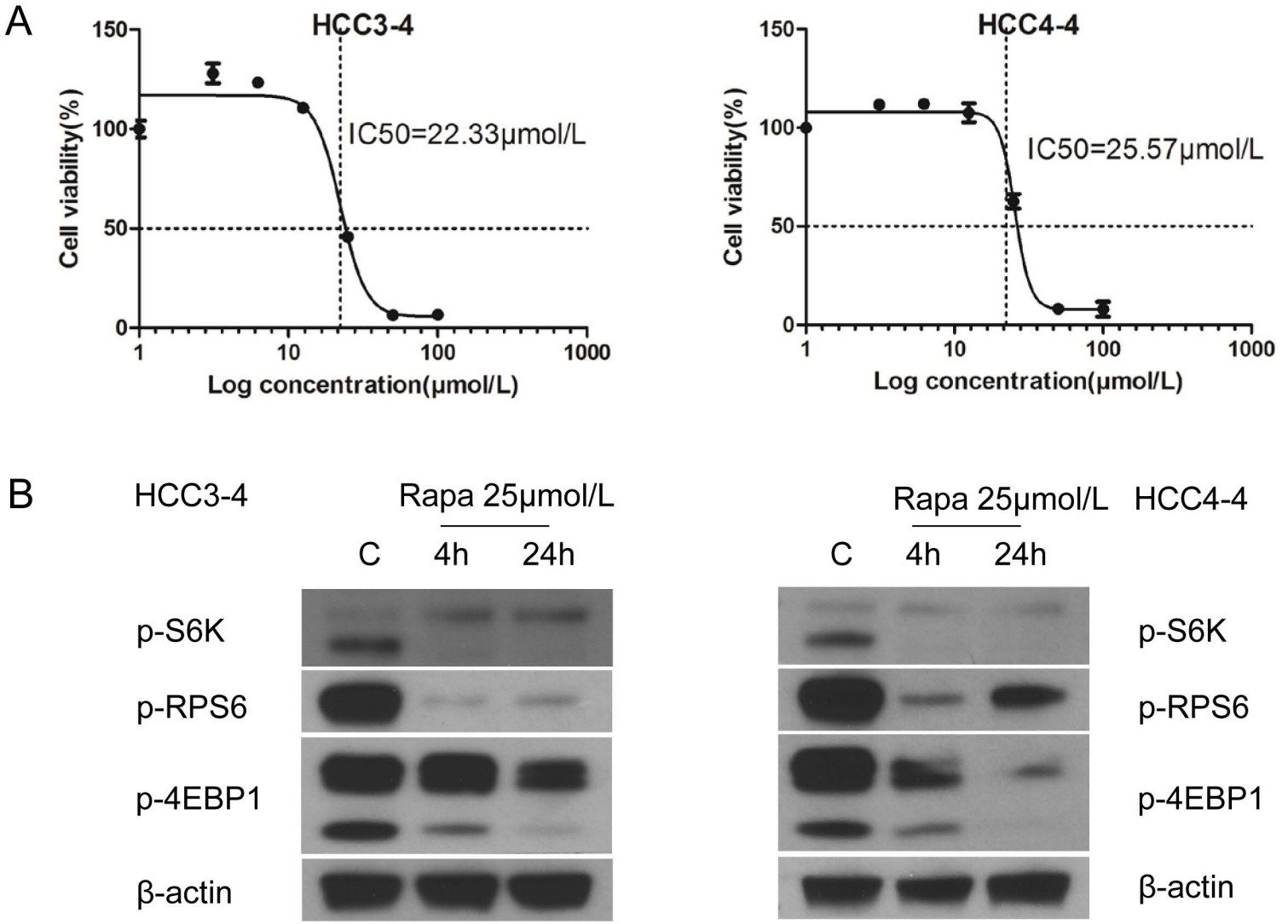
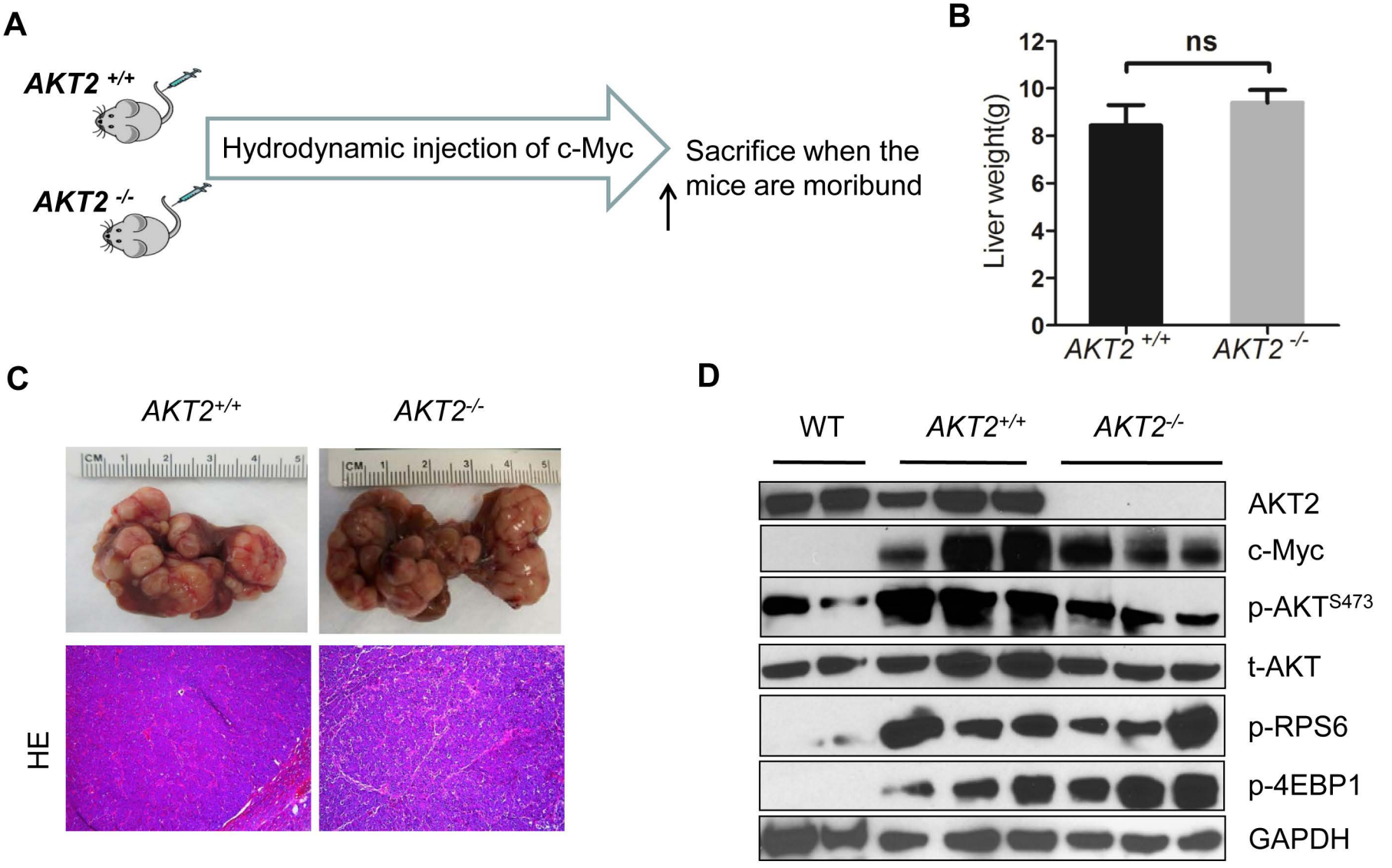
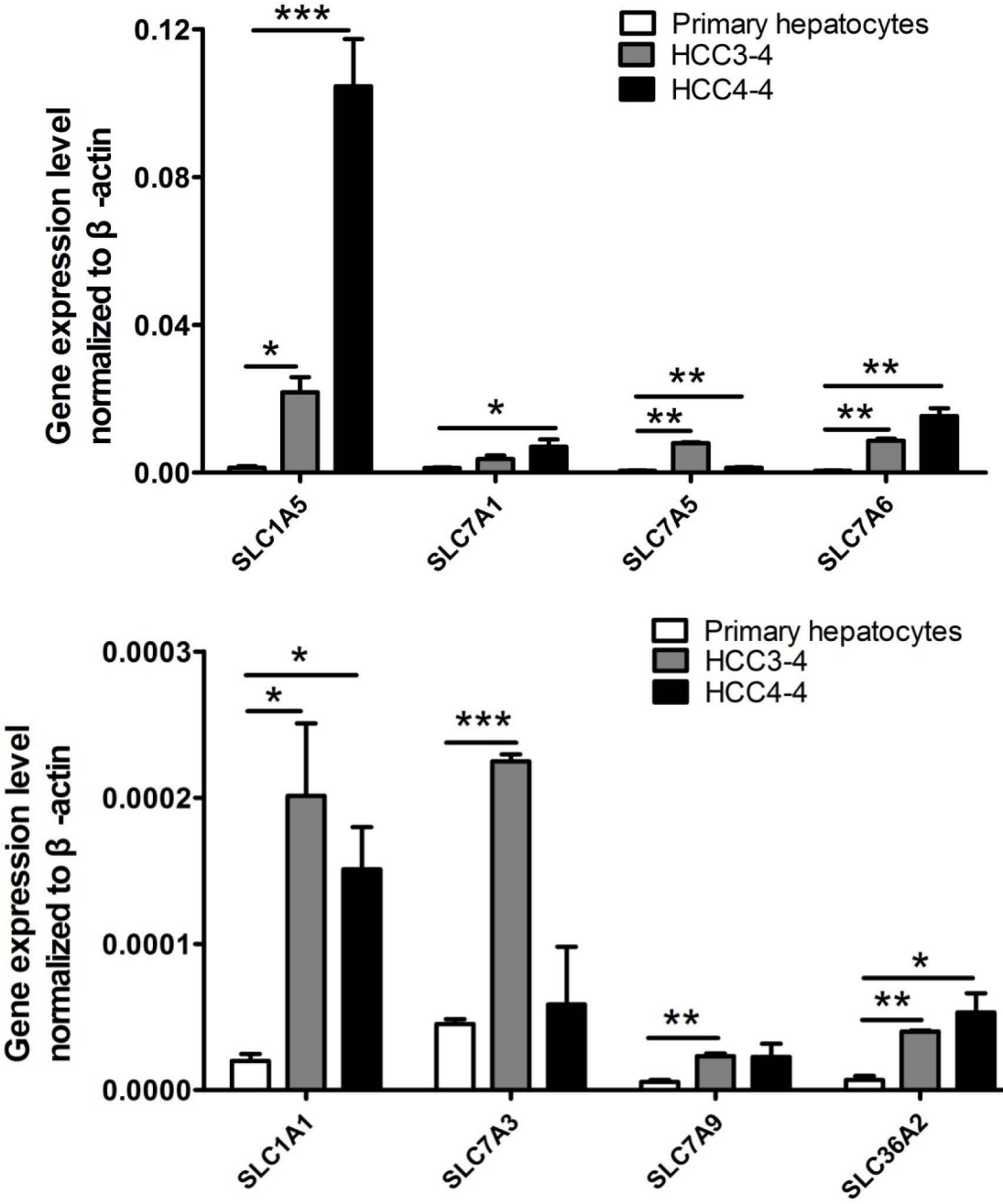


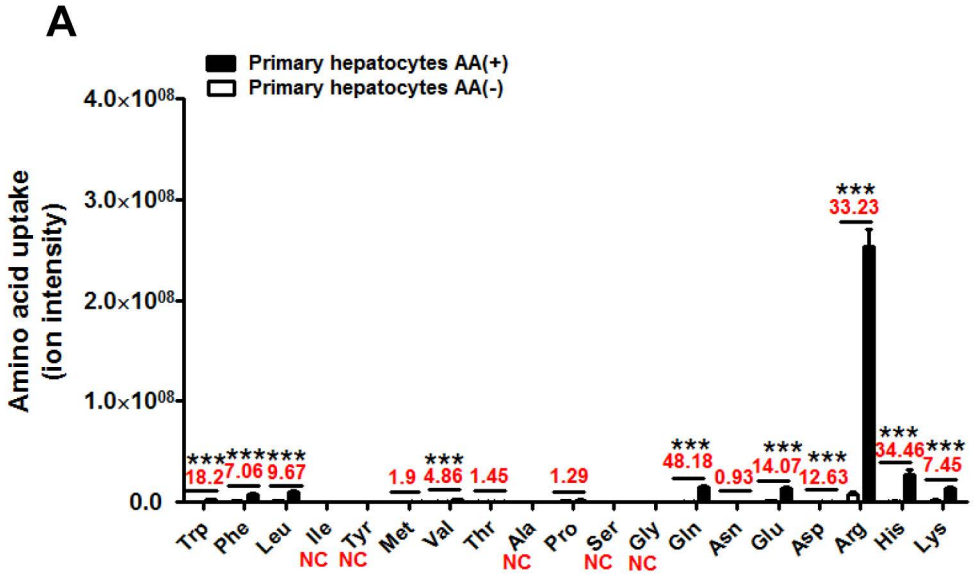
Supplementary Fig. 1. mTORC1 pathway is efficiently inhibited by Rapamycin in HCC3-4 and HCC4-4 c-Myc mouse liver tumor cell lines. (A) IC50 of Rapamycin in HCC3-4 and HCC4-4. **(B)** Western blotting showing the downregulation of mTORC1 in HCC3-4 and HCC4-4 cell upon Rapamycin treatment.

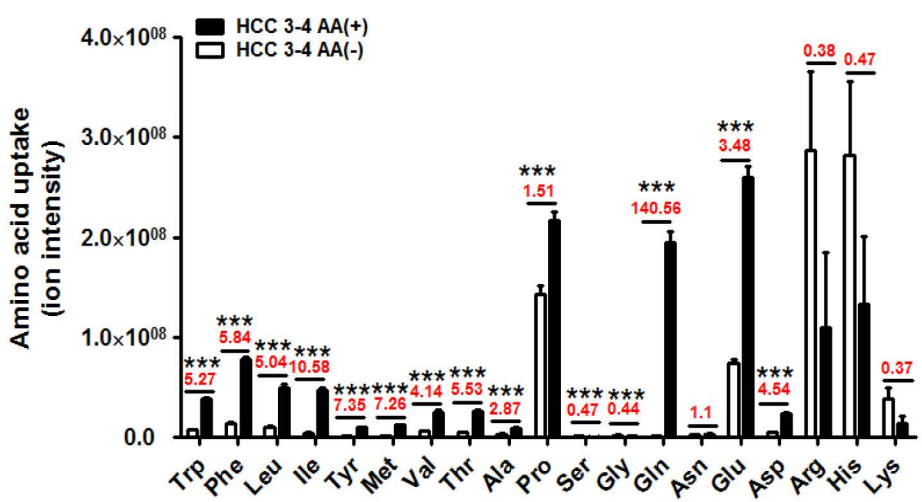


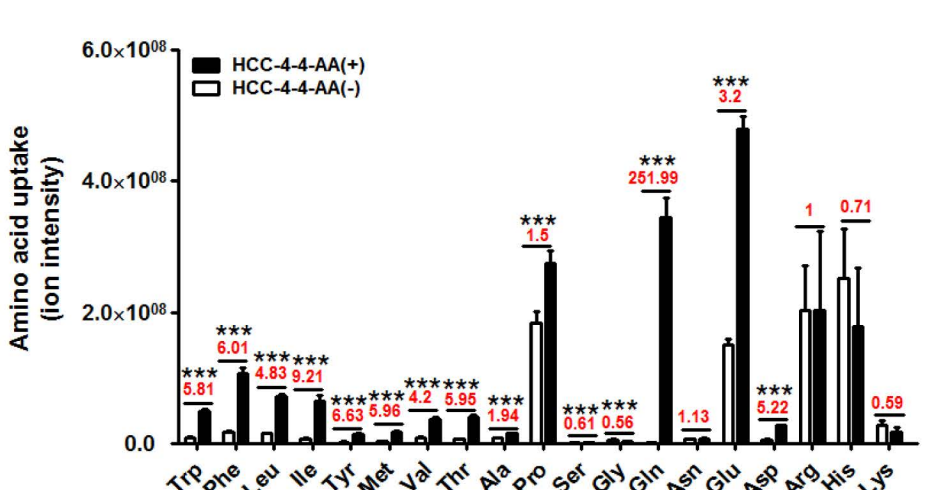
Supplementary Fig. 2. c-Myc driven hepatocarcinogenesis is independent of *AKT2*. **(A)** Study design. **(B)** Liver weight of *AKT2*$^{+/+}$ and *AKT2* $^{-/-}$ mice. Data are presented as mean ± SEM. ns, not significant. **(C)** Gross and HE images from *AKT2*$^{+/+}$ and *AKT2* $^{-/-}$ mouse livers. Magnifications:100× (HE). **(D)** Representative Western blotting from WT, *AKT2*$^{+/+}$ and *AKT2* $^{-/-}$ liver tissues.



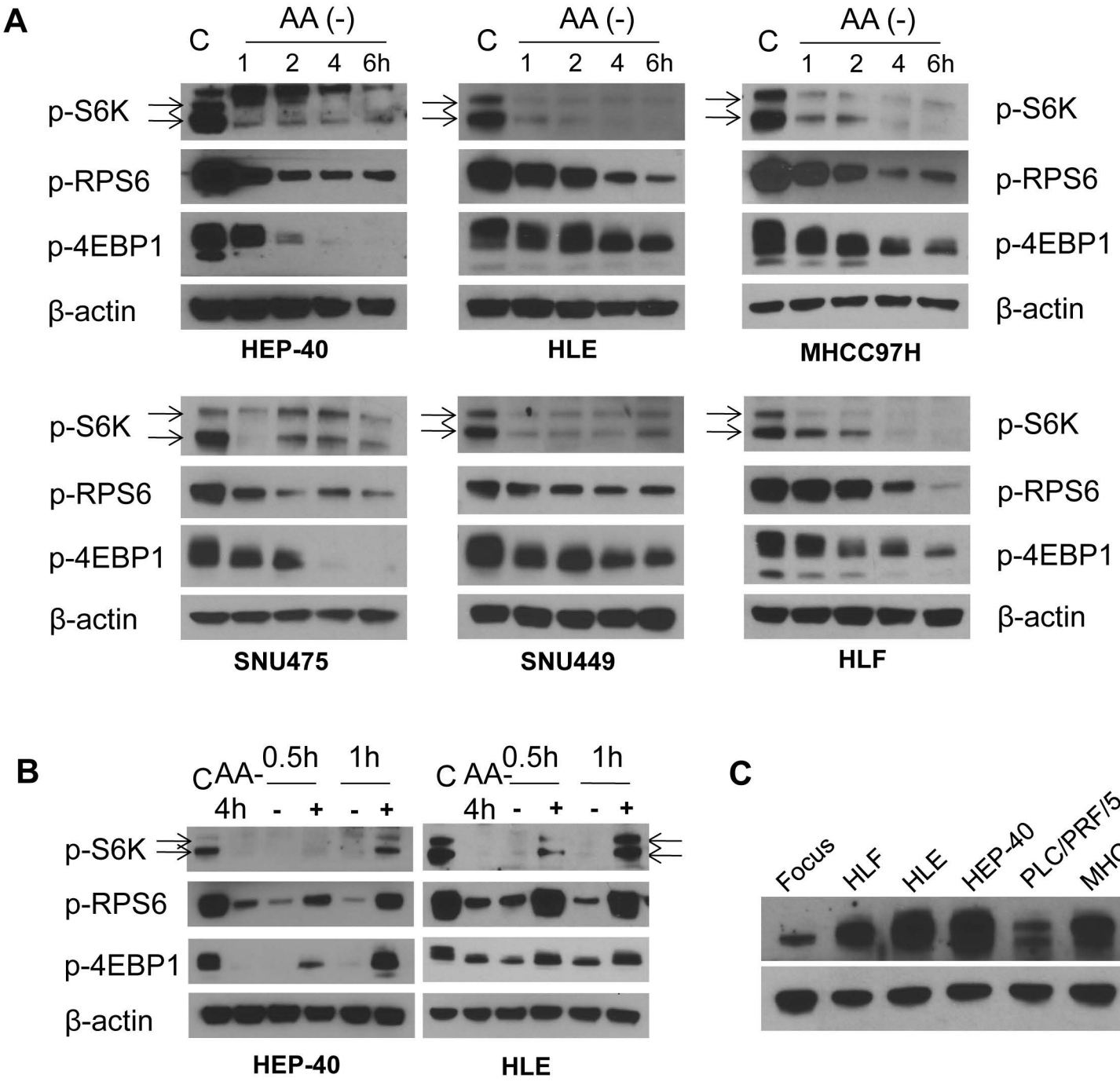
Supplementary Fig. 3. c-Myc driven tumorigenesis is dependent on Amino acids transporters activity. The mRNA levels of SLC transporters in the expression array determined by qRT-PCR in primary hepatocyte and c-Myc tumors (HCC3-4/HCC4-4 cells). Data are presented as mean ± SEM. *P<0.05; **P<0.01; ***P<0.001.



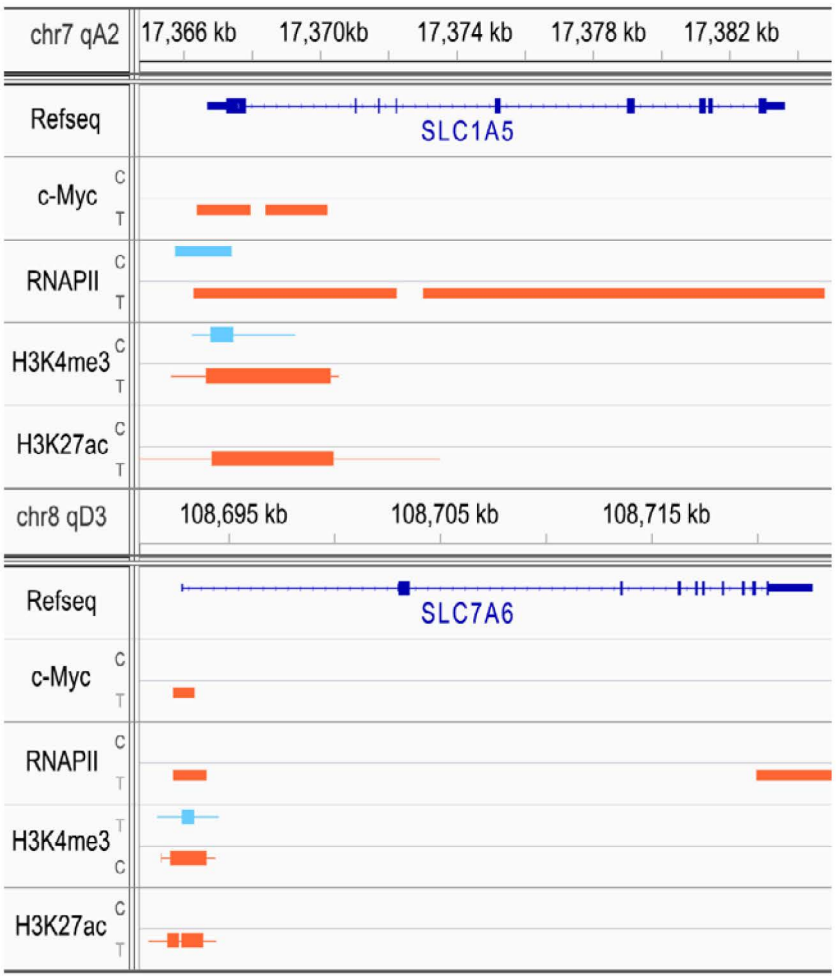


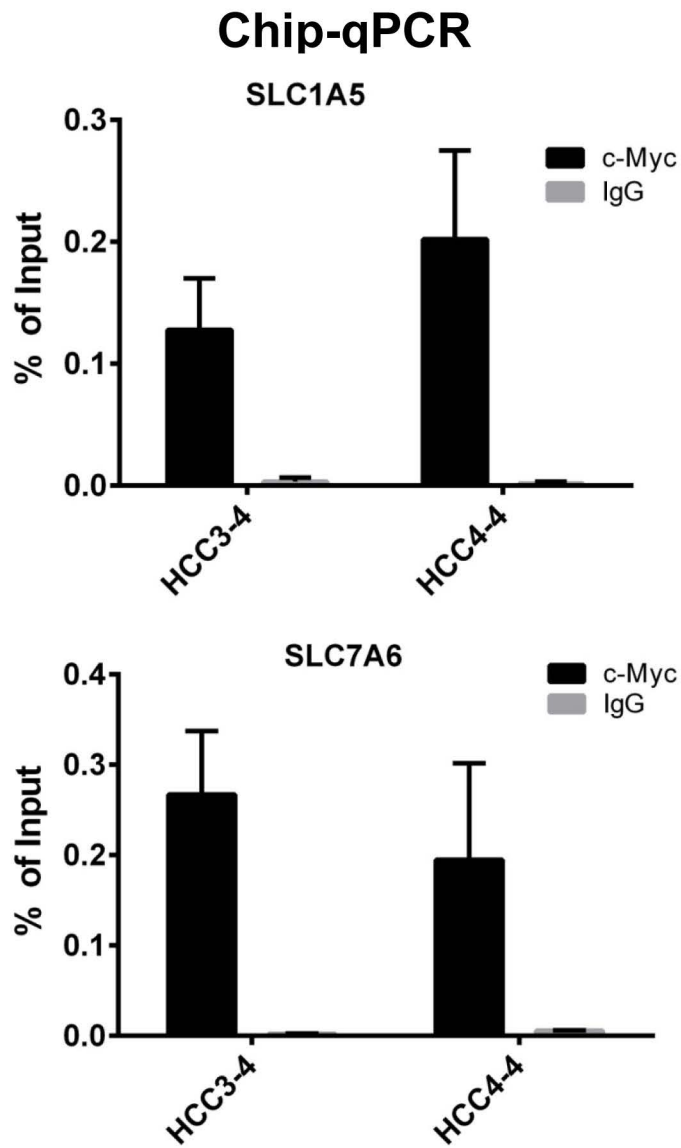


Supplementary Fig. 4 . c-Myc driven tumorigenesis is dependent on glutamine uptake. (A) Amino acid uptake was measured using LC-MS in the absence or in the presence amino acid and L-Glutamine in primary hepatocytes. **(B)&(C)** HCC3-4 and HCC4-4 cells were incubated with or without Amino acid and L-Glutamine for 0.5h as previously described to assess amino acid uptake. Data are presented as mean ± SEM. ***P<0.001

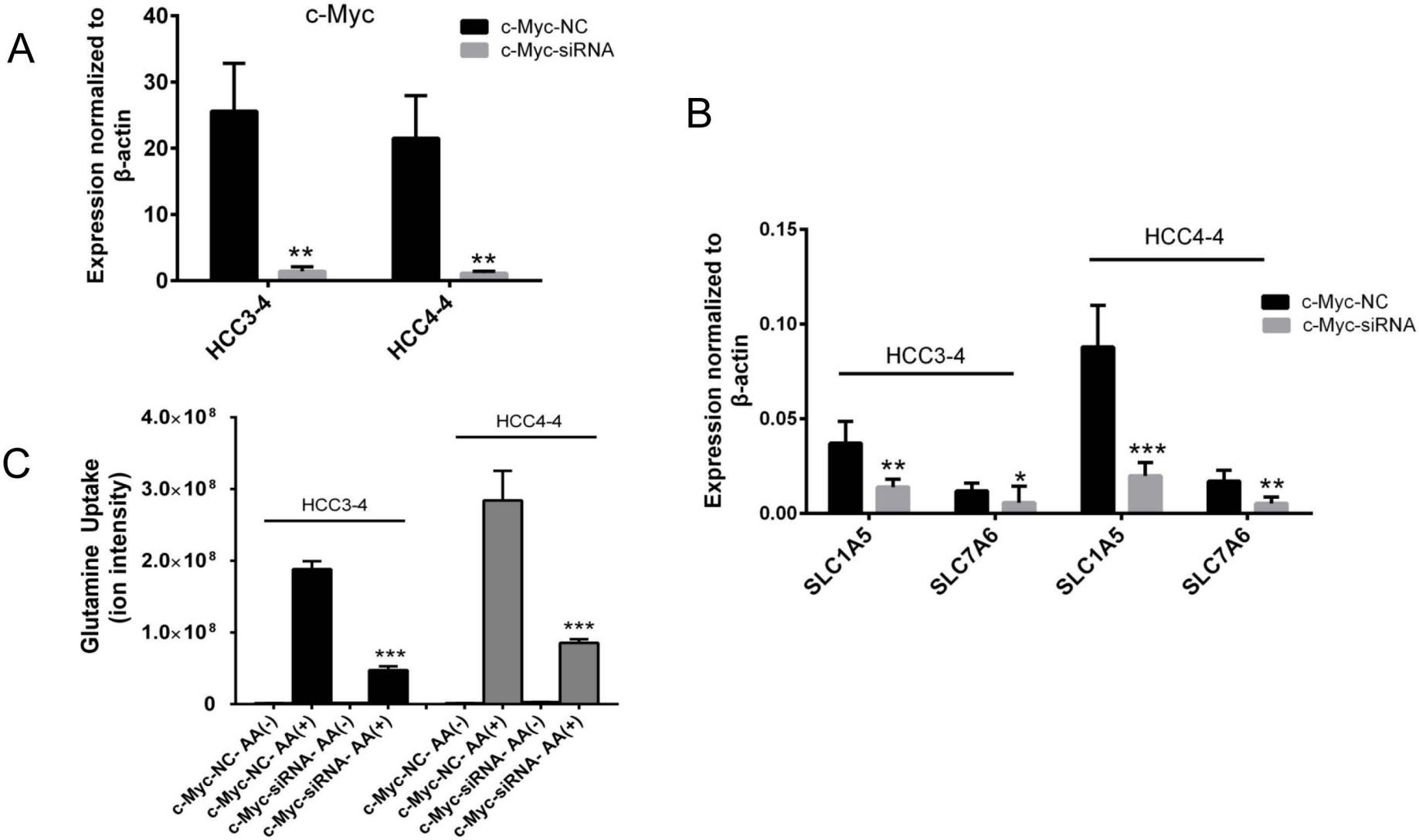


Supplementary Fig. 5. Amino acids are the major factors promoting mTORC1 activation in human HCC cell lines with high c-Myc expression. (A) Western blotting of mTORC1 pathway genes in Hep40, HLE, MHCC97H, SNU475, SNU449 and HLF cells after amino acid deprivation in various time points (1h, 2h, 4h and 6h). **(B)** Amino acid were added back after deprivation of 4 hours, cells were collected in 0.5h and 1h for protein analysis. **(C)** Western blotting showing c-Myc expression in 9 human HCC cell lines.

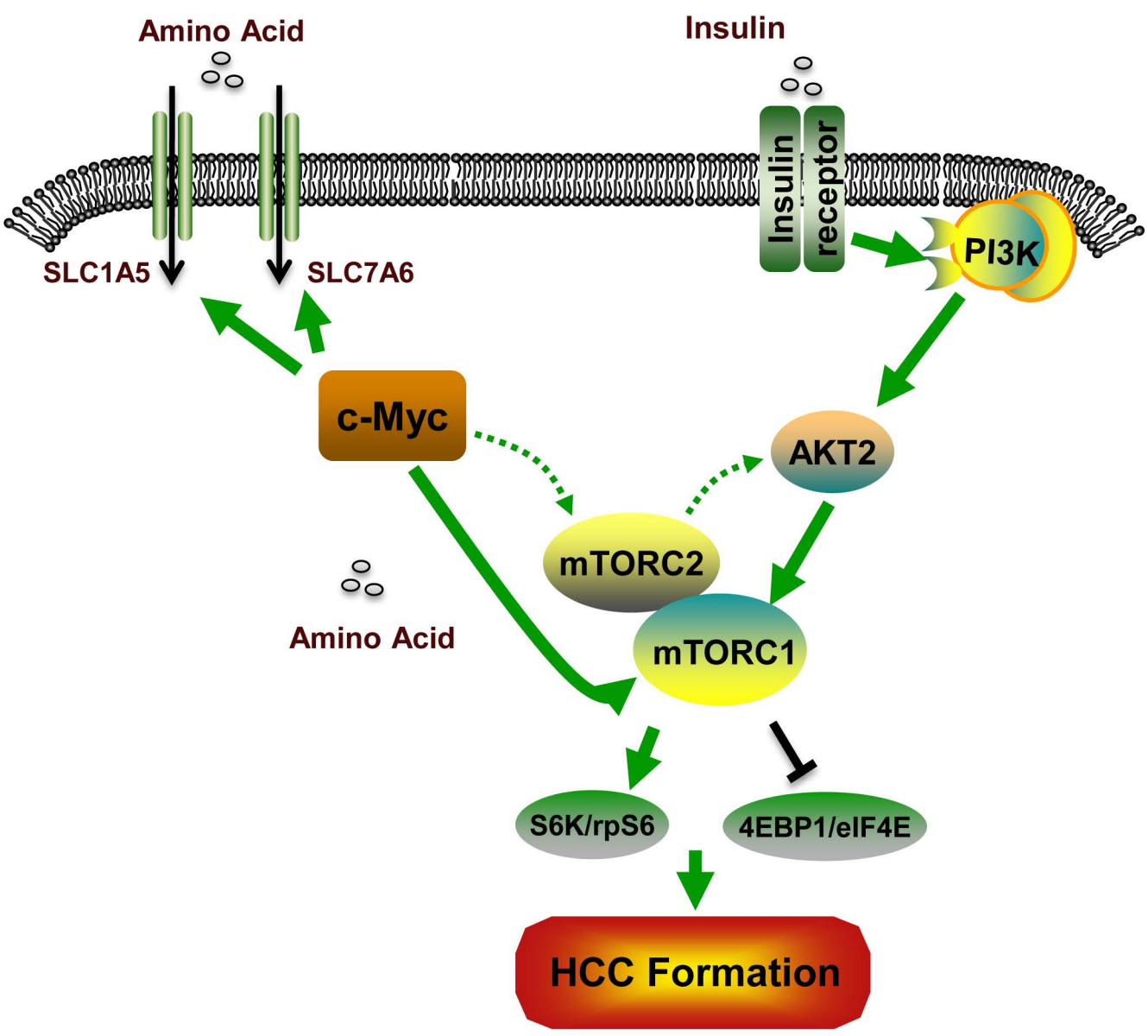




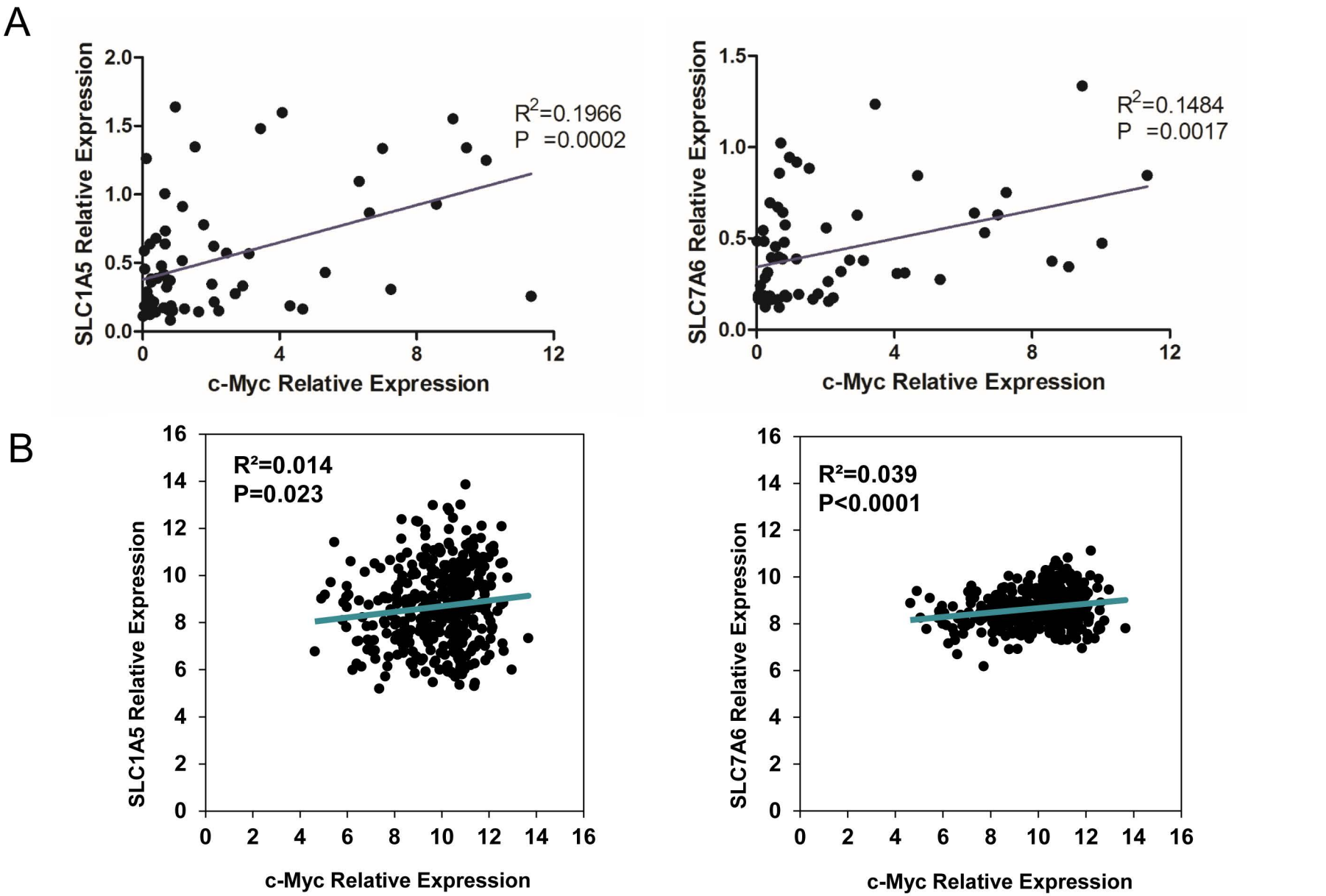
Supplementary Fig. 6. (A) ChIP-Seq data show that c-Myc interacts with SLC1A5 and SLC7A6 promoter sequence. Binding regions of c-Myc, RNA polymerase II (RNAPII), as well as active histone markers H3K4me3 and H3K27ac in C (control liver, light blue) and T (c-Myc liver tumor, orange) are shown. **(B)** Direct binding of c-Myc to the promoters of SLC1A5 and SLC7A6 examined by ChIP-qPCR in HCC3-4 and HCC4-4 cells.



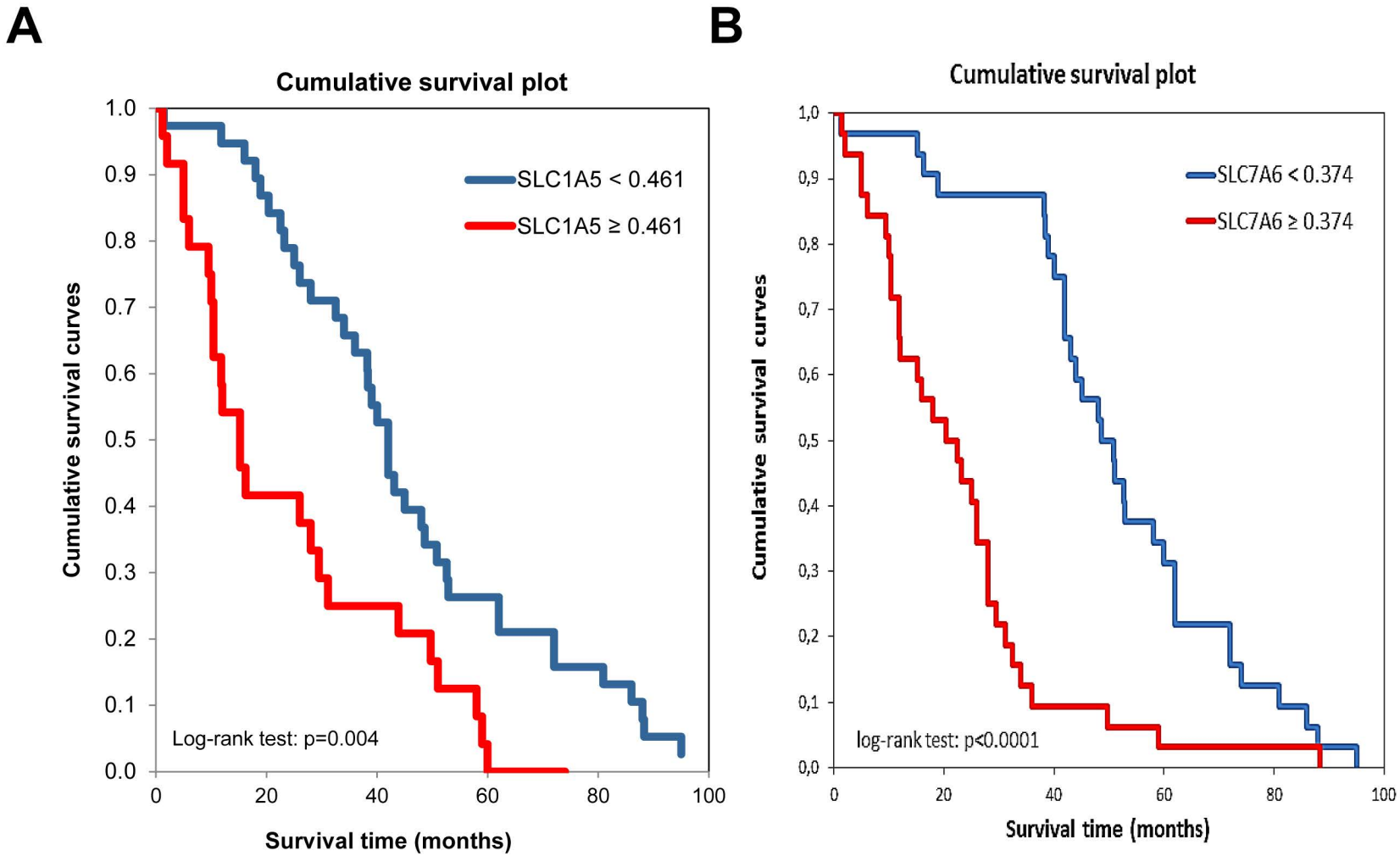
Supplementary Fig. 7. Silencing of c-Myc reduces the expression of SLC1A5 and SLC7A6 as well as the uptake of glutamine in HCC3-4 and HCC4-4 cells. (A) c-Myc expression levels with or without human c-Myc specific siRNA; (**B)** Expression levels of SLC1A5 and SLC7A6 before and after c-Myc knockdown; **(C)** Uptake of glutamine before and after c-Myc knockdown. *P<0.05; **P<0.01; ***P<0.001.



Supplementary Fig. 8. Pathway illustration.



Supplementary Figure. 9. **(A)** mRNA levels of SLC1A5 and SLC7A6 correlate with those of c-Myc in the collection of human HCC samples analyzed by qRT-PCR. **(B)** Correlation between c-Myc and SLC1A5/SLC7A6 mRNA expression using TCGA human HCC data set.



Supplementary Figure. 10. Kaplan-Meier survival analysis of HCC patients with high and low mRNA expression of SLC1A5. (A) and SLC7A6 **(B)**. Of note, high levels of the two genes are associated with shorter survival of HCC patients.

Supplementary Table 1. Clinicopathological features of HCC Patients

Variables	Features	
	HCCB[a]	HCCP[b]
No. of patients	32	32
Male	24	23
Female	8	9
Age (Mean ± SD)	66.7 ± 9.9	68.2 ± 10.2
Etiology		
HBV	15	14
HCV	11	13
Ethanol	3	3
Wilson's disease	2	1
Hemochromatosis	1	1
Cirrhosis		
+	24	24
-	8	8
Tumor size		
> 5 cm	22	26
< 5 cm	10	6
Edmondson and Steiner grade		
II	9	7
III	15	14
IV	8	11
Alpha-fetoprotein secretion		
> 300 ng/ml of serum	21	23
< 300 ng/ml of serum	11	9
Survival after partial liver resection		

(months)	56.9 ± 16.7	17.2 ± 9.6
Means ± SD		

[a]HCCB, HCC with better outcome/longer survival (survival >3 years following partial liver resection)

[b]HCCP, HCC with poorer outcome/shorter survival (survival <3 years following partial liver resection)

Supplementary Table 2. Multivariate Cox regression analysis of factors contributing to overall survival of HCC patients

Covariates	**Full model (HR and 95% CI)**	**Stepwise backward elimination (HR and 95% CI)**
Age	1.013 (0.985–1.043)	–
Male sex	0.536 (0.249–1.154)	–
Cirrhosis (y/n)	1.397 (0.648–3.014)	–
Etiology		
HCV	Reference	–
HBV	1.002 (0.459–2.191)	–
Ethanol	0.296 (0.101–0.865)*	–
Wilson's disease	0.293 (0.080–1.080)	–
Hemochromatosis	0.170 (0.027–1.077)	–
Diameter > 3 cm	1.519 (0.659–3.503)	–
AFP > 300 ng/ml	1.034 (0.546–1.960)	–
Grade		
II	Reference	Reference
III	1.389 (0.607–3.179)	1.653 (0.824–3.313)

IV	2.960 (1.328–6.595)*	2.936 (1.365–6.313)**
c-Myc	1.404 (1.207–1.635)**	1.286 (1.118–1.480)**
SLC1A5	3.095 (1.343–7.129)**	3.673 (1.692–7.976)**
SLC7A6	4.726 (2.140–10.439)**	3.473 (1.913–6.304)**

* $p<0.05$; ** $p< 0.01$

SUPPLEMENTARY MATERIALS AND METHODS

Histology and Immunohistochemistry

Mouse liver lesions were fixed in 4% paraformaldehyde overnight at 4°C and embedded in paraffin by two board-certified pathologists (S.R. and F.D.) in accordance with the criteria by Frith et al.[1] For immunohistochemistry, antigen retrieval was performed in 10mM sodium citrate buffer (pH 6.0) by placement in a microwave on high for 10 min, followed by a 20-min cool down at room temperature. After a blocking step with the 5% goat serum and Avidin-Biotin blocking kit (Vector Laboratories, Burlingame, CA), the slides were incubated with primary antibodies overnight at 4°C. Slides were then subjected to 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity and subsequently the biotin conjugated secondary antibody was applied at a 1:500 dilution for 30 min at room temperature. Anti-c-Myc antibody (sc-40) was purchased from Santa Cruz Biotechnology; anti-HA tag (3724), anti-phospho-RPS6 (2211), and anti-phospho-4EBP1 (2855) antibodies were obtained from Cell Signaling Technology Inc (Danvers, MA); and anti-Ki67 antibody (IHC-00375) was purchased from Bethyl Laboratories (Montgomery, TX). These primary antibodies were selected since they have been extensively validated by the manufacturers for immunohistochemistry. The immunoreactivity was visualized with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), using Vector NovaRED™ (Vector Laboratories) as the chromogen. Slides were counterstained with Mayer's hematoxylin.

Western blotting and mTORC1 activity

Mouse livers tissues 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 2mM EDTA] containing the Complete Protease Inhibitor Cocktail (ThermoFisher Scientific, Waltham, MA). Protein concentrations were determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA)

using bovine serum albumin as standard. For Western blotting, aliquots of 40 µg were denatured by boiling in Tris-Glycine SDS Sample Buffer (Bio-Rad), separated by SDS-PAGE, and transferred onto nitrocellulose membranes (Bio-Rad) by electroblotting. Membranes were blocked in in 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and probed with following specific antibodies: AKT (9272, Cell Signaling Technology), Phospho-AKTS473 (3787, Cell Signaling Technology), AKT2 (3063, Cell Signaling Technology), Phospho-mTOR (2971, Cell Signaling Technology), c-Myc (32072, Abcam), Phospho-p70 S6 Kinase (9205, Cell Signaling Technology), Phospho-RPS6 (4858, Cell Signaling Technology), 4E-BP1 (9644, Cell Signaling Technology), Phospho-4E-BP1 (2855, Cell Signaling Technology), eIF4E (2067, Cell Signaling Technology), HA-tag (2367, Cell Signaling Technology), SLC1A5 (D260740, BBI Life Sciences, Amherst, NY),SLC7A6 (sc-136885, Santa Cruz), GPNA (CG5901, Coolaber, a glutamine analogue), Anti-GAPDH (MAB374, EMD Millipore, Billerica, MA) and anti-β-Actin (A5441, Sigma-Aldrich) were used as loading controls. Each primary antibody was followed by incubation with horseradish peroxidase-secondary antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1:5000 for 30 min and proteins were revealed with the Super Signal West Dura (ThermoFisher Scientific, Waltham, MA). The mTORC1 activity was determined on human HCC protein lysates with the K-LISA mTOR activity kit (EMD Millipore) following the manufacturer's protocol.

Quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR)

Validated Gene Expression Assays for human *c-Myc* (Hs00153408_m1), *SLC1A5* (Hs01056542_m1), *SLC7A6* (Hs00187757_m1), and *β- Actin* (ID: 4333762T) genes were purchased from Life Technologies-ThermoFisher Scientific. PCR reactions were performed with 100 ng of cDNA on the whole sample collection and cell lines, using an ABI Prism 7000 Sequence Detection System and TaqMan Universal PCR Master Mix (ThermoFisher Scientific). Cycling conditions were: 10 min of denaturation at 95°C and 40 cycles at 95°C for 15 s and at

60°C for 1 min. Quantitative values were calculated by using the PE Biosystems Analysis software and expressed as N target (NT). $NT = 2^{-\Delta Ct}$, wherein ΔCt value of each sample was calculated by subtracting the average Ct value of the target gene from the average Ct value of the *β- Actin* gene.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) analysis was performed by using bacterial artificial chromosome (BAC) probes, covering the MYC (RP11-440N18) locus at the 8q24.21 genomic region and a reference locus (CEP7) at the 7q11.23 chromosome as control, onto 4-micron sections of formalin-fixed paraffin-embedded tissue from each tumor. The BAC clones were chosen according to their genomic location as defined in the UCSC genome browser (http://genome.ucsc.edu). The BAC clones were obtained from Life Technologies-ThermoFisher Scientific (Waltham, MA, USA). The BAC DNA was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution. Two hundred interphase nuclei were counted from each tumor by using a Olympus BX61 microscope (Olympus Life Science, Center Valley, PA), equipped with a Imaging QICAM controlled by Genikon software (Nikon, Melville, NY). MYC amplification was defined as a MYC/CEP7 ratio of 1.5 or higher.

Microarray analysis and ChIP-Seq data processing

Liver tissues from control FVB/N mice (n=4) and tumor bearing c-Myc mice (n=4) were used to isolate mRNA and preparation of probes for hybridization on the Affymetrix GeneChip® Mouse 1.0 ST arrays following the manufacturer's instruction. Array data were normalized using the Robust Multiarray Average (RMA) approach. Differentially expressed genes（DEGs）between different group were screened by Bioconductor package LIMMA. The associated P values were

adjusted to false discovery rates (FDR) based on the Benjamini and Hochberg method. DEGs were cut-off by |log2 FC| >1 and FDR<0.05. heatmap analysis was carried out using atrix2png online software. The up-regulated genes in the c-Myc tumors, the heatmap codes the color as red; if expression of a gene was down-regulated in the c-Myc tumors, the heatmap codes the color as green. ChIP-seq data was analyzed based on a previous study (GSE76078),2 and the reads were aligned using the BWA aligner using default settings. Peaks were called with a model-based analysis of MACS2 software.

ChIP-qPCR

ChIP was performed as previously described[3], with modifications. Cells were crosslinked in 1% formaldehyde (Thermo, #28908) for 5-10 min at room temperature. After glycine quenching, cells pellets were lysed with lysis buffer, and then subjected to sonication with Diagenode Bioruptor (S220). The sonicated sample was subjected to immunoprecipitation using magnetic beads (Anti-rabbit IgG, already binding antibody) at 4°C overnight (c-Myc CST9402, Normal Rabbit IgG CST2729). DNA was purified using the Axygen Kit (Tewksbury, MA) and quantified by qPCR. For ChIP-qPCR, the primer sequences and the ChIP sequence positions are: SLC7A6F:CGACCGAGCATCCTGGAAGCT; SLC7A6R: AGCTTCCAGGATGCTCGGTCG; The ChIP sequence position: chr8:106168086-106169721. SLC1A5F: TCTGAGTGGCTGGGACTTGGCCA; SLC1A5R: TGGCCAAGTCCCAGCCACTCAGA. The ChIP sequence position: chr7:16780494-16784814.

Cell line experiments

HEP40, HLE, SNU475, MHCC97H, SNU449 and HLF human HCC cell lines were cultured as monolayer cultures in Dulbecco's modified Eagle medium or RPMI 1640 medium supplemented

with 10% fetal bovine serum (FBS). HCC3-4 and HCC4-4 HCC cell lines, isolated from c-Myc mouse liver tumors,[4] were kindly provided by Dr. Felsher of Stanford University and were confirmed to express high levels of c-Myc. For amino acid deprivation experiments, amino acid free DMEM (US Biological, Salem, MA) was used and added to the cells. For amino acid add back experiment, after 1 hour (for HCC3-4 and HCC4-4 cells) and 4 hours (HEP40 and HLE cells) amino acid deprivation, respectively, the complete amino acid solution (ThermoFisher Scientific) was added to the cells. Subsequently, cells were collected at different time points, as indicated in the figures. For Amino acid uptake analysis, after amino acid starvation, cells treated with the same medium with or without amino acid and L-glutamine for 0.5h. Cells were collected into precooled EP tube, and make it dry by evaporating in a decompression evaporator. Ready for LC-MS analysis. All siRNA sequences were designed using the BLOCK-iT™ RNAi Designer and custom synthesized. (GenePharma, Shanghai, China). HCC3-4 and HCC4-4 cell lines were transfected with short interfering RNAs (siRNAs) targeting SLC1A5 and SLC7A6 or control siRNA (negative control) using Lipofectamine 2000 (Life Technologies, Carlsbad, CA). Cells were harvested 48 h for western blot assays after transfection. For chemical inhibitor of glutamine transporter, HCC3-4 and HCC4-4 cells were treated with DMEM media in the presence or absence of 1mM GPNA (the SLC1A5 inhibitor) for 48h. To silence human c-MYC (NM_002467), siRNAs targeting exon 2 (5'-GCTTGTACCTGCAGGATCT-3') and a non-silencing negative control siRNA (C-siRNA) were purchased from GenePharma (Shanghai, China).

References

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