

Supplementary Materials: Canopy Homolog 2 as a Novel Molecular Target in Hepatocarcinogenesis

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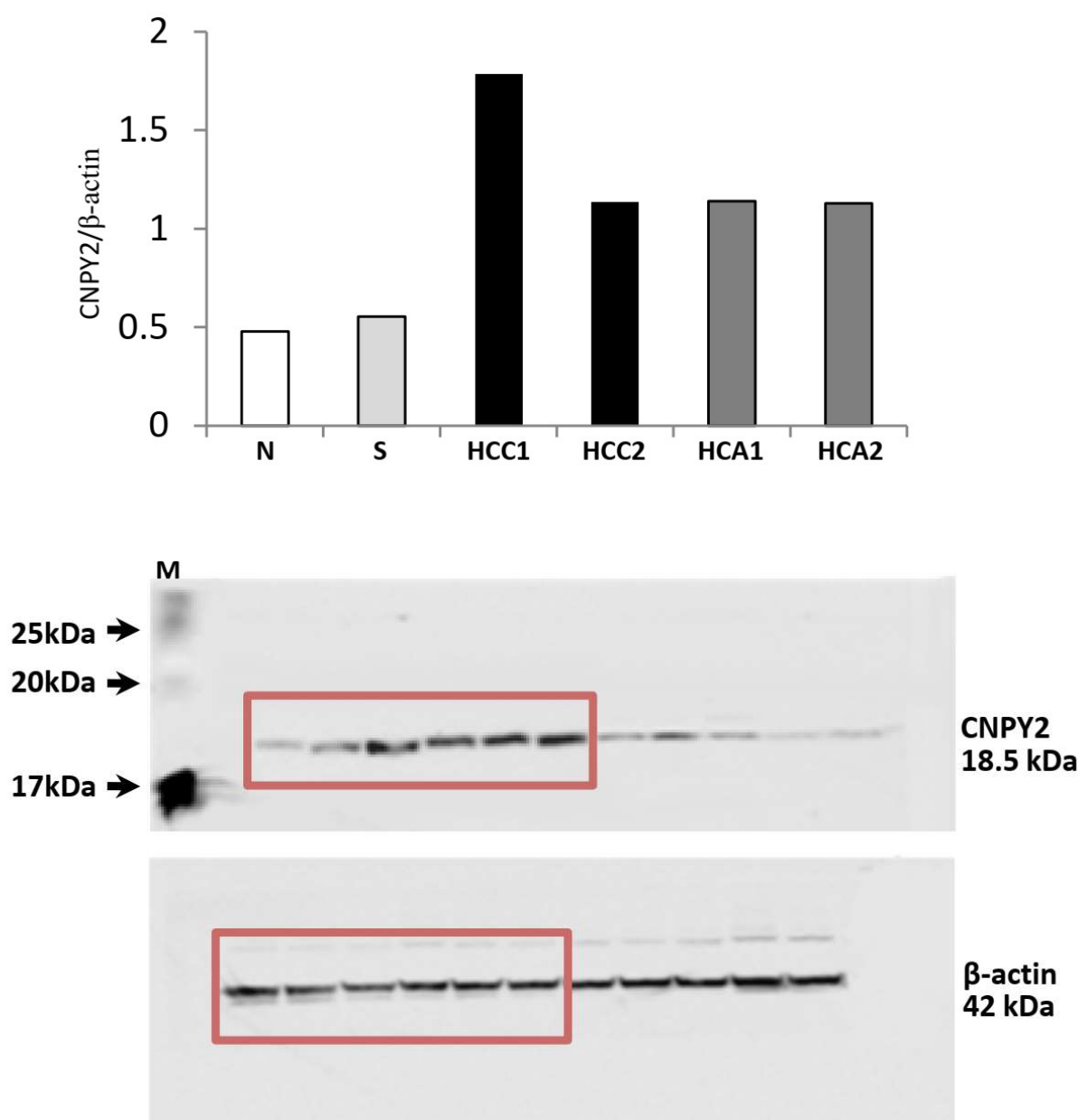


Figure S1. Overexpression of CNPY2 in HCCs of DEN-treated C57Bl/6J mice detected by Western blot analysis. Note very low expression of CNPY2 in non-treated mice livers, small increase in non-healthy surrounding livers and high expression in HCCs. N: normal liver; S: surrounding liver.

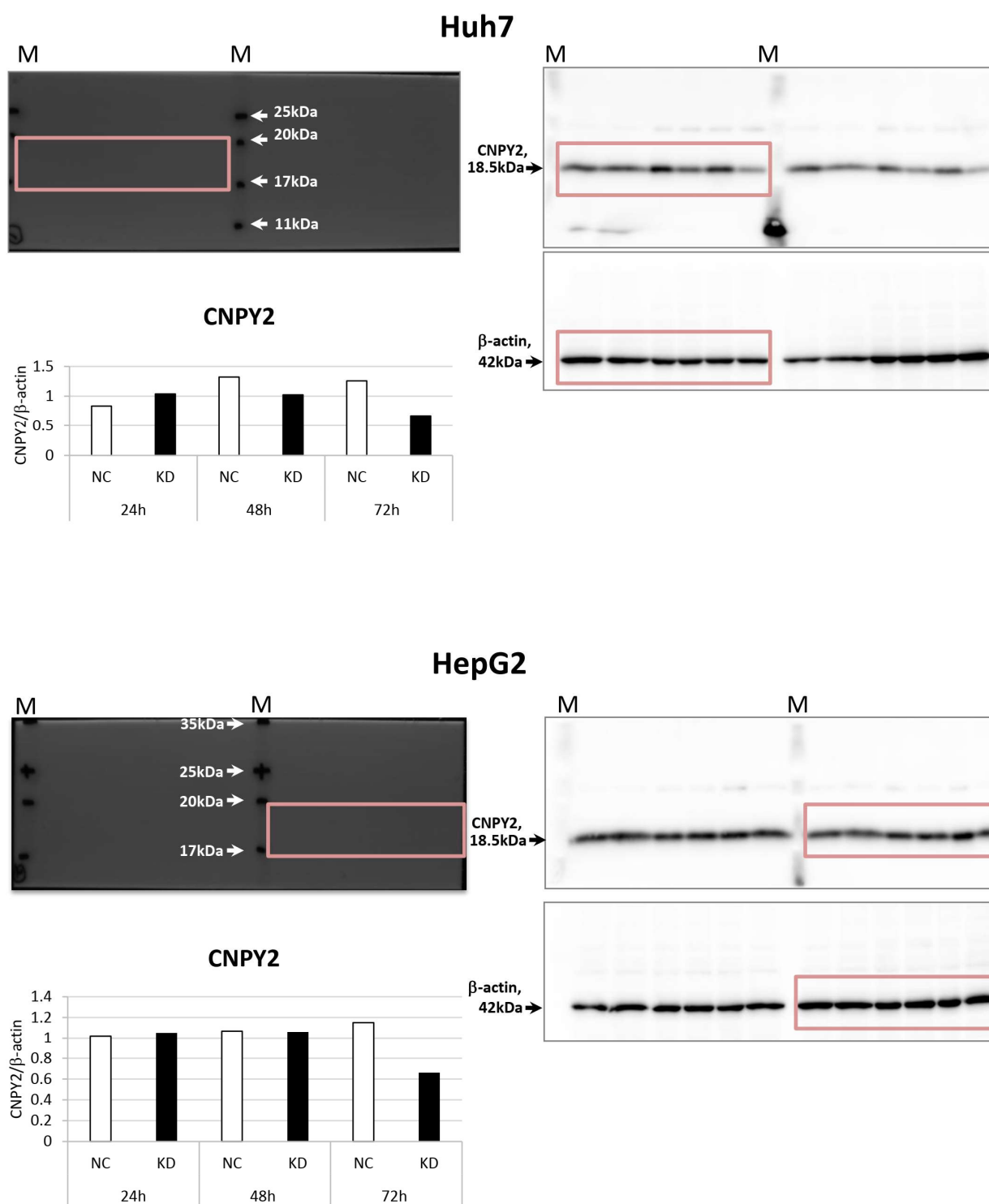


Figure S2. CNPY2 knockdown in Huh7 and HepG2 cells detected by Western Blot analysis.

Liver cancer cell

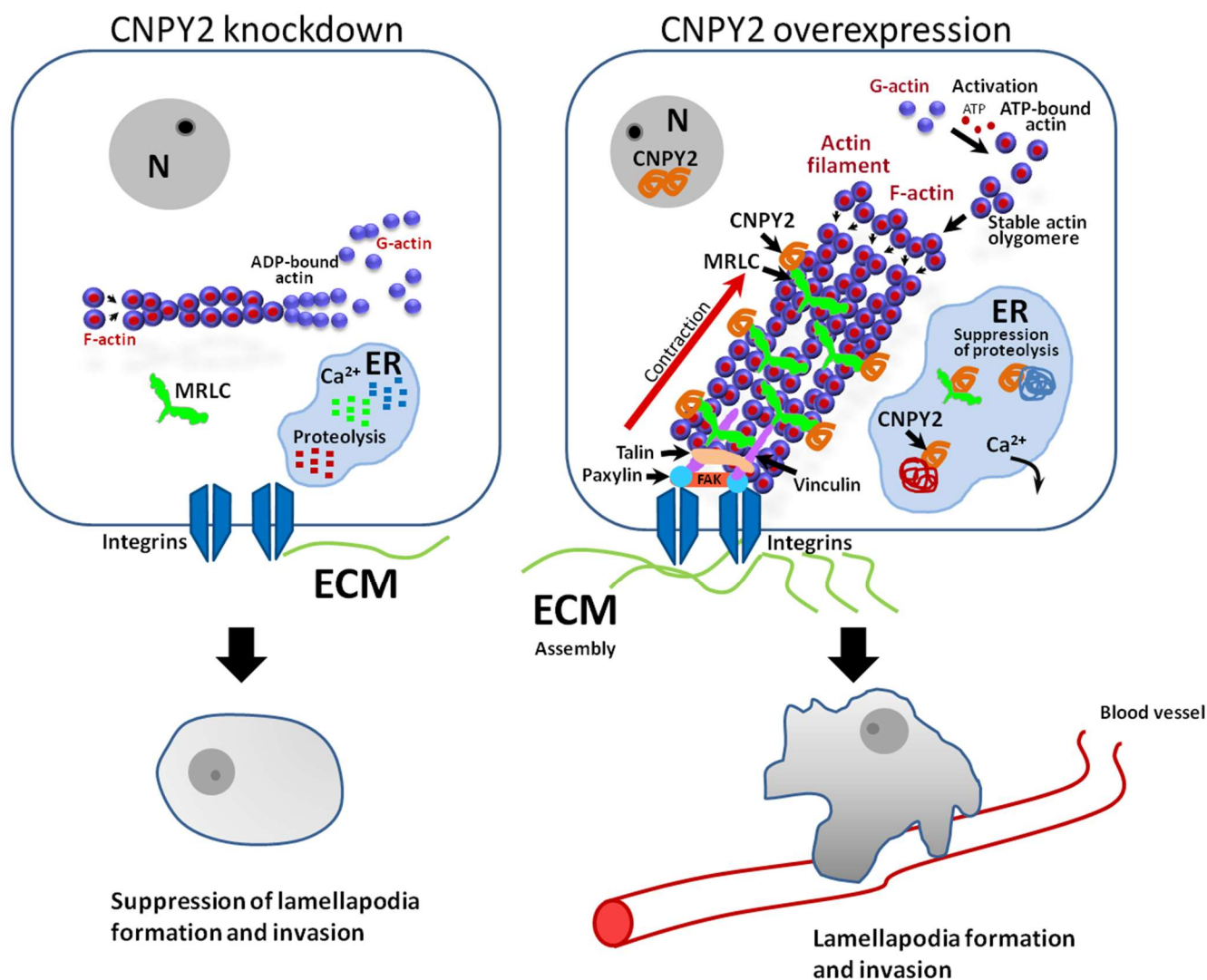


Figure S3. A proposed effect of CNPY2 knockdown on ER stress, actomyosin-ECM system and invasion activity of liver cancer cells. N: nuclear; ER: endoplasmic reticulum, ECM: extracellular matrix; G-actin, globular actin; F-actin, filamentous actin; MRLC, myosin regulatory light chain.

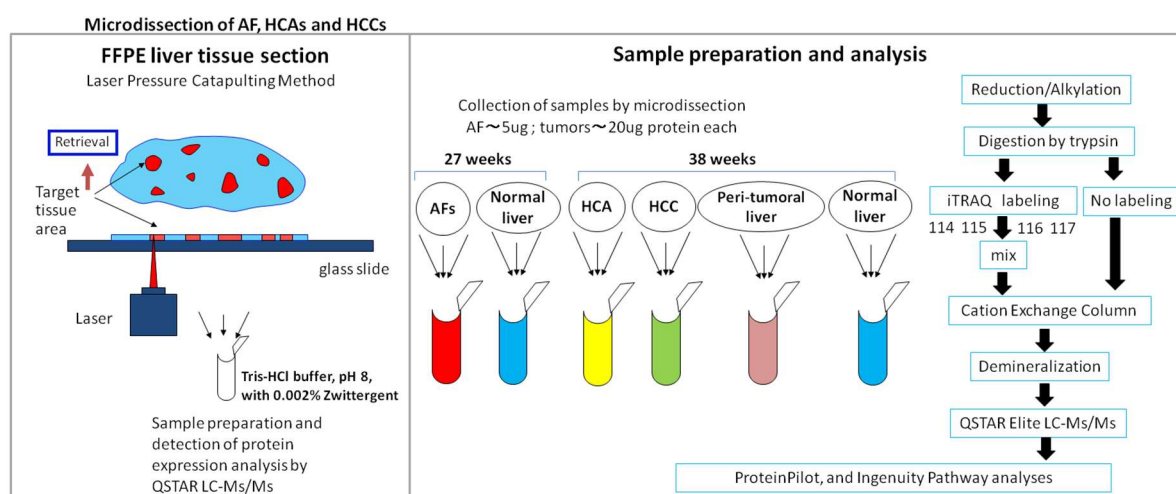


Figure S4. Developed method for sensitive proteome analysis in laser-microdissected samples of altered foci and liver tumors of DEN-treated and control C57Bl/6J mice.

Table S1. Up-stream regulator analysis in CNPY2kn Huh7 cell line (IPA).

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-Score	p-Value of Overlap	Target Molecules in Dataset
TP53	transcription regulator	Activated	2.019	2E-09	ATP5MC1,CALU,CLTA, COL3A1,COMT, DDX39A,EPHX1,G6PD,GAPDH,H2AX,HS P90AA1,HSPA5, IDH2,KRT8,LDHA,M DH2,MYH9,PFN1,PK M, PRDX3,RPS12,SOD2, TALDO1,TIMM44,T MED7, TRPV1,TTN,UBC,UG DH
SP1	transcription regulator	Inhibited	-2.392	0.01	ATP5F1B,BACE2,HS PA5,KRT19,PKM,SO D2,UBC,UGDH
Ins1	other	Inhibited	-2.377	0.0058	ALB,G6PD,GAPDH, LDHA,SOD2,TF
CEBPA	transcription regulator	Inhibited	-2.19	0.0317	AKR1C1/AKR1C2,A LB,EPHX1,GAPDH, HSPA5, SOD2 AKR1C1/AKR1C2,A LDOA,COL3A1,EPH X1,G6PD,HSP90AA1, HSPA9,HYOU1,LM NA,PRDX1,PREP,S10 0P,SOD2, TALDO1,TXN,UBC, UGDH
NFE2L2	transcription regulator	Inhibited	-2.112	1E-10	AFP,AHSG,AKR1C1/ AKR1C2,ALB,CBX3, CLTA, CUX2,EPHX1,G6PD, GAPDH,HSPA5,KH
HNF4A	transcription regulator	Inhibited	-2.042	0.0003	

MYC	transcription regulator	Inhibited	−2.343	1E-10	DRBS1, KRT10, KRT8, LDHA, MDH2, PKM, PRDX5, RTF2, SCFD1, SERPINA1, TEX10, TF, TXN, AFP, ALB, ALDOA, C, ANX, COL3A1, G6PD, GAPDH, GOT2, HSP90AA1, HSPA9, IDH2, KIF16B, LDHA, NCL, PKM, PRDX3, PRDX4, PREP, RPL13, RPL28, RPL4, RPLP2, RPS12, SERPINA1, SOD2, TF, TXN, AFP, AHSG, AKR1C1/ AKR1C2, ALB, COL3A1, HSPA5, SERPINA1, HSPA9, MARCKSL1, PFN1, PRDX1, ALDOA, G6PD, HSPA5, SERPINA1, TF, TTN, COMT, HSPA2, S100P, SERPINA1, SLC9A3R1, RSF7, AKR1C1/ AKR1C2, COL3A1, GAPDH, H2AX, HSPA5, LDHA, PKM, TF, TRPV1, LDHA, MDH2, PFN1, PKM, PRDX5, SERPINA1, SND1, ALDOA, G6PD, GAPDH, IDH2, LDHA, PKM, TALDO1
HNF1A	transcription regulator		−1.981	0.0142	
Ige	complex		−1.98	0.0947	
SREBF1	transcription regulator		−1.969	0.0021	
PGR	ligand-dependent nuclear receptor		−1.969	0.0037	
IGF1	growth factor		−1.813	0.001	
MTOR	kinase		−1.725	0.0027	
PCGEM1	other		−1.704	2E-09	

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Table S2. Patient clinicopathological characteristics, tumor characteristics, and treatment characteristics for HCV+ HCC patients (N = 90).

Factor	Median (Range)	N	Percentage
Patient Demographics			
Gender	Male	62	69
	Female	28	31
Cirrhosis stage	1	22	24
	2	19	21
	3	17	19
	4	32	36
Age (y)	>70	74.04 (71–84)	24
	≤70	63.83 (45–70)	86
BMI	15–24	21.00 (15.7–24.7)	83
	>25	26.80 (25.2–28.8)	17
Recurrence	Positive	54	60
	Negative	36	40

Stage	I		25	28
	II		41	45
	III		21	24
	IV		3	3
Tumor characteristics				
Differentiation	Well		8	9
	Moderate		35	39
	Poor		47	52
Venous invasion	Positive		25	28
	Negative		65	72
Depth of tumor invasion	T1		25	28
	T2		42	47
	T3		20	22
	T4		3	3
Distant metastasis	Positive		1	99
	Negative		89	1
Tumor size (cm ³)	≤2	0.7 (0.03–1.95)	55	61
	>2	13.5 (2.02–70.31)	35	39
Formation of capsule	Present		67	74
	Absent		23	26
Infiltration to capsule	Positive		54	60
	Negative		36	40
Treatment characteristics				
Neoadjuvant therapy	Yes		26	29
	No		64	71
Radiation therapy	Yes		14	16
	No		76	84
Surgical resection	Lobe		9	10
	Wedge		81	90

Tumor volume was counted as $\text{length}/2 * (\text{width}/2) * (\text{width}/2)$ and expressed in cm³.