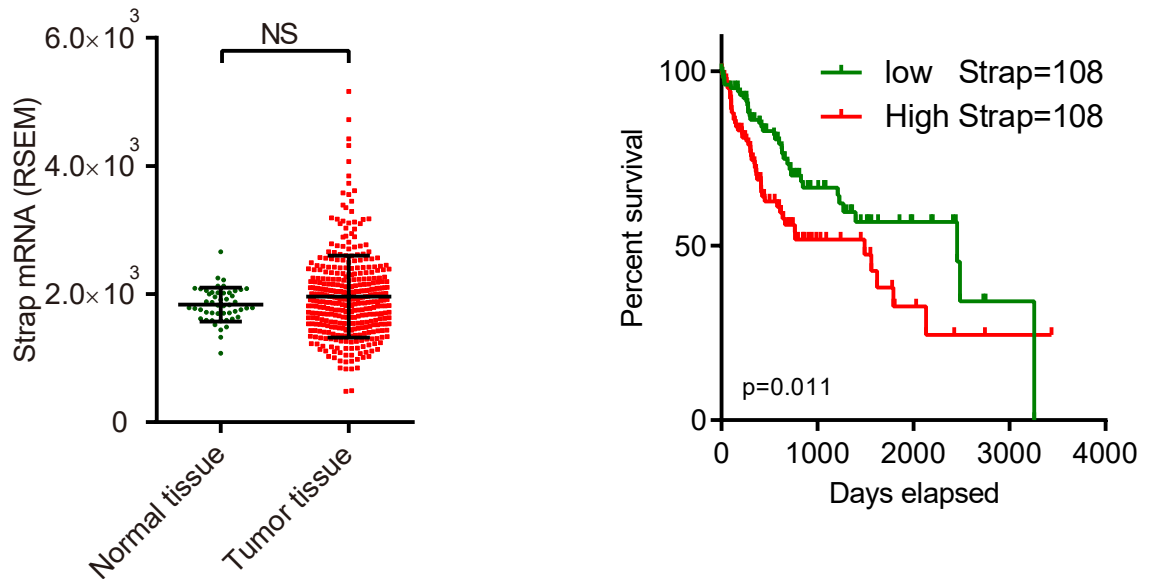


Figure S4. Analysis of the TCGA liver cancer cohort reveals that no significant difference in average *STRAP* RNA levels is observed between normal and tumor samples. Kaplan-Meier survival analysis and a log-rank test of the top and bottom 30% *STRAP* expressors revealed a significant trend ($p=0.011$) of reduced survival in the high expressing group.

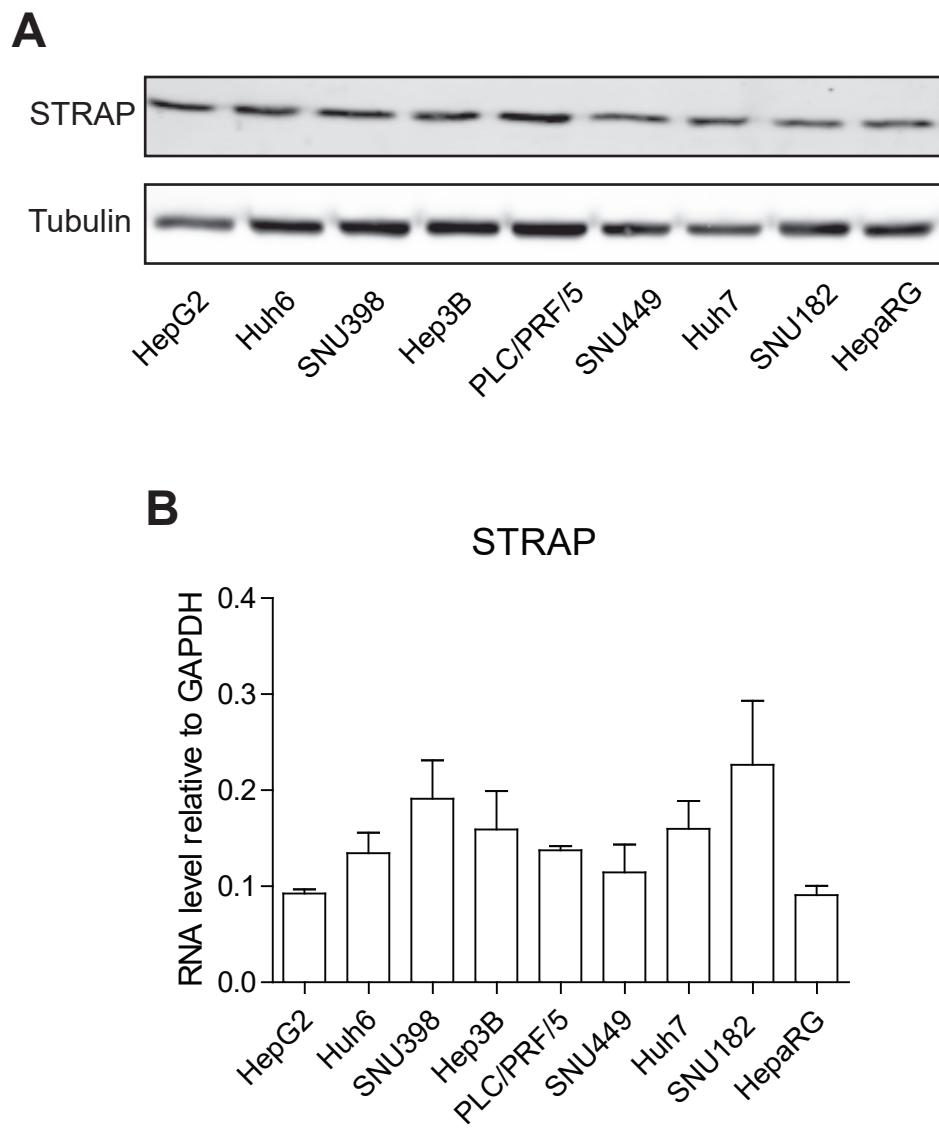


Figure S5. Baseline levels of STRAP protein and RNA in a panel of 9 HCC cell lines.

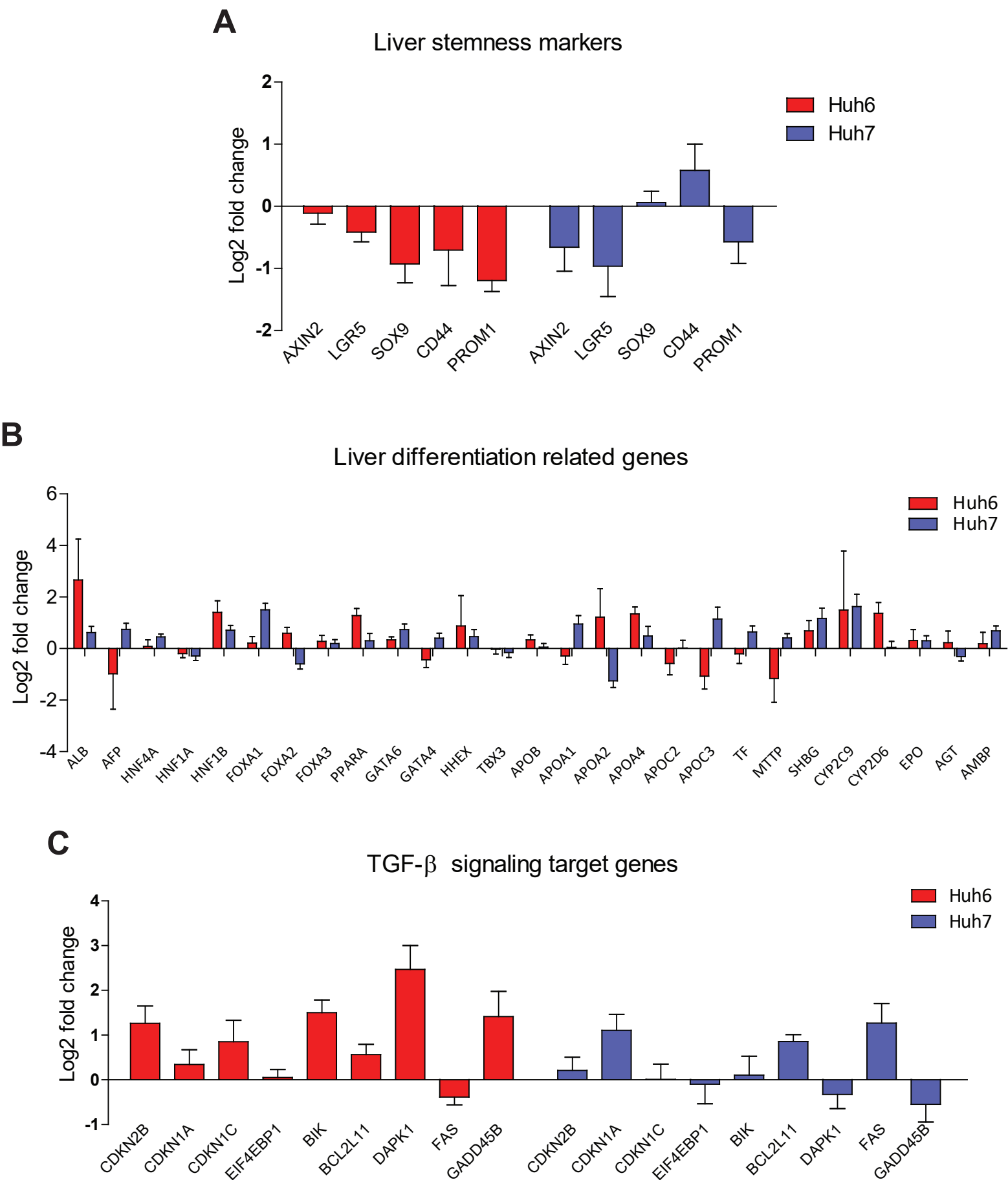


Figure S6. Log2 fold change of liver stemness markers (A), liver differentiation related genes (B) and TGF- β signaling target genes (C) in STRAP knock-out clones compared to controls. The results are presented as log2 fold change \pm Standard Error, n=3.

Supplemental Table S1. Gene mutations of Wnt/ β -catenin signaling components in HCC cell lines

Cell line	Gene	AA alteration	Zygoty
HepG2	<i>CTNNB1</i>	p.W25_I140 del	Heterozygous
Huh6	<i>CTNNB1</i>	p.G34V	Heterozygous
SNU398	<i>CTNNB1</i>	p.S37C	Heterozygous
Hep3B	<i>AXIN1</i>	p.R146*	Homozygous
PLC/PRF/5	<i>AXIN1</i>	p.(R373_M418 del)	Homozygous
SNU449	<i>AXIN1</i>	p.R712*	Homozygous
Huh7			
HepaRG			
SNU182			

Supplemental Table S2. Clinicopathologic characteristics of HCC TMA

Characteristic	N=109
Age (median, range)	60.3 (23-87)
Male/Female (n, %)	80/29 (73/27)
Hepatitis-Ba/Hepatitis-Cb (n, %)	20/17 (18/16)
Cirrhosis (n, %)	59 (46)
Tumor differentiation (n, %) (well, moderate, poor)	33/57/18 (31/53/17)
Vascular invasion (n, %)	57 (61)
Single vs multiple lesions (n, %)	73/36 (67/33)
Median size (median, range)	4.5 cm (0.5-25.0)
Median AFP (median, range)	8.5 ug/l (1-63.000)
Recurrence (n, %)	41 (38)
HCC related death (n, %)	27 (25)

Supplemental Table S3. Selected *STRAP* sgRNAs

Species	Gene target	gRNA	Sequence
Human	STRAP exon2	sgRNA1	TTGGGGTGCAACACTGAATA
	STRAP exon1	sgRNA2	AATCAACCACGGGTCGCGTG
	STRAP exon1	sgRNA3	CACGCGACCCGTGGTTGATT

Supplemental Table S4. Primer sequences of *STRAP* used for Sanger sequencing

Exons	Forward Sequence (5'~3')	Reverse Sequence (5'~3')
<i>Exon1</i>	CCCTTCTTTTCCTGTTGCC	GTGTTGGCTCTCATCTCAG
<i>Exon2</i>	GGTGGTAGTTAAATAGCTG	TGGGATCAAACATGCGTTC

Supplemental Table S5. Primer sequence used for Ion Torrent sequencing

Gene	Forward Sequence (5'~3')	Reverse Sequence (5'~3')
<i>STRAP</i>	Adapter A-Barcode-CCCTTCTTTTCCTGTTGCC	Adapter PI-Barcode-GTGTTGGCTCTCATCTCAG

Supplemental Table S6. Primer sequences used for qRT-PCR

Gene	Forward Sequence (5'~3')	Reverse Sequence (5'~3')
<i>DCDC2</i>	ACTTGGACATAGGAGAAATCAAGA	CGAGCTGACACGTTGATCCT
<i>NTHL1</i>	TATGAGGGCTCGGACAGTGA	TTTGGTTTGGCTGGAGAGCA
<i>RPS26</i>	AAACATAGTGGAGGCCGCAG	CACATACAGCTTGGGAAGCAC
<i>OLR1</i>	CCTTGCTCGGAAGCTGAATG	TCTCCATGCCAGATCCAGTC
<i>ABHD13</i>	CCGGCGACACCCGAG	ACAAAGTTCCACAGCATCCAG
<i>KLHL42</i>	GGCCTCCATGAACCAGAAGA	GTTCCGGTCTCTGGTAGTGTAT
<i>ZNF853</i>	AGCAGGAAATGCTCCACCAG	GTGGAAGTCTGTTCTCTCTCC
<i>LGR5</i>	ACACGTACCCACAGAAGCTC	CTAAAAGCCTGGACGGGGAT

Supplemental Table S7. Gene information

Gene	Protein	
<i>STRAP</i>	STRAP	A scaffolding protein without enzymatic function exerting regulatory functions on a variety of cellular processes
<i>CTNNB1</i>	β -catenin	A dual function protein involved in regulation and coordination of cell–cell adhesion and gene transcription
<i>AXIN1</i>	AXIN1	To form a destruction complex with APC, GSK3 and CK1 α leading to the degradation of β -catenin
<i>AXIN2</i>	AXIN2	To form a destruction complex with APC, GSK3 and CK1 α leading to the degradation of β -catenin
<i>APC</i>	APC	A tumor suppressor, negatively regulating β -catenin by forming a destruction complex with AXIN1/2, GSK3 and CK1 α
<i>GSK3</i>	GSK3	A Ser/Thr kinase
<i>CSNK1A1</i>	CK1 α	Kinase with preferential acidic protein targets
<i>LGR5</i>	LGR5	The receptor of R-spondin family of stem cell factors to potentiate Wnt/ β -catenin signaling
<i>ASK1</i>	MAP3K5	Mitogen-activated protein kinase
<i>PI3K</i>	PIK3CA	Phosphatidylinositol 3-kinase
<i>PDK1</i>	PDK1	Pyruvate dehydrogenase kinase
<i>DCDC2</i>	DCDC2	A protein with two doublecortin peptide domains binding to tubulin and enhancing microtubule polymerization
<i>NTHL1</i>	NTHL1	A bifunctional DNA glycosylase that has an associated beta-elimination activity
<i>RPS26</i>	40S ribosomal protein S26	A ribosomal protein as a component of the 40S subunit
<i>OLR1</i>	OLR1	The protein binds, internalizes and degrades oxidized low-density lipoprotein
<i>ABHD13</i>	ABHD13	Unknown
<i>KLHL42</i>	KLHL42	Unknown
<i>ZNF853</i>	Zinc finger protein 853	A protein contains the zinc finger, a structural motif, for the coordination of one or more zinc ions in order to stabilize the fold.
<i>MYC</i>	MYC	A multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation
<i>CCND1</i>	Cyclin D1	A member of highly conserved cyclin family, whose members are

		characterized by a dramatic periodicity in protein abundance throughout the cell cycle
<i>GLUL</i>	glutamate-ammonia ligase	It catalyzes the synthesis of glutamine from glutamate and ammonia in an ATP-dependent reaction
<i>RGN</i>	Regucalcin	It may have an important role in calcium homeostasis
<i>BIRC5</i>	BIRC5/ Survivin	This protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death
<i>SOX9</i>	SOX-9	A transcription factor
<i>CD44</i>	CD44	<i>CD44 participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis.</i>
<i>PROM1</i>	Prominin-1/CD133	The precise function of CD133 remains unknown, it has been proposed to act as an organizer of cell membrane topology
<i>ALB</i>	Albumin	Its main function is to regulate the Oncotic pressure of blood
<i>AFP</i>	alpha fetoprotein	Alpha-fetoprotein expression in adults is often associated with hepatoma or teratoma
<i>HNF4A</i>	HNF4A/ NR2A1	HNF4A is a nuclear transcription factor
<i>HNF1A</i>	HNF1A	A transcription factor expressed in organs of endoderm origin
<i>HNF1B</i>	HNF1B	<i>HNF1B is a nuclear transcription factor</i>
<i>FOXA1</i>	FOXA1/ HNF-3A	A transcriptional activator for liver-specific transcripts such as albumin and transthyretin
<i>FOXA2</i>	FOXA2/ HNF-3B/ TCF-3B	A transcriptional activator for liver-specific transcripts such as albumin and transthyretin
<i>FOXA3</i>	FOXA3/ HNF-3G/ TCF-3G	A transcriptional activator for liver-specific transcripts such as albumin and transthyretin
<i>PPARA</i>	PPARα/NR1C1	A transcription factor and a major regulator of lipid metabolism in the liver
<i>GATA6</i>	GATA6	This protein preferentially binds (A/T/C)GAT(A/T)(A) of the consensus binding sequence.
<i>GATA4</i>	GATA4	A member of the GATA family of zinc finger transcription factors
<i>HHEX</i>	HHEX	A member of the homeobox family of transcription factors, many of which are involved in the development of liver, thyroid, forebrain etc.
<i>TBX3</i>	TBX3	A member of T-box family which are the transcription factors involved in the regulation of developmental processes
<i>APOB</i>	Apolipoprotein B	Apolipoprotein B is the primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles
<i>APOA1</i>	Apolipoprotein A1	Apolipoprotein A1 is the major protein component of high density lipoprotein particles in plasma.

<i>APOA2</i>	Apolipoprotein A2	The second most abundant protein of the high density lipoprotein particles
<i>APOA4</i>	Apolipoprotein A4	Apolipoprotein A4 is secreted into circulation on the surface of newly synthesized chylomicron particles
<i>APOC2</i>	Apolipoprotein C2	A component of very low density lipoproteins and chylomicrons
<i>APOC3</i>	Apolipoprotein C3	A component of very low density lipoprotein
<i>TF</i>	Transferrin	Transferrins are iron-binding blood plasma glycoproteins that control the level of free iron (Fe) in biological fluids
<i>MTTP</i>	MTTP	This protein plays a central role in lipoprotein assembly
<i>SHBG</i>	SHBG/SSBG	A glycoprotein that binds to the two sex hormones: androgen and estrogen
<i>CYP2C9</i>	CYP2C9	An important cytochrome P450 enzyme with a major role in the oxidation of both xenobiotic and endogenous compounds
<i>CYP2D6</i>	CYP2D6	A member of the cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body
<i>EPO</i>	Erythropoietin/hematopoietin	A hormone that induces red blood cell production
<i>AGT</i>	Angiotensinogen	Angiotensin is a peptide hormone that causes vasoconstriction and a subsequent increase in blood pressure
<i>AMBP</i>	AMBP	AMBP interacts with CD79A
<i>CDKN1A</i>	p21 ^{Cip1}	A cyclin-dependent kinase inhibitor (CKI) that is capable of inhibiting all cyclin/CDK complexes
<i>CDKN2B</i>	CDKN2B	A cyclin-dependent kinase inhibitor
<i>CDKN1C</i>	CDKN1C	A tight-binding inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation
<i>EIF4EBP1</i>	4E-BP1	Interaction of this protein with eIF4E inhibits complex assembly and represses translation
<i>BIK</i>	Bcl-2-interacting killer	Interaction of this protein with cellular and viral survival-promoting proteins, such as BCL2 and the Epstein-Barr virus enhances programmed cell death
<i>BCL2L11</i>	BCL2L11	Interaction of this protein with other members of the BCL-2 protein family, including BCL2, BCL2L1/BCL-X(L), and MCL1, activates apoptosis
<i>DAPK1</i>	DAPK1	A positive mediator of gamma-interferon induced programmed cell death
<i>FAS</i>	Fas cell surface death receptor	The Fas receptor is a death receptor on the surface of cells that leads to programmed cell death (apoptosis)

<i>GADD45B</i>	GADD45B	GADD45B responds to environmental stresses by mediating activation of the p38/JNK pathway and is involved in the regulation of growth and apoptosis
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