

Supplemental data

Gluconeogenic enzyme PCK1 deficiency promotes CHK2 O-GlcNAcylation and hepatocellular carcinoma growth upon glucose deprivation

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Supplementary materials & methods

Cell culture. A549, HCT116, and HCF-7 cells were cultured in DMEM (HyClone, Logan, UT, USA) supplemented with 10 % FBS (Gibco, Rockville, MD, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (HyClone) at 37 °C in 5 % CO₂.

Plasmid constructs. For recombinant plasmid construction, DNA fragments (PCK1 NM_002591, CHEK2 NM_007194.4, Δ N truncated CHK2 69-543aa, Δ C truncated CHK2 1-221aa, and OGT NM_181672.2) were amplified by PCR and separately cloned into the pSEB-3Flag, pBudCE4.1-3HA, or pBudCE4.1-5Myc vector. PCK1 G309R, CHK2 T378A, and CHK2 T383A were constructed by overlapping PCR. To construct plasmids expressing shRNAs, two pairs of oligonucleotides encoding shRNAs targeting OGT, OGA, AMPK, or GOT2 (Supplemental Table S3) were cloned into the pLL3.7 vector (from Prof. Bing Sun, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China).

Cell lysis and Western blotting. Protein lysates from cells or liver samples were extracted using cell lysis buffer (Beyotime Biotechnology, Jiangsu, China) containing 1 mM phenylmethanesulfonyl fluoride (Beyotime). Equal volumes of protein samples were separated by 10% SDS/PAGE and electro-transferred to PVDF membranes (Millipore, Billerica, MA, USA). The immunoblots were probed with the indicated antibodies. Proteins bands were visualized with Chemiluminescent Substrate (Bio-Rad, USA) and exposed to CareStream Film (Kodak, USA). Quantification of bands in Western blots was performed using Image-Pro Plus software.

RNA extraction, reverse transcription PCR, and quantitative real-time PCR. Total RNA was extracted from cells using the TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA)

and reverse-transcribed into cDNA using the PrimeScript™ RT Reagent Kit (RR047A, TaKaRa, Japan). Quantitative real-time PCR analysis of target mRNA-expression levels was achieved using SYBR Green qPCR Master Mix (Bio-Rad, Hercules, CA, USA) and the CFX Connect Real-time PCR Detection System (Bio-Rad). The specific primers used are shown in Supplemental Table S3. All samples were analyzed in triplicate for each experiment. The relative gene-expression levels were normalized to β -actin and calculated using the $2^{-\Delta\Delta C_t}$ method.

Cell-proliferation assay. Cells were re-seeded in 96-well plates at a density of 1×10^3 cells/well and cultured in low-glucose medium containing 5 mM glucose for 5 days. The plates were scanned and phase-contrast images were acquired in real time every 24 h post-treatment. Quantified time-lapse curves were generated using IncuCyte ZOOM software (ESCO, Changqi South Street, Singapore). For clone formation capacity, cells were seeded into 6-well plate at 800/well. After 7-10 days on 5 mM glucose condition, the cells were fixed with paraformaldehyde and then incubated in crystal violet. The clones were photographed and the number of clones was counted by Image-Pro Plus software, version 6.0 (Media Cybernetics, Inc., Bethesda, MD, USA).

IP-MS assay. PKO cells transfected with Flag-tagged OGT before cultured in 5 mM glucose were collected and incubated overnight at 4°C with ANTI-FLAG M2 affinity gel (Sigma, A2220). Immunoprecipitated complexes were eluted and stained with Coomassie blue. Excised protein gel bands were sent to Shanghai Applied Protein Technology Co., Ltd. to identify OGT-binding proteins. After performing the reduction reaction and alkylation treatment, the samples were incubated with trypsin (mass ratio, 1:50). The tryptic products were desalted, lyophilized, and re-dissolved in 0.1% formic

acid solution. MS analysis was performed using a Q Exactive MS instrument (Thermo Fisher) coupled to an Easy-nLC 1000 LC instrument (Thermo Fisher) in Shanghai Applied Protein Technology Co., Ltd. The raw MS data were analyzed using Mascot 2.2 software to identify immunoprecipitating proteins. Further, pathway-enrichment analysis was performed using the KEGG database (<http://kobas.cbi.pku.edu.cn/>), p values < 0.05.

Immunofluorescence assays. CHK2 and OGT staining were performed with anti-Flag (mouse 1:400) and anti-HA (rabbit 1:400) antibodies, using PKO cells co-transfected with CHK2-Flag and OGT-HA vectors. Endogenous CHK2 and OGT staining were used by anti-CHK2 (mouse 1:200) and anti-OGT (rabbit 1:100) in SK-Hep1 cells. Specific signals were visualized using secondary antibodies (goat anti-rabbit IgG/TRITC or goat anti-mouse IgG/FITC). For nuclear staining, cells were treated with 1 µg/mL DAPI (10236276001, Roche Diagnostics GmbH, Mannheim, Germany). Stained sections were detected by a laser-scanning confocal microscope (Leica TCS SP8, Solms, Germany).

Ubiquitination assay by immunoprecipitation. protein ubiquitination assays were performed by immunoprecipitation, as described previously (1).

CHK2 protein-stability assay. PCK1-OE SK-Hep1 cells or PKO cells transfected with CHK2 WT or mutant plasmids were treated with 40 µM cycloheximide and harvested after 0, 12, 24, or 36 h. The cells were lysed, and CHK2 was detected using an anti-Flag antibody (Sigma).

CHK2-oligomerization assay. The PKO cells were incubated with HEPES buffer (40 mM HEPES pH 7.5, 150 mM NaCl, 0.1% NP-40) supplemented with protease (Roche) and phosphatase (Beyotime) inhibitor cocktails for 15 min and centrifuged at 14,000 × *g* for 20 min at 4°C. Whole-cell extracts were cross-linked with 0.025% glutaraldehyde for 5 min at 37°C and then quenched with 50 mM Tris-HCl (pH 8.0). The samples were then separated on a 4–20% precast gel and analyzed by Western blotting using the indicated antibodies.

Molecular dynamic simulation. Missing loops and side chains of the dimeric CHK2 structure were complemented using SWISS-model with a previously reported CHK2 dimer structure as template (PDB code: 3I6U). The O-GlcNAcylation was added onto the predicted structure using PyMOL 2.3 software. Molecular dynamic simulation was conducted using GROMACS 2019 package (2). Topologies were generated by ACPYPE with Amber99sb force field parameters (3). After an initial energy minimization, 10 ns of unrestrained NPT MD simulation was performed under constant temperature of 300 K and constant pressure of 1 atm.

Cell cycle analysis. Cell cycle analysis using a FACSCalibur instrument (BD Biosciences, Franklin Lakes, NJ, USA) and CellQuest software, as described previously (4).

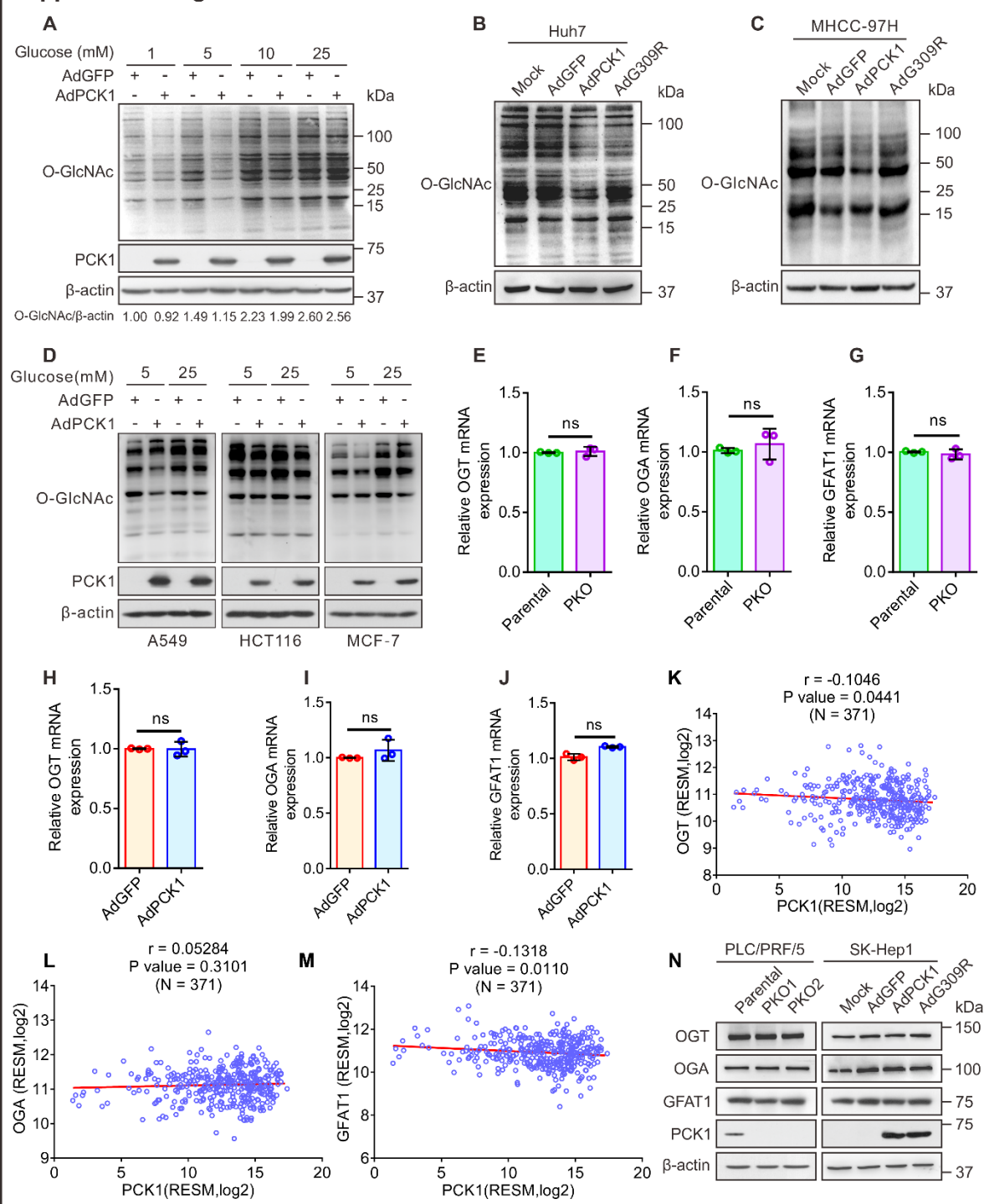
Immunohistochemical (IHC) assay. Human or mouse liver tissues were fixed in 4% paraformaldehyde for at least 24 h and embedded in paraffin, according to standard procedures. The sections were incubated overnight at 4°C with primary antibodies against PCK1 (1:500), p-GFAT (1:200), O-GlcNAc (1:100), or Ki67 (1:100).

Subsequently, the slides were incubated with secondary anti-rabbit or anti-mouse IgG (ZSGB-BIO, Beijing, China) and visualized using 3, 3'-diaminobenzidine (ZSGB-BIO). The stained slides were scanned with a Panoramic Scan 250 Flash or MIDI system, and images were acquired using Panoramic Viewer software, version 1.15.2 (3DHistech, Budapest, Hungary). The mean staining intensity was analyzed using Image-Pro Plus software.

References

1. Xie Y, Lv X, Ni D, Liu J, Hu Y, Liu Y, et al. HPD degradation regulated by the TTC36-STK33-PELI1 signaling axis induces tyrosinemia and neurological damage. *Nature communications*. 2019;10(1):4266.
2. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1-2:19-25.
3. Sousa da Silva AW, and Vranken WF. ACPYPE - AnteChamber PYthon Parser interfacE. *BMC Research Notes*. 2012;5(1):367.
4. Tuo L, Xiang J, Pan X, Hu J, Tang H, Liang L, et al. PCK1 negatively regulates cell cycle progression and hepatoma cell proliferation via the AMPK/p27(Kip1) axis. *Journal of experimental & clinical cancer research*. 2019;38(1):50.

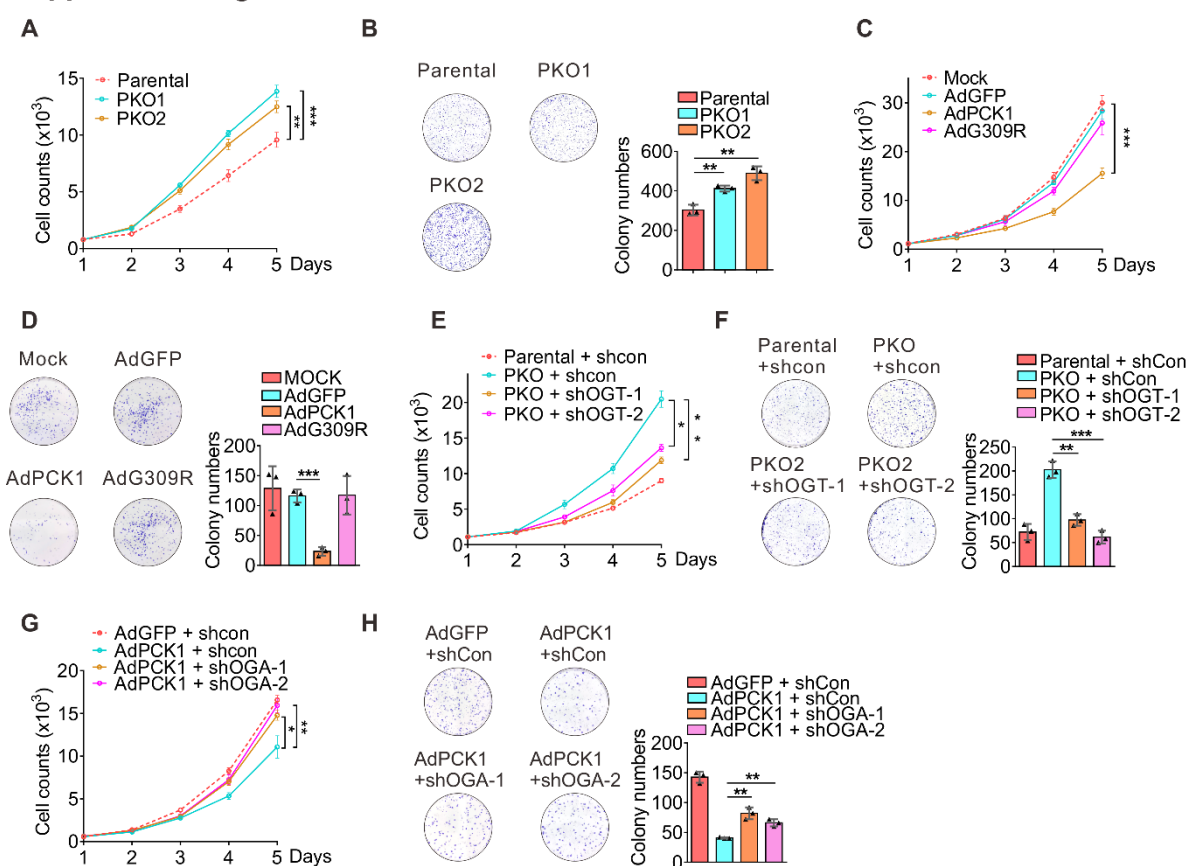
Supplemental Figure 1



Supplemental Figure 1. The expression of OGT, OGA and GFAT are not regulated by PCK1. (A) Global O-GlcNAcylation levels in PCK1-expressing SK-Hep1 cells cultured in medium containing different concentrations of glucose for 12 h. Densitometric analysis was shown as indicated. (B and C) Protein O-GlcNAcylation in Huh7 (B) and MHCC-97H cells (C) overexpressing PCK1 or G309R, and then cultured with 5mM glucose medium for 12 h. (D) Representative Western blots analysis of the indicated proteins in A549, HCT116, MCF-7 cells overexpressing GFP and PCK1 after incubation in medium containing 5 or 25 mM glucose for 12 h. (E-J) OGT, OGA and GFAT1 mRNA expression in PKO cells (E-G) and PCK1-OE cells in

SK-Hep1 (**H-J**) at 5 mM glucose. Data are represented mean $\pm$ SD ($n \geq 3$), 2-tailed Student's t test was used to compare two groups. ns, not significant. (**K-M**) Correlation of PCK1 and OGT (**K**), OGA (**L**) or GFAT1 (**M**) mRNA expression in HCC patient samples from TCGA database. To compare the correlation of gene expression levels, we analyzed the RNA-sequencing data by Expectation Maximization (RSEM), two genes were normalized to transcript abundance of genes. Pearson' correlation coefficient (r) was used to test linear correlations. (**N**) The protein expression levels of OGT, OGA and GFAT1 in PKO cells (left) and SK-Hep1 cells overexpressing PCK1 or G309R (right) under low-glucose concentration (5mM).

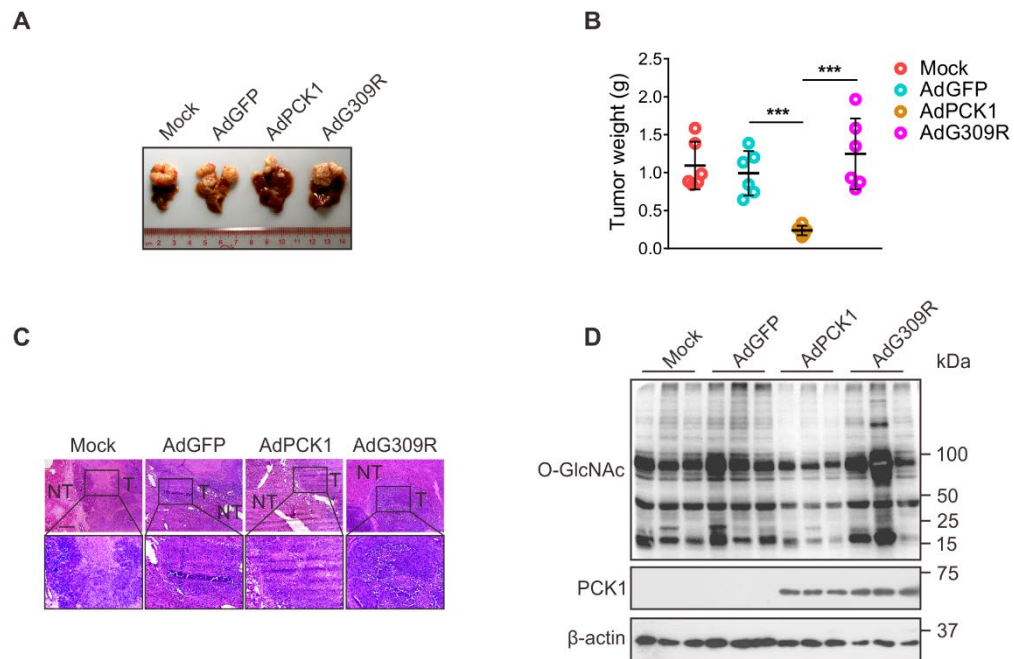
Supplemental Figure 2



Supplemental Figure 2. PCK1 inhibits hepatoma cells proliferation via the HBP.

(A-D) The growth curve and colony formation capacity of PKO cells (A and B) and PCK1-OE cells (C and D). (E-H) PKO cells were transfected with OGT shRNA1/2 plasmid (E and F) and PCK1-OE cells were transfected with OGA shRNA1/2 plasmid (G and H), respectively. Values are shown as indicated (n≥3), *p < 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by the Tukey's test.

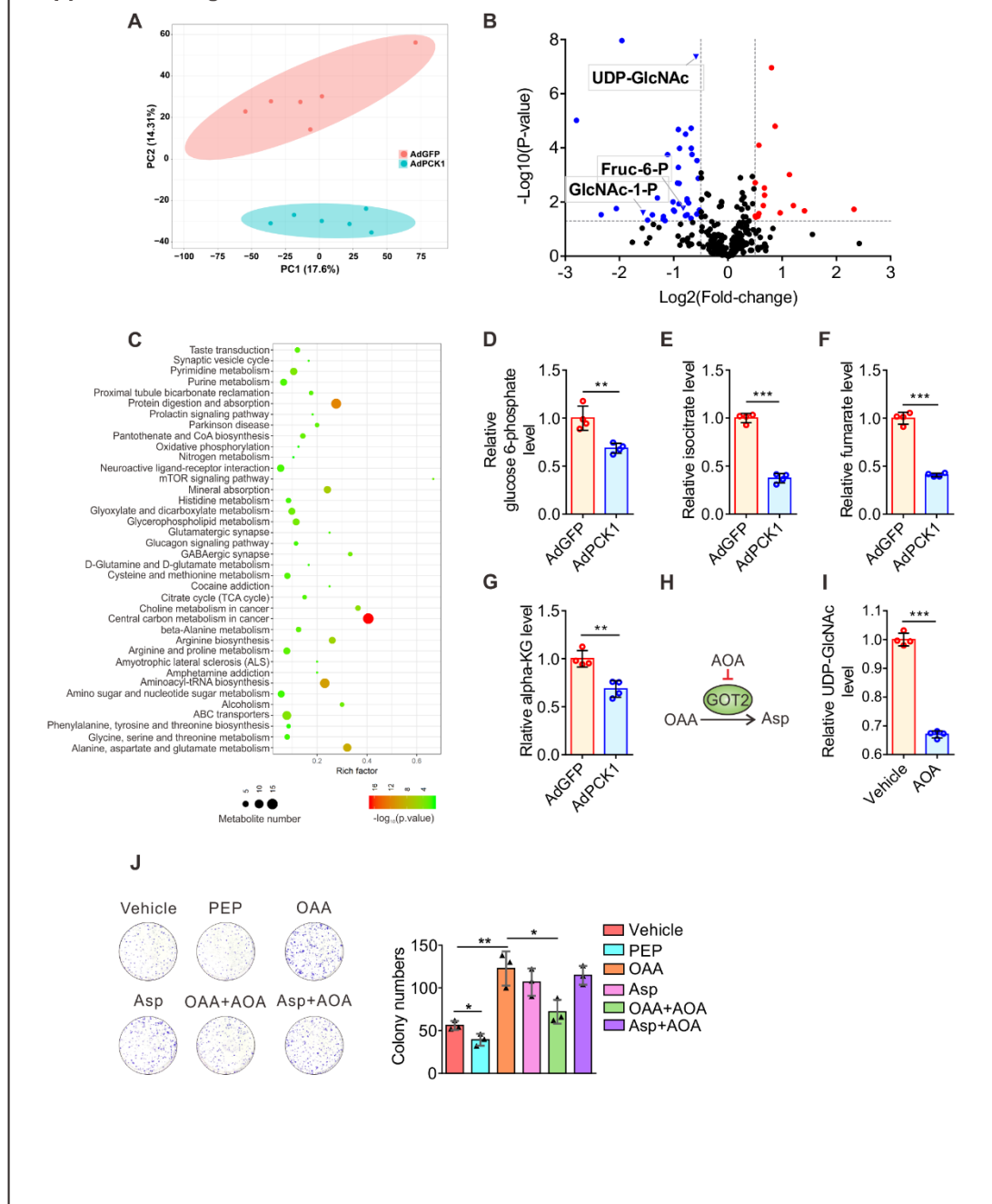
Supplemental Figure 3



Supplemental Figure 3. Overexpression of PCK1 inhibits HCC growth in vivo.

An orthotopic implantation tumor model established with MHCC-97H cells. Cells were infected with AdGFP, AdPCK1, or AdG309R, and implanted into the left liver lobes of nude mice. $n = 6/\text{group}$. **(A)** Representative gross appearance of orthotopic implantation tumors. **(B and C)** Tumor weight **(B)** and H&E staining analysis **(C)**, Scale bars: 100 μm). Data are represented as mean $\pm$ SD, *** $p < 0.001$, one-way ANOVA followed by the Tukey's test. **(D)** Protein expression of PCK1 and total O-GlcNAcylation in liver tumors.

Supplemental Figure 4

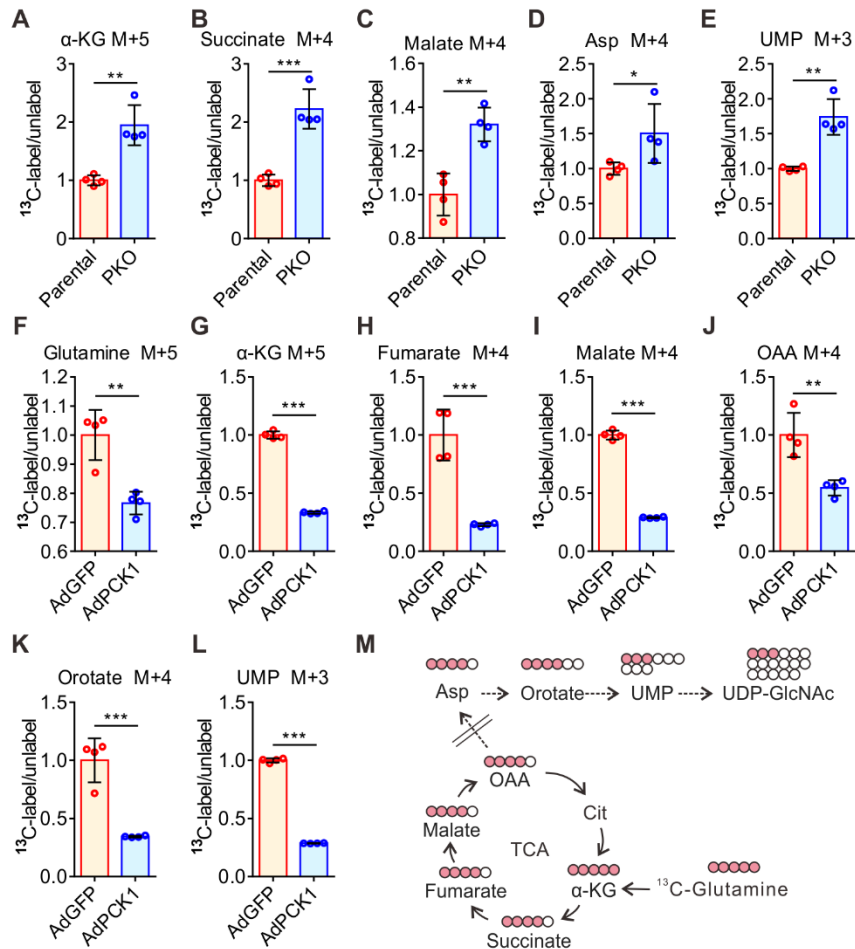


Supplemental Figure 4. Overexpression of PCK1 inhibits pyrimidine metabolism.

(A-C) Principal component analysis (A), volcano plot of fold-change including HBP metabolites (B), and pathway-enrichment analysis (C) of metabolite profiles were obtained using a metabolomics assay in SK-Hep1 cells infected with AdCPK1 or AdGFP and cultured in medium containing 5 mM of glucose for 12 h. (D-G) Targeted LC-MS analysis of intermediate metabolites of glycolytic pathway and TCA cycle. (H) OAA is converted to Asp by the mitochondrial GOT2 enzyme, which can be prevented by the GOT2 inhibitor, AOA. (I) Relative UDP-GlcNAc levels were measured by LC-MS in PKO cells treated with 20 μ M AOA. (J) Colony formation capacity of SK-Hep1 cells treated with 1 mM PEP, 1 mM OAA, 1 mM Asp or 20 μ M AOA for 10 days. Values are shown as indicated, *p < 0.05, **p < 0.01, ***p < 0.001, 2-tailed

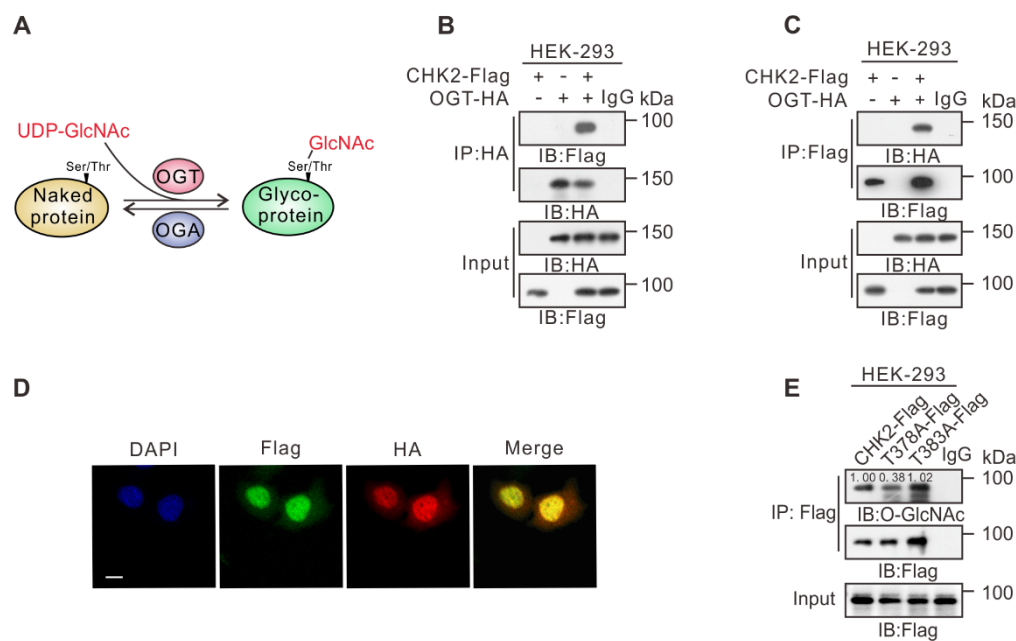
Student's t test (two groups) or one-way analysis of variance (ANOVA) followed by Tukey's test (more than two groups).

Supplemental Figure 5



Supplemental Figure 5. PCK1 deletion promotes glutamine utilization toward pyrimidine and UDP-GlcNAc synthesis. (A-L) PKO cells (A-E) and PCK1 overexpressing SK-Hep1 cells (F-L) were incubated with $^{13}\text{C}_5$ -glutamine and LC-MS analysis was performed to detect m+5 labeled glutamine and aketoglutarate; m+4 labeled succinate, malate, fumarate, oxaloacetate, aspartate and orotate; m+3 labeled UMP. (M) Schematic for conversion of $^{13}\text{C}_5$ -glutamine into UDP-GlcNAc. Values are shown as indicated, *p < 0.05, **p < 0.01, ***p < 0.001, followed by 2-tailed 2-tailed Student's t test.

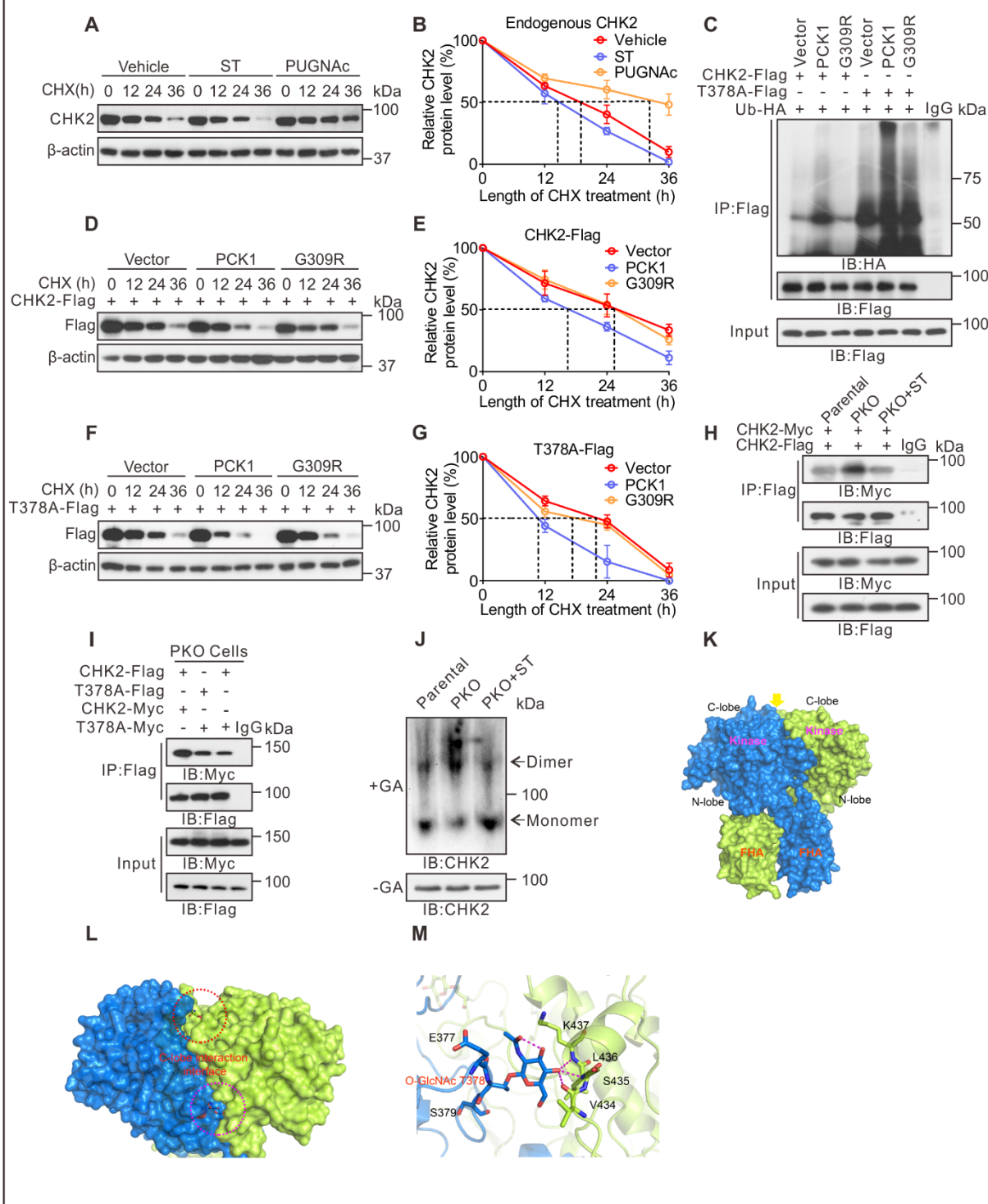
Supplemental Figure 6



Supplemental Figure 6. OGT interacts with CHK2 and mediates its O-GlcNAcylation. (A) A pictorial representation of protein O-GlcNAcylation regulated by OGT and OGA. OGT and OGA catalyze the addition and removal of GlcNAc on proteins, respectively. (B and C) Co-IP of OGT-HA and CHK2-Flag was examined using an anti-HA antibody (B) or an anti-Flag antibody (C) in HEK-293 cells. (D) Subcellular co-localization of OGT-HA and CHK2-Flag in PKO cells was determined using immunofluorescence staining (scale bars: 50 μ m). (E) HEK-293 cells were transfected with vectors encoding Flag-tagged versions of WT CHK2, T378A CHK2, or T383A CHK2. Cell lysates were purified using anti-Flag M2 agarose beads and

probed with an anti-Flag or anti-O-GlcNAc antibody. Quantitative analysis of the O-GlcNAcylation/Flag (IP) band was performed using the Image-pro plus software.

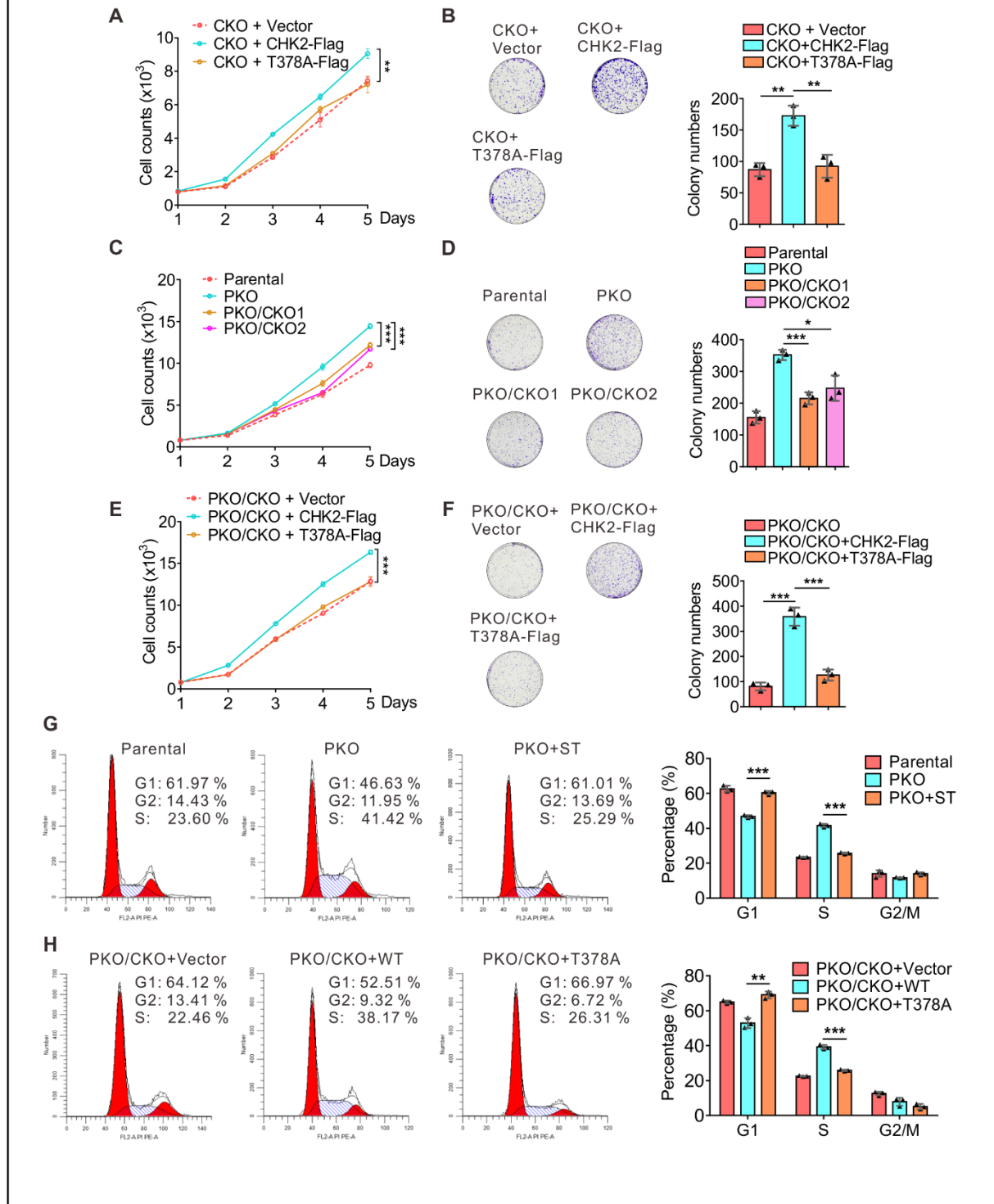
Supplemental Figure 7



Supplemental Figure 7. PCK1 promotes CHK2 degradation and reduces the formation of CHK2 dimer. (A and B) Half-life of endogenous CHK2 (A) in PLC/PRF/5 treated with PUGNAc or ST, CHK2 level was analyzed by using densitometric software (B). (C) CHK2 ubiquitination in PCK1-OE cells in the presence of HA-tagged ubiquitin (Ub-HA). (D-G) Half-life of CHK2-Flag (D) and T378A-Flag (F) in PCK1-OE cells, densitometric analyses are shown in E and G. (H) The co-IP of CHK2-Myc and CHK2-Flag was determined by anti-Flag antibody. (I) Flag/Myc-tagged CHK2 WT and T378A were overexpressed in PKO cells and used for co-IP experiment. (J) The oligomer states of CHK2 in PKO cells. Cell lysis were

treated with glutaraldehyde (GA) and probed with an anti-CHK2 antibody. (**K-M**) Structural modeling of the dimeric CHK2 structure with O-GlcNAcylated T378. Dimeric CHK2 structures are covered with surface (**K**). Interaction interface of the C-lobe of the kinase domain (**L**). O-GlcNAcylated T378s are shown as sticks in the circles. Interactions between O-GlcNAcylated T378 and VSLK motif (**M**). E377, O-GlcNAcylated T378, and S379 are shown as marine blue sticks. The amino acids VSLK of the C-lobe of kinase domain are shown as light green sticks. Red dashed lines indicate hydrogen bonds.

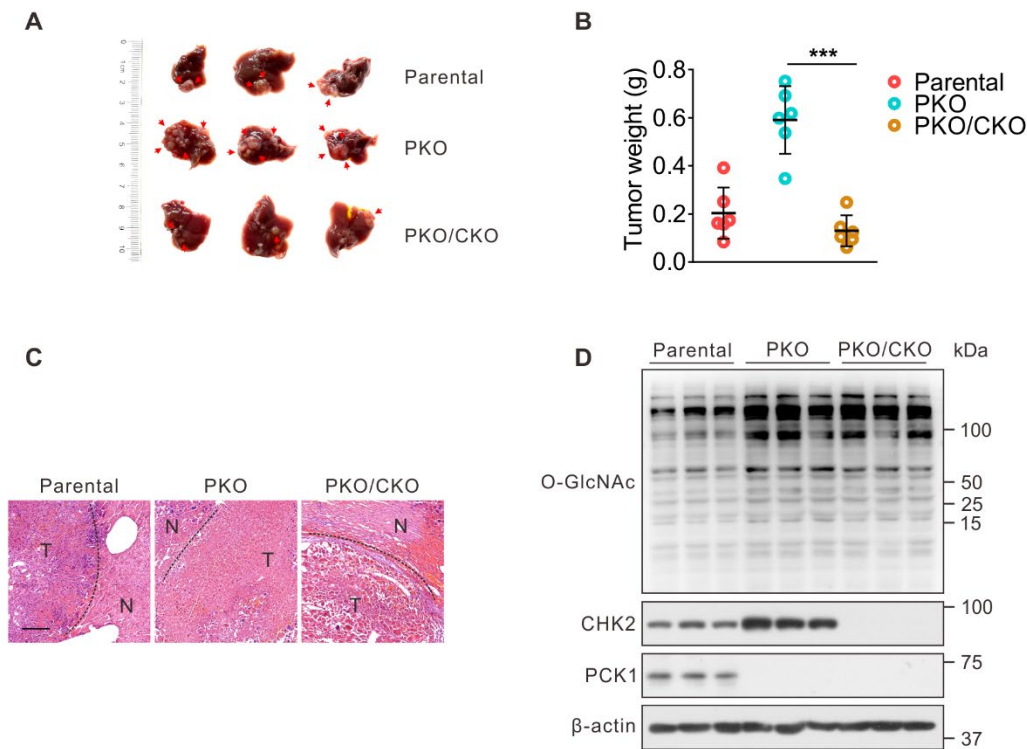
Supplemental Figure 8



Supplemental Figure 8. CHK2 O-GlcNAcylation promotes HCC proliferation.

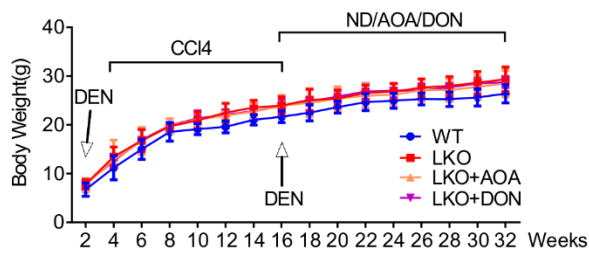
(A-F) Cell proliferation and Colony formation analysis in CKO cells rescued by CHK2-Flag or T378A-Flag (A and B), and PKO/CKO cells (C and D) or rescue assay as indicated (E and F). (G and H) Flow cytometric analysis. PKO cells were treated with ST (G), or PKO/CKO cells were transfected with CHK2 WT or CHK2 T378A mutant (H), and then subjected to flow cytometry. Percentage (%) of cells in the G1 and S phases is indicated in each graph. Values are shown as indicated (n ≥ 3), *p < 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by the Tukey's test (more than two groups).

Supplemental Figure 9



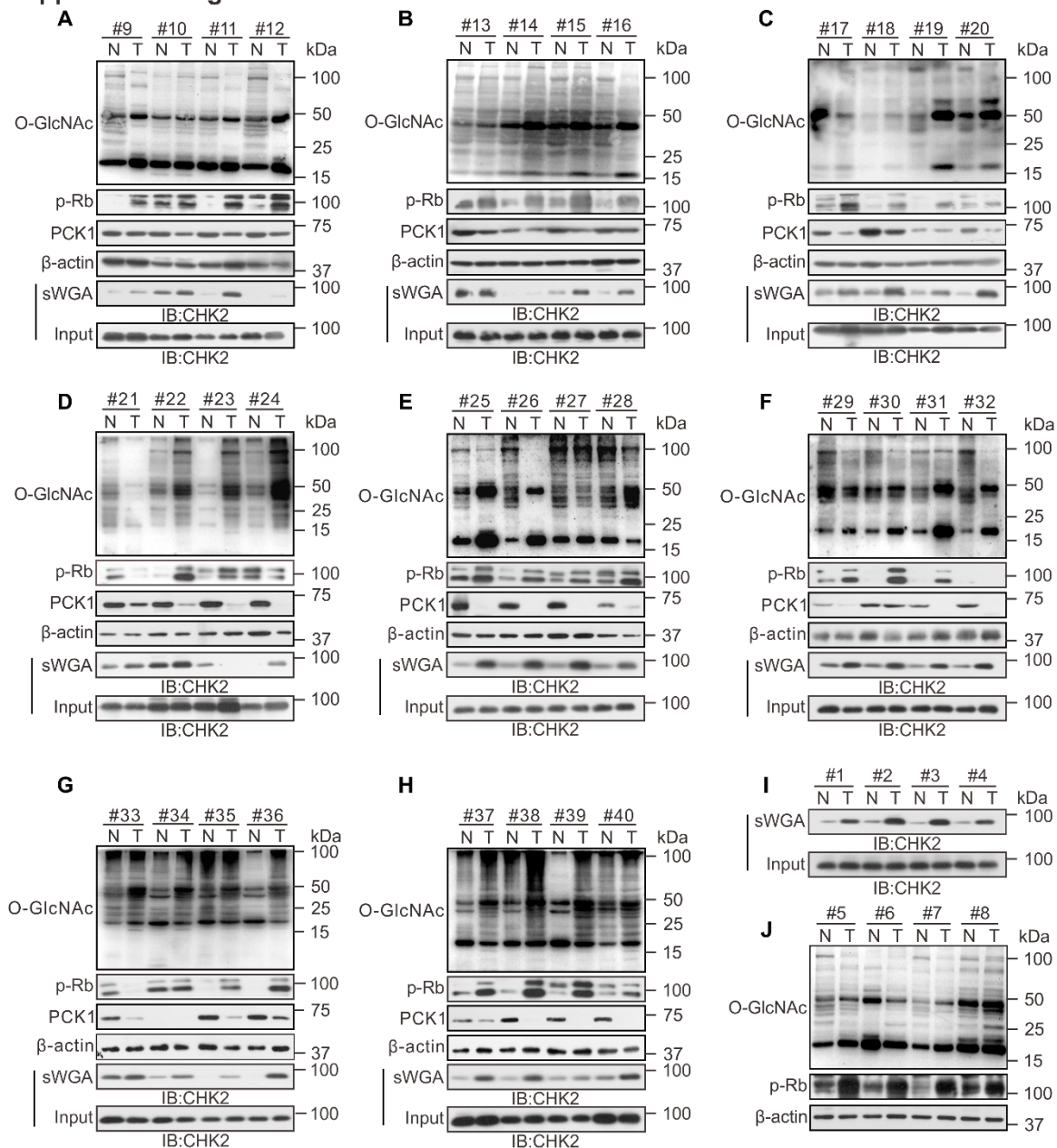
Supplemental Figure 9. CHK2 is required for the PCK1 loss-induced liver tumor growth in vivo. (A) Representative gross appearance of orthotopic implantation tumors. An orthotopic implantation tumor model was established with PLC/PRF/5 cells. Cells were implanted into the left liver lobes of nude mice. n=6/group. (B and C) Tumor weight (B) and H&E staining analysis (C) of each group as indicated. Data are represented mean $\pm$ SD, ***p < 0.001, one-way ANOVA followed by the Tukey's test. (D) Protein expression of PCK1, CHK2, and total O-GlcNAcylation in liver tumors.

Supplemental Figure 10



Supplemental Figure 10. The body weights of *Pck1*-Knockout Mice. The line chart of the body weights in *Pck1*-knockout mice. The liver tumors were induced through combined injection with DEN (75 mg/kg) and CCl₄ (2 ml/kg), when the mice were 2 weeks old. At 16 weeks after DEN/CCl₄ treatment, the LKO mice were administered 5 mg/kg AOA or 1 mg/kg DON through intraperitoneal injection, twice a week for 16 weeks.

Supplemental Figure 11



Supplemental Figure 11. Downregulation of PCK1 but upregulation of O-GlcNAc in 40 cases of HCC. (A-J) Protein expression of PCK1, global O-GlcNAcylation, p-Rb, and CHK2 O-GlcNAcylation in human HCC (See also Figure 7).

Supplemental Table 1: 618 candidate OGT-binding proteins identified by IP-MS analysis.

(See Separate Excel documents)

Supplemental Table 2, Clinical Correlation between Relative Expression of PCK1 and Clinicopathologic Parameters of HCC Patients (n=40)

Variable	Frequency (%)	PCK1 relative expression		P Value
		Low(n=31)	High(n=9)	
Sex				
Male	33(82.5)	25	8	0.5666
Female	7 (17.5)	6	1	
Age				
≤ 55	24(60)	19	5	0.7572
> 55	16(40)	12	4	
Tumor size, cm*cm				
> 25	22(55)	21	1	0.0038*
≤ 25	18(45)	11	8	
Alpha fetoprotein, ng/mL				
≤ 100	20(50)	13	7	0.0583
> 100	20(50)	18	2	
Hepatitis B surface antigen				
Positive	25(62.5)	20	5	0.6250
Negative	15(37.5)	11	4	
Histological differentiation				
Well	15(37.5)	9	6	0.0401*
Moderate/Poor	25(62.5)	22	3	
Recurrence				
Absent	19(47.5)	12	7	0.0388*
Present	21(52.5)	19	2	

A total of 40 paired HCC tissues with defined clinical data were chosen for this correlation study. * $P < 0.05$.

Supplemental Table 3, related to KEY RESOURCES TABLE. Sequence of Oligonucleotides.

Name	Sequence(5'-3')
Primer sequences for quantitative RT-PCR	
OGT	Forward: AGAGGCAGTTCGCTTGTATCG Reverse: AGTAGGCATCAGCAAAGGTAGG
OGA	Forward: AGCAGCCTTGAGTGGTGAGC Reverse: TGGGGATTTTGATTGAGCTATG
GFAT1	Forward: CCAGTCCTGTCAATAGCCACC Reverse: CACAAGTGCAAAAGCACCTTC
β-actin	Forward: AGGCCAACCGCGAGAAGATGACC Reverse: GAAGTCCAGGGCGACGTAGCAC
Primer sequences for knockdown	
shOGT	Forward1: TGCATGTTATTTGAAAGCAATTCAAGAGATTGCTTTC AAATAACATGCTTTTTTC Reverse1: TCGAGAAAAAAGCATGTTATTTGAAAGCAATCTCTT GAATTGCTTTCAAATAACATGCA Forward2: TGCCCTAAGTTTGAGTCCAATTCAAGAGATTGGACT CAAAGTTAGGGCTTTTTTC Reverse2: TCGAGAAAAAAGCCCTAAGTTTGAGTCCAATCTCTT GAATTGGACTCAAAGTTAGGGCA
shOGA	Forward1: TGGACAATTCTTTATGACATTTCAAGAGAATGTCATA AAGAATTGTCCTTTTTTC Reverse1: TCGAGAAAAAAGGACAATTCTTTATGACATTCTCTT GAAATGTCATAAAGAATTGTCCA Forward2: TGCCTTTGTACACTGCGGAATTCAAGAGTTCCGCAG TGACAAAGGCTTTTTTC Reverse2: TCGAGAAAAAAGCCTTTGTACACTGCGGAATCTCTT GAATTCCGCAGTGTACAAAGGCA
shAMPK	Forward1: TGCAGGCCCGAGAGGTAGATATTCAAGAGATATCTA CCTCTGGGCCTGCTTTTTTC Reverse1:

	TCGAGAAAAAAGCAGGCCAGAGGTAGATATCTCT TGAATATCTACCTCTGGGCCTGCA Forward2: TGCAGAAGTATGTAGAGCAATTCAAGAGATTGCTCT ACATACTTCTGCTTTTTTC Reverse2: TCGAGAAAAAAGCAGAAGTATGTAGAGCAATCTCTT GAATTGCTCTACATACTTCTGCA
shGOT2	Forward1: TGCTACAAGGTTATCGGTATTTCAAGAGAATACCGA TAACCTTGTAGCTTTTTTC Reverse1: TCGAGAAAAAAGCTACAAGGTTATCGGTATTCTCTT GAAATACCGATAACCTTGTAGCA Forward2: TGCCAATCATATGCCAAGAATTCAAGAGATTCTTGG CATATGATTGGCTTTTTTC Reverse2: TCGAGAAAAAAGCCAATCATATGCCAAGAATCTCTT GAATTCTTGGCATATGATTGGCA
Primer sequences for molecular cloning	
pAdTrack-TO4- GFP-PCK1	Forward: CGCGGATCCACCATGGGCCCTCCTCAGCTGCAAAA CGGCC Reverse: CCCAAGCTTCTACATCTGGCTTATTCTTTGCTTCAA G
pSEB-3Flag-PC K1	Forward: CGGGGTACCATGGGCCCTCCTCAGCTGCAAAACGG C Reverse: CTGGATATCCATCTGGCTTATTCTTTGCTTCAAG
pBu-3HA-PCK1	Forward: CGGGGTACCATGGGCCCTCCTCAGCTGCAAAACGG C Reverse: CTGGATATCCATCTGGCTTATTCTTTGCTTCAAG
pSEB-3Flag-OG T	Forward: ACGCGTCGACACCATGGCGTCTTCCGTGGGC Reverse: CGCGGATCCTGCTGACTCAGTGACTTCAACAGG
pBu-3HA-OGT	Forward: ATAGCGGCCGCACCATGGCGTCTTCCGTGGGC Reverse: CGCGGATCCTGCTGACTCAGTGACTTCAACAGG
pSEB-3Flag-CH EK2	Forward: CCCAAGCTTACCATGGGCTCTCGGGAGTCGGATGT TG Reverse: ATAGCGGCCGCACAACACAGCAGCACACACAGCT

pAdTrack-TO4-GFP-3Flag-CHEK2	Forward: CCCAAGCTTACCATGGGCTCTCGGGAGTCGGATGTG Reverse: ATAGCGGCCGCGACAACACAGCAGCACACACAGCT
pBudCE4.1-5My c-CHEK2	Forward: CGGGGTACCATGGGCTCTCGGGAGTCGGATGTTG Reverse: ATAGCGGCCGCGCAACACAGCAGCACACACAGCT
Primer sequences for mutagenesis	
pAdTrack-TO4-GFP-G309R (PCK1 mutation 925G>A)	Forward: ATCTTGAGTAAGTGCCTTGGGGGAGA Reverse: TCTCCCCAAGGCACTTACTCCAAGAT
pSEB-3Flag-G309R	Forward: GAAGGTTGAGTGCGTCAGGGATGACATTGCCT Reverse: AGGCAATGTCATCCCTGACGCACTCAACCTTC
pSEB-3Flag-ΔC (CHEK2 1aa-221aa)	Forward: CCCAAGCTTACCATGGGCTCTCGGGAGTCGGATGTG Reverse: ATAGCGGCCGCGACATGATGTATTCATCTCTTAATGC
pSEB-3Flag-ΔN (CHEK2 69aa-543aa)	Forward: CCCAAGCTTACCATGGAACCTATTCTATTCTCCTGAG Reverse: ATAGCGGCCGCGACAACACAGCAGCACACACAGCT
pSEB-3Flag-T378A (CHEK2 mutation 1132A>G)	Forward: GATTTTGGGAGAGGCCTCTCTCATGAGAAC Reverse: GTTCTCATGAGAGAGGCCTCTCCCAAATC
pBudCE4.1-5My c-T378A (CHEK2 mutation 1132A>G)	Forward: GATTTTGGGAGAGGCCTCTCTCATGAGAAC Reverse: GTTCTCATGAGAGAGGCCTCTCCCAAATC
pSEB-3Flag-T383A (CHEK2 mutation 1147A>G)	Forward: CCTCTCTCATGAGAGCCTTATGTGGAACC Reverse: GGTTCCACATAAGGCTCTCATGAGAGAGG
PCK1, CHEK2-targeted single gRNAs	
PCK1-sgRNA-1	Forward: CACCGGCTGAAGAAGTATGACAAC Reverse: AAACGTTGTCATACTTCTTCAGCC
PCK1-sgRNA-2	Forward: CACCGTAGGCTGTCCAGGCTTCCC Reverse:

	AAACGGGAAGCCTGGACAGCCTAC
CHEK2-sgRNA-1	Forward: CACCGACTGGGTAACGCTGCCATG Reverse: AAACCATGGCAGCGTTACCCAGTC
CHEK2-sgRNA-2	Forward: CACCGTTGAGGCTCAGCAGTCTCA Reverse: AAACTGAGACTGCTGAGCCTCAAC
lentiCRISPR-v2	Forward: GGTTTATTACAGGGACAGCAG Reverse: ACACGACATCACTTTCCCAG

Supplemental Table 4, Antibodies used for Western blotting, IP, IHC and IHF.

Western blotting			
Antibodies	Source	Antibody dilution	Identifier
Rabbit anti-PCK1	Abcam	1:1000	Cat# ab28455
Mouse anti-O-GlcNAc	Abcam	1:2000	Cat# ab2739
Rabbit anti-OGT	Abcam	1:2000	Cat# ab96718
Rabbit anti-OGA	Abcam	1:5000	Cat# ab124807
Rabbit anti-GOT2	Abcam	1:5000	Cat# ab171739
Rabbit anti-GFAT1	Abcam	1:500	Cat# ab125069
Rabbit anti-p-Rb (S780)	Abcam	1:1000	Cat# ab184702
Goat anti-rabbit, secondary	Abcam	1:10000	Cat# ab6721
Goat anti-mouse, secondary	Abcam	1:10000	Cat# ab6789
Rabbit anti-CHK2	Cell Signaling	1:1000	Cat# 6334
Rabbit anti-p-CDK2 (Thr160)	Cell Signaling	1:1000	Cat# 2561
Rabbit anti-p27	Cell Signaling	1:1000	Cat# 3686
Rabbit anti-AMPK	Cell Signaling	1:1000	Cat# 5832
Rabbit anti-p-AMPK(Thr172)	Cell Signaling	1:1000	Cat# 2535
Rabbit anti-HA	Cell Signaling	1:2000	Cat# 3724
Rabbit anti-Myc-Tag	Cell Signaling	1:1000	Cat# 2272S
Rabbit anti-Rb	Bioworld	1:2000	Cat# BS1310
Rabbit anti-CDK2	Bioworld	1:2000	Cat# BS1050
Rabbit anti-p-GFAT1(Ser243)	Immuno-Biological Laboratories	1:5000	Cat# S243
Mouse monoclonal anti-FLAG M2	Sigma-Aldrich	1:5000	Cat# F3165,
Mouse anti- β -actin	ZSGB-BIO	1:5000	Cat# TA-09
IP			

ANTI-FLAG® M2 Affinity Gel	Sigma-Aldrich	1:10	Cat# A2220
sWGA-conjugated agarose beads	Vector Laboratories	1:10	Cat# AL-1023S
Rabbit anti-HA	Cell Signaling	1:100	Cat# 3724
Mouse monoclonal anti-FLAG M2	Sigma-Aldrich	1:100	Cat# F3165
Rabbit anti-CHK2	Cell Signaling	1:100	Cat# 6334
Rabbit anti-OGT	Abcam	1:100	Cat# ab96718
IHC			
Rabbit anti-PCK1	Abcam	1:500	Cat# ab28455
Rabbit anti-p-GFAT1(Ser243)	Immuno-Biological Laboratories	1:200	Cat# S243
Mouse anti-O-GlcNAc	Abcam	1:100	Cat# ab2739
Rabbit anti-Ki67	Thermo	1:100	Cat# PA516785
IHF			
Rabbit anti-HA	Cell Signaling	1:400	Cat# 3724
Mouse monoclonal anti-FLAG M2	Thermo	1:400	Cat# MA1-91878
Mouse anti-CHK2	Cell Signaling	1:200	Cat# 3440S
Rabbit anti-OGT	Abcam	1:100	Cat# ab96718
Goat anti-rabbit IgG/TRITC, secondary	ZSGB-BIO	1:100	Cat# ZF-0316
Goat anti-mouse IgG/FITC, secondary	ZSGB-BIO	1:100	Cat# ZF-0312

Supplemental Table 5: Chemicals, Critical Commercial Assays and Experimental Models.

RESOURCE	SOURCE	IDENTIFIER
Chemicals		
(+)-Glucose solution	Sigma-Aldrich	G8769
2× Taq PCR Green Mix	Dinguo	PER 007
Carbon tetrachloride (CCl ₄)	Macklin	C805332
Diethylnitrosamine (DEN)	Sigma	N0756
DMEM	HyClone	SH30243.01
ST045849	Tim Tec	MFCD03308174
Lipofectamine 2000	Invitrogen	11668019
Thiamet G	Selleck	S7213
UDP-GlcNAc	BBL Life Sciences	7512-17-6
Phospho(enol)pyruvic acid monopotassium salt	Sigma-Aldrich	P7127
Oxaloacetate	Sigma-Aldrich	O4126
Aspartate	Sigma-Aldrich	V900407
Opti-MEM	Gibco	31985070
Penicillin-Streptomycin Solution	HyClone	SV30010
AOA hemihydrochloride	Selleck	S4989
Polybrene	Yeasen	40804ES76
6-diazo-5-oxo-L-norleucine	Sigma-Aldrich	D2141
Metformin HCl	Selleck	S1950
Suero fetal bovino esteril	Natocor	SFBE
ANTI-FLAG® M2 Affinity Gel	Sigma-Aldrich	A2220
Trypsin	Gibco	15050057
Trypsin-EDTA	Gibco	25200072
Cycloheximide	MCE	HY-12320
4',6-diamidino-2-phenylindole	Invitrogen	D1306
Matrigel basement membrane matrix	Corning	354234
Critical Commercial Assays		
BCA protein assay Kit	Dinguo	BCA02
Cell lysis buffer for Western and IP	Beyotime	P0013
Histostain™ - SP Kits	ZSGB-BIO	SPN-9001
cOmplete™, EDTA-free Protease Inhibitor Cocktail Tablets	Roche	4693132001

DAB kit	Zhongshan Jinqiao	ZLI-9019
DAPI	Roche	10236276001
Elivision™ plus Polyer HRP (Mouse/Rabbit) IHC Kit	Maxim	KIT-9901
Genomic DNA Purification Kit	Genloci	GP0155
iTaq™ Universal SYBR® Green Supernix	Bio-Rad	1725121
PrimeScript® RT reagent Kit	Takara	RR047A
TRIzol™ Reagent	Invitrogen	15596026
Experimental Models		
BALB/c nude mice (male, 4 weeks)	Beijing, China	BEIJING HFK BIOSCIENCE CO.,LTD
<i>Pck1</i> ^(flox/flox) 129S6/SvEv Tac mice (male and female)	EMMA: 011950-UNC	the Mutant Mouse Resource & Research Centers
<i>AlbCre</i> ^(+/+) C57 mice (male and female)	Nanjing, China	Model Animal Research Center of Nanjing University
Cell Lines		
HEK293	Lab stock	
Huh7	Lab stock	
MHCC97H	Lab stock	
PLC/PRF/5	Lab stock	
A549	Lab stock	
HCT116	Lab stock	
MCF-7	Lab stock	
SK-Hep1	ATCC	HTB-52

Supplemental Table 6: Software and Algorithms.

ACPYPE	Sousa da Silva and Vranken, 2012	http://github.com/Ilazzaro/acpype
GraphPad Prism 7.0	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
GROMACS 2019	Abraham et al., 2015	http://www.gromacs.org/
Image-pro plus 7.0	Media Cybernetics	http://www.mediacy.com/imageproplus/
MaxQuant (1.5.2.8)	Maxquant	http://www.maxquant.org/
PyMOL 2.3	Schrodinger	https://pymol.org/

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