The mTORC2-Akt cascade is crucial for c-Myc to promote hepatocarcinogenesis in mice and men

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Supplementary Materials and Methods

Plasmid and reagents

The plasmids including pT3EF1a-c-Myc, pT3-EF1α, pCMV-Cre, and pCMV/sleeping beauty transposase (SB) involved in this study have been described in our previous publications.[1, 2] To stabilize the effect of c-Myc, pT3EF1a-MCL1 was cloned from pCDNA3-HA-Mcl1 (Addgene, 25714) using a standard molecular cloning approach and was injected together with pT3EF1a-c-Myc in relevant transgenic mice. Plasmids were purified using the Endotoxin free Maxi prep kit (Sigma-Aldrich, St.Louis, MO). PLKO.1 puro plasmid (8453), human Rictor shRNA (1853), and mouse Rictor shRNA (21341) were obtained from Addgene. Subsequently, the plasmids were packaged into lentivirus. Short interfering RNAs (siRNAs) targeting Akt1 (162427) and Akt2 (162213) were purchased from Thermo Fisher Scientific. Short interfering RNA targeting mouse c-Myc (SR414450) was obtained from Origene Technologies (Rockville, MD). A scramble small interfering RNA (4390846, Life Technologies, Carlsbad, CA) was used as negative control.

MLN0128 (I-3344) and Rapamycin (R-5000) were purchased from LC Laboratories (Woburn, MA). Wortmannin (S2758) was purchased from Adooq BioScience (Irvine, CA). A-674563 (S2670) and CCT128930 (S2635) were obtained from Selleck Chemicals (Houston, TX).

MLN0128 was first dissolved in NMP (1-methyl-2-pyrrolidinone) (328634, Sigma-Aldrich) to make a stock solution of 20mg/ml and the aliquots were stored at -80℃. It was then 1:100 diluted into 15% PVP/H2O (polyvinylpyrrolidone K 30, Diluted in H2O at a 15.8:84.2 W/V ratio) (81420 Sigma-Aldrich). The diluted solution was stored at 4℃ in dark before administration.

Rapamycin was dissolved 100% ethanol to make 50 mg/ml Rapamycin stock and keep them at -80℃, and was mixed with 10% PEG400 and 10% Tween 80 to make 1mg/ml working solution and was stored at 4℃ in dark before administration.

The BrdU Cell Proliferation Assay Kit (6813) was purchased from Cell Signaling Technology (Danvers, MA) and Cell Death Detection Elisa Kit (11544675001) was obtained from Roche Molecular Diagnostics (Indianapolis, IN). Cell proliferation and apoptosis analyses were conducted following the manufacturers' instructions.

Hydrodynamic tail vein injection and mouse treatment

Sleeping beauty–mediated hydrodynamic injection was performed as described.[3] The plasmid mixture compounds, which induced c-myc HCC, are depicted in Supplement Table1.

MLN0128 (1mg/kg/day) was orally administered via gavage. Rapamycin was intraperitoneally injected in a dose of 6 mg/kg/day, 6 days a week for 3 weeks.

All the animal experiments were performed according to protocols approved by the committee for animal research at the University of California, San Francisco (San Francisco, CA).

Histology and immunohistochemistry

Liver specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were cut at 5 μm in thickness. For immunohistochemistry, antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) or EDTA buffer (pH 9.0) by placement in a microwave heated for 10 min, followed by 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. The following antibodies were used: the anti-Ki67 (RM-9106), anti-c-Myc (ab32072, Abcam), anti- Cleaved Caspase-3 (Asp175) (9661, Cell Signaling Technology) antibody, anti-p-AKT1 (ab81283, Abcam), and anti-p-AKT2 (ab38513, Abcam). Slides were then subjected to 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity and subsequently the biotin conjugated secondary antibody

(B2770, Thermo Fisher Scientific) was applied at a 1:500 dilution for 30 min at room temperature. Finally, the signal was visualized using the Vectastain ABC Elite Kit (Vector Laboratories In, Burlingame, CA) and developed with 3,3′-diaminobenzidine. Sections were counterstained with hematoxylin (Sigma-Aldrich). Negative controls were performed with the same procedure, and PBS was incubated as a substitute of the primary antibodies. In human specimens, immunoreactivity for p-AKT1 and p-AKT2 was estimated semi-quantitatively: upregulation of p-AKT1 and/or p-AKT2 was defined when immunolabeling for these proteins was stronger in HCC when compared to corresponding surrounding non-neoplastic livers. Nuclear accumulation of the c-Myc protein was instead used to establish c-Myc activation.

Protein extraction and Western blotting

Mouse liver tissue and cells were homogenized in Mammalian Protein extraction reagent supplemented with the Complete Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Indianapolis, IN). The lysate was clarified by centrifugation for 10 minutes at 4°C, and stored at -80°C until assayed. Concentrations were detected with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) using BSA (bovine serum albumin) as standard. Aliquots of 25 µg were denatured by boiling in Tris-Glycine SDS Sample Buffer (Invitrogen), separated by SDS-PAGE, and transferred onto nitrocellulose membranes (Invitrogen). Membranes were blocked in 5% non-fat dry milk in TBST (Tris-buffered saline containing 0.1% Tween 20) for 1 hour and probed with the following specific antibodies: Rictor (9476, Cell Signaling Technology), t-AKT (9272, Cell Signaling Technology), Phospho-AKT(Ser473) (3787, Cell Signaling Technology), Phospho-AKT(Thr308) (13038, Cell Signaling Technology), Phospho-AKT1 (Ser473) (9018, Cell Signaling Technology), Phospho-AKT2 (Ser474) (8599, Cell Signaling Technology), AKT1 (75692, Cell Signaling Technology), AKT2 (3063, Cell Signaling Technology), Phospho-FoxO1 (Ser256) (84192, Cell Signaling Technology), SGK3 (8573, Cell Signaling Technology), Phospho-mTOR (Ser2448) (2971, Cell Signaling Technology), mTOR (2983, Cell Signaling Technology),

Phospho-S6 Ribosomal Protein (Ser235/236) (4858, Cell Signaling Technology), S6 Ribosomal Protein (2217, Cell Signaling Technology), Phospho-4E-BP1 (Thr37/46) (2855, Cell Signaling Technology), Phospho-4E-BP1 (Ser65) (9451, Cell Signaling Technology), 4E-BP1 (9644, Cell Signaling Technology), Phospho-PRAS40 (Thr246) (13175, Cell Signaling Technology), PCNA (2586, Cell Signaling Technology), Cleaved Caspase-3 (Asp175) (9661, Cell Signaling Technology), Anti-Cyclin D1 antibody (ab16663, Abcam), and c-Myc (ab32072, Abcam). 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 diluted 1:5000 for 1 hour and then revealed the signal with the Super Signal West Pico Chemiluminescent Substrate (Pierce Chemical Co., New York, NY).

RNA extraction and quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR)

Total mRNA from mouse liver tissues as well as human and mouse cell lines was extracted by using the Quick RNA Miniprep kit (Zymo Research, Irvine, CA, USA). For assessment of the levels of human and mouse *c-MYC*, *AKT1*, and *AKT2* genes, validated Gene Expression Assays for human *c-MYC* (ID # Hs00153408_m1), *AKT1* (ID # Hs00178289_m1), *AKT2* (ID # Hs01086099_m1), and *β-Actin* (ID # 4333762T) genes as well as for mouse *c-Myc* (ID # Mm00487804_m1), *Akt1* (ID # Mm01331626_m1), *Akt2* (ID # Mm02026778_g1), and *β-Actin* (ID # Mm00607939_s1) genes were purchased from Applied Biosystems (Foster City, CA, USA). PCR reactions were performed with 100 ng of cDNA of the collected samples or cell lines, using an ABI Prism 7000 Sequence Detection System with TaqMan Universal PCR Master Mix (Applied Biosystems). Cycling conditions were: denaturation at 95°C for 10 min, 40 cycles at 95°C for 15 s, and then extension 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.

Cell culture

HCC3-4 and HCC4-4 HCC cell lines, isolated from c-Myc mouse liver tumors, were kindly provided by Dr. Felsher of Stanford University and were confirmed to express high levels of c-Myc. HLE, HLF, Huh7, Hep3B, SK/Hep1, PLC5, MHCC97L, SNU449 and SNU182 human HCC cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were maintained as monolayer cultures in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) at 37°C in a 5% CO2 atmosphere.

Colony forming assay

When cells reached about 30%-40% confluency in wells, pLKO.1 or shRictor lentivirus was added into culture medium. 48-72 hours later, cells were trypsinized and cultured in 100 × 20 mm culture dishes in culture medium containing 2μg/ml puromycin. Cells transfected with corresponding lentivirus were plated in 6-well culture plates at a density of 1×10^3 cells/well in triplicate. 2 weeks later, cells were fixed for 5 min with 10 % formalin and stained for 30 min with 0.05 % crystal violet dye, washed and analyzed.

Cell transfection

HCC3-4 and HCC4-4 cells were transfected with siRNAs targeting Akt1 or Akt2 or both or c-Myc or control siRNA (negative control) using Lipofectamine™ RNAiMAX Transfection Reagent (13778150, Thermo Fisher Scientific) according to the manufacturer's instructions, respectively. Transient transfection experiments with untagged c-Myc (ID # SC112715) cDNA in pCMV6-XL5 plasmid (OriGene Technologies, Rockville, MD, USA) were conducted in the Huh7

human HCC cell line using the Lipofectamine 2000 Reagent (Life Technologies) following the manufacturer's protocol.

Cell proliferation and apoptosis detection

Treated cells were stained with crystal violet. After washed and dried, stained cells were treated with lysate solution and shaken gently on a rocking shaker for 20-30 minutes. Diluted lysate solutions were added into 96-well plates and the OD was measured with BioTek ELx808 Absorbance Microplate Reader.

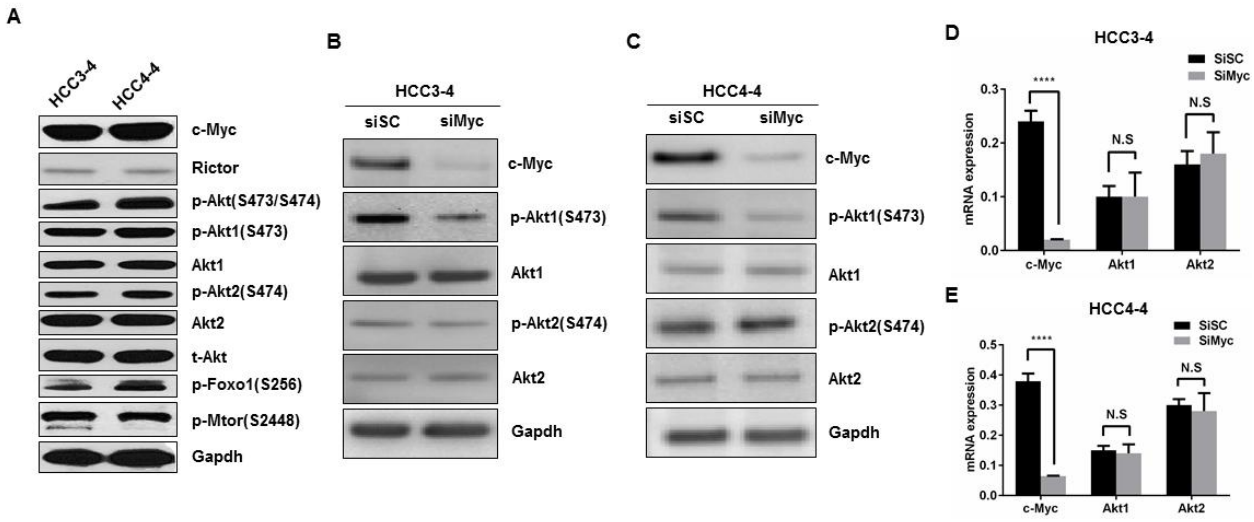
BrdU Cell Proliferation Assay was performed to detect cell proliferation using a second method. Cell apoptosis was detected via the Cell Death Detection Elisa Kit. The OD value was measured with the BioTek ELx808 Absorbance Microplate Reader.

Inhibition of Akt1 or Akt2 by selective inhibitors

HCC3-4, HCC4-4 as well as c-Myc high or low expressing human HCC cells were seeded in 6-well plate grown for 12h and treated with 5μM A-674563, CCT128930, or both for 24-48 hours. Dimethyl sulfoxide (DMSO) was diluted and used as control. Subsequently, cell proliferation and apoptosis were assessed.

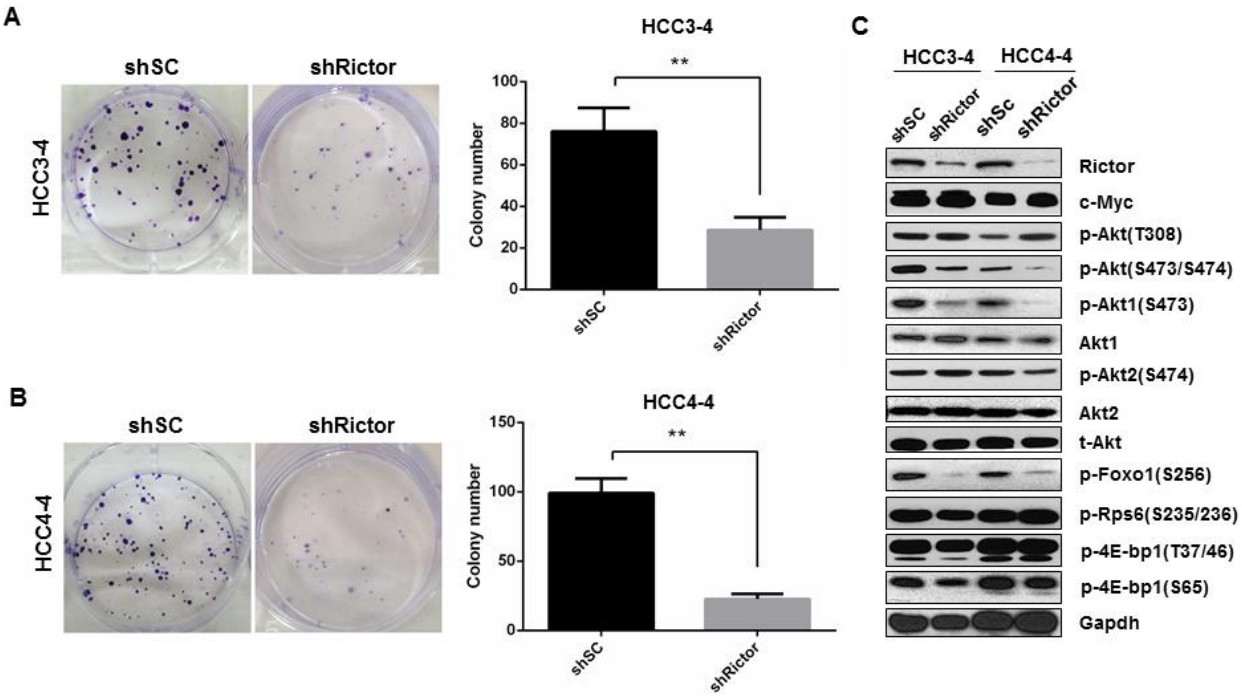
PI3K inhibition

HCC3-4 and HCC4-4 cells were seeded in 6-well plate grown for 12h and treated with 5μM wortmannin in triplicate for 48 hours. DMSO was diluted and used as control. Subsequently, cells were stained with crystal violet. The OD value was measured with the BioTek ELx808 Absorbance Microplate Reader. Cells were harvested for Western blotting after 48 hours-treatment.



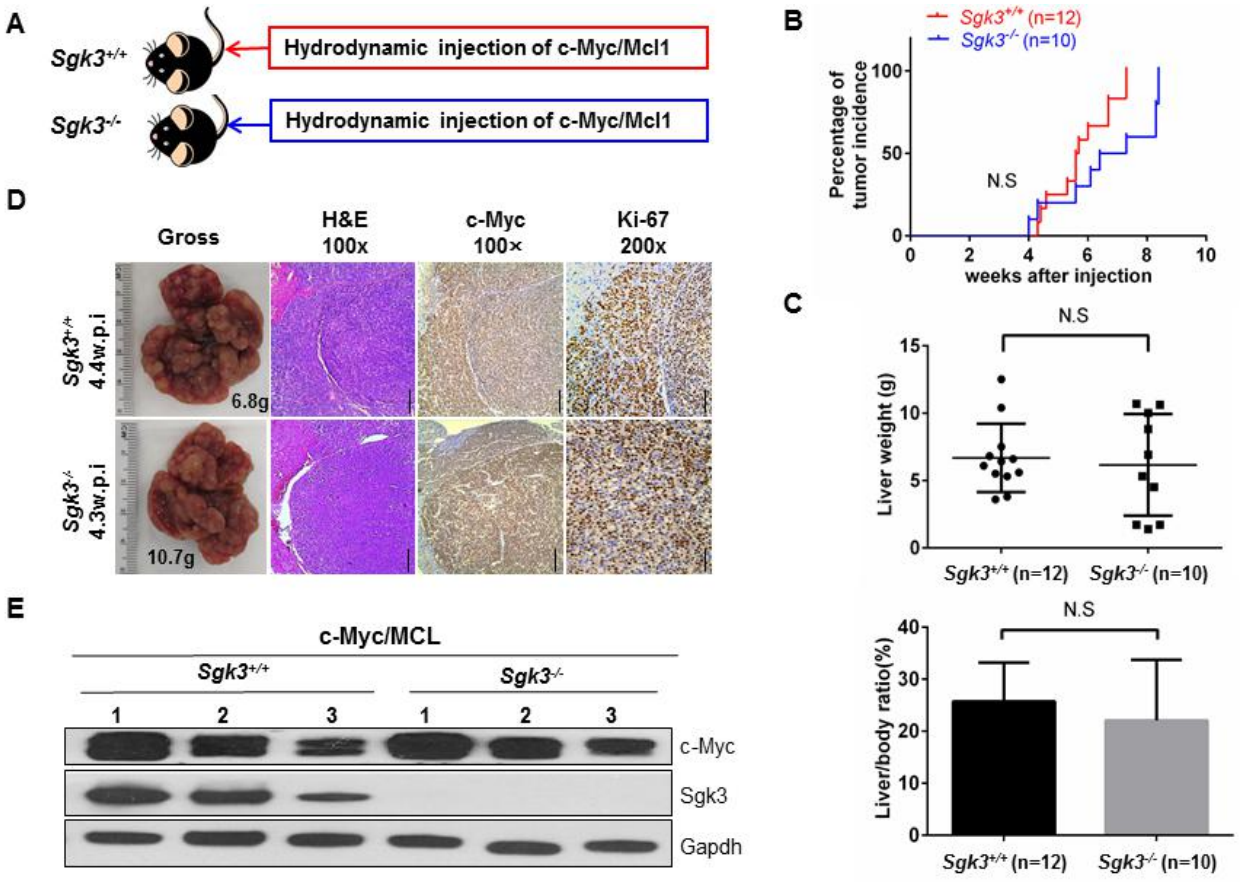
Supplementary Figure 1. Modulation of c-Myc affects the levels of activated/phosphorylated p-Akt1, but not those of p-Akt2, in mouse HCC3-4 and HCC4-4 cell lines, as assessed by Western blotting and quantitative real-time RT-PCR.

(A) Representative Western blotting from HCC3-4 and HCC4-4 HCC cell lines showing upregulated levels of c-Myc. (B) Western blotting from the HCC3-4 HCC cell line transfected with control and c-Myc siRNA. (C) Western blotting from the HCC4-4 cell line transfected with control and c-Myc siRNA. (D) Real-time quantitative RT-PCR analysis showing lack of changes in the levels of *Akt1* and *Akt2* mRNA in HCC3-4 cells transfected with control and c-Myc siRNA. (E) Real-time quantitative RT-PCR analysis showing lack of changes in the levels of *Akt1* and *Akt2* mRNA in HCC4-4 cells transfected with control and c-Myc siRNA. ****P<0.0001. Abbreviations: siSC, control (scramble) siRNA, siMyc, c-Myc siRNA; N.S., not significant.



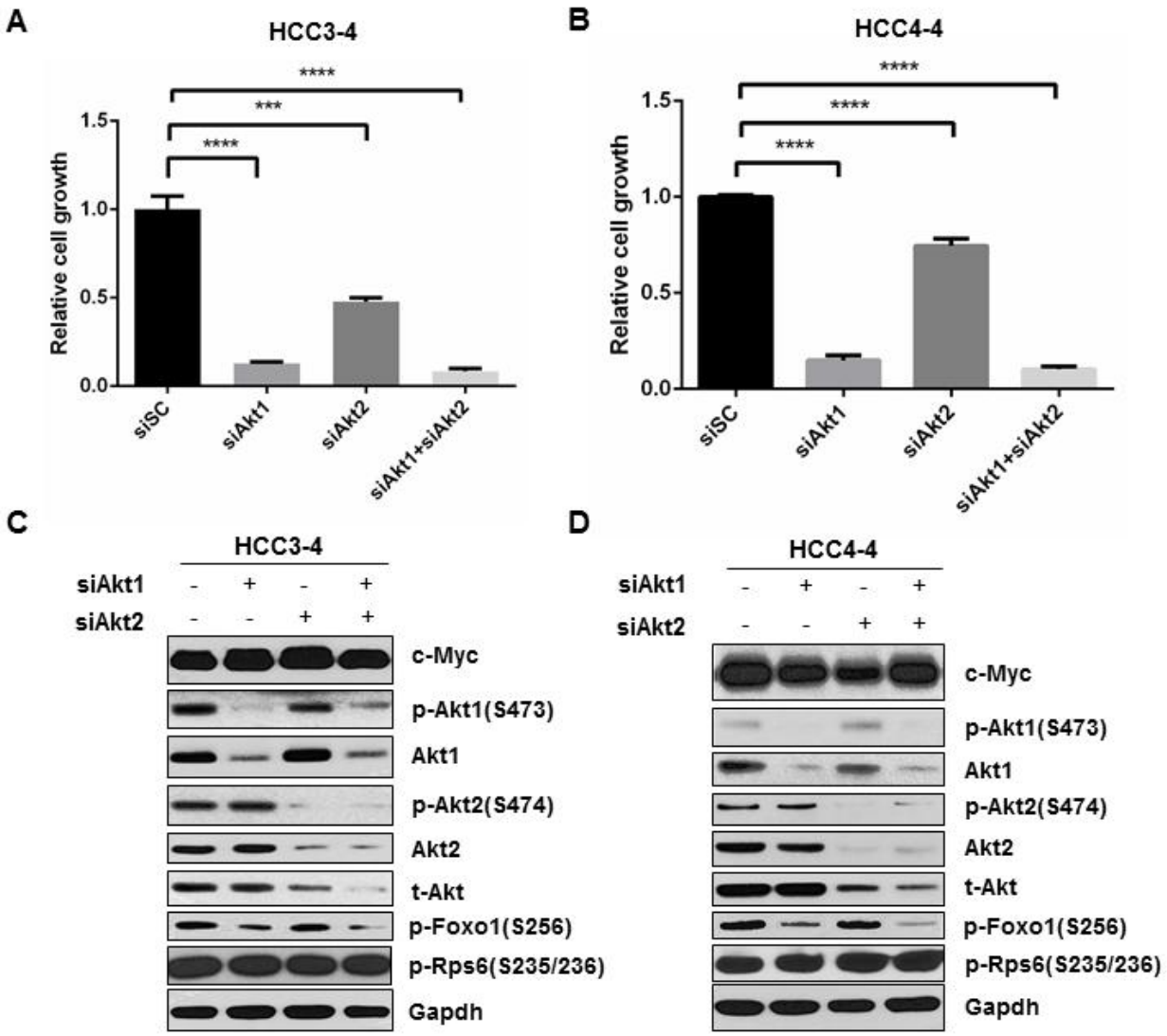
Supplementary Figure 2. Inhibition of Rictor expression suppresses the clonogenic capacity of HCC3-4 and HCC4-4 HCC cell lines.

(A) (B) HCC3-4 and HCC4-4 c-Myc induced HCC cell lines were transfected via lentivirus with either pLKO.1 or shRictor. Colony forming assay was performed in triplicate. Representative photos are shown. The colonies were counted, and data are presented as mean ± standard error of the mean. ***P*<0.01. (C) Western blotting was performed in HCC3-4 and HCC4-4 cell lines transfected via lentivirus with either pLKO.1 or shRictor.



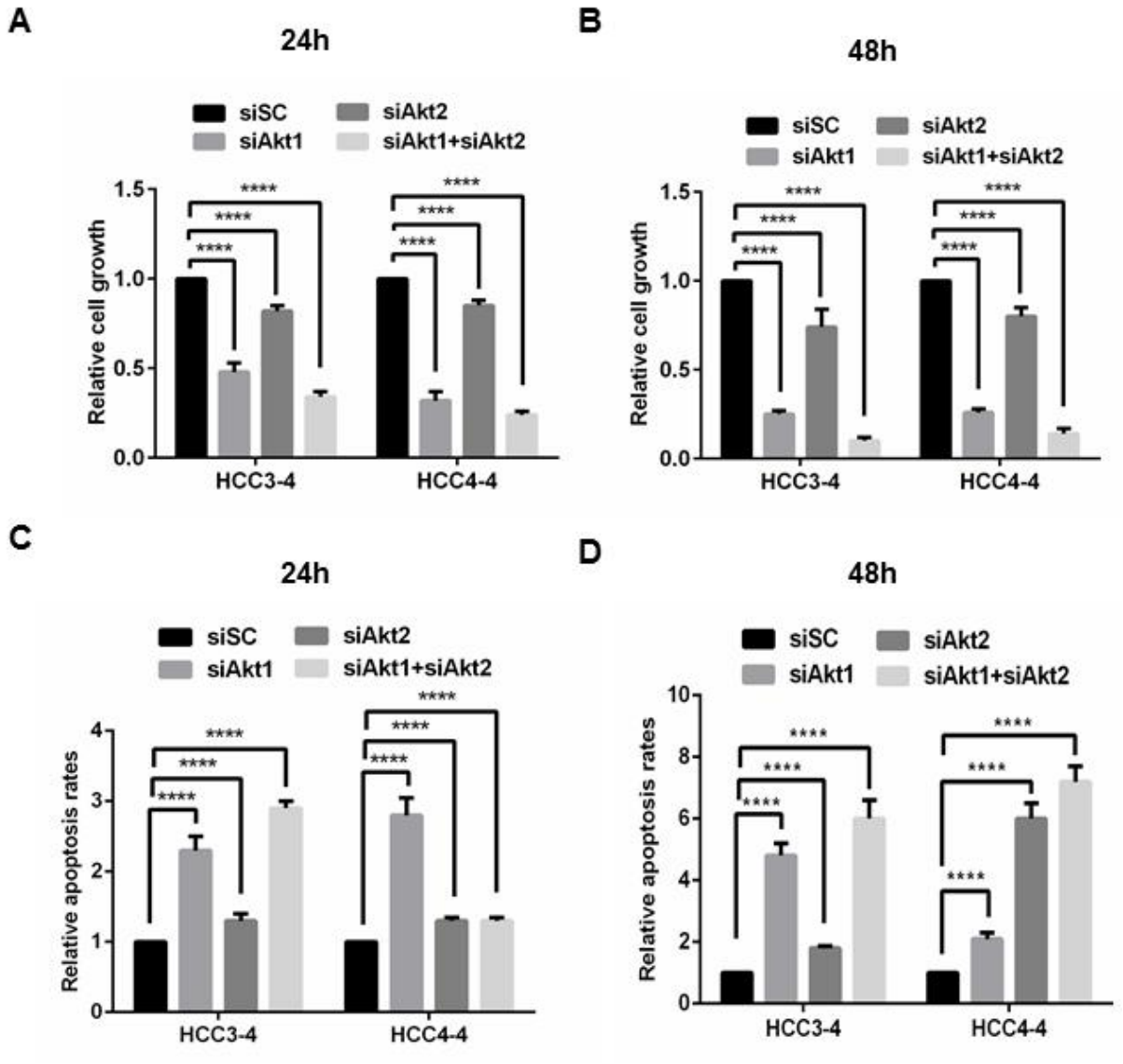
Supplementary Figure 3. Sgk3 status does not influence c-Myc dependent hepatocarcinogenesis.

(A) Study design. (B) Tumor incidence curve. (C) Liver weight and liver/body ratio of *Sgk3*$^{-/-}$ and *Sgk3*$^{+/+}$ mice injected with the c-Myc/MCL1 construct, respectively. (D) Gross images of livers, hematoxylin and eosin, c-Myc, and Ki-67 staining in *Sgk3*$^{-/-}$ and *Sgk3*$^{+/+}$ mice injected with c-Myc/MCL1 construct at specific weeks post injection, respectively. Magnifications: ×100 (hematoxylin and eosin, c-Myc), scale bar=200μm; ×200 (Ki-67), scale bar=100μm. (E) Representative Western blotting image from *Sgk3*$^{-/-}$ and *Sgk3*$^{+/+}$ mouse liver tissues injected with the c-Myc/MCL1 construct. Abbreviations: N.S, no significance; HE, hematoxylin and eosin; w.p.i., weeks post injection.



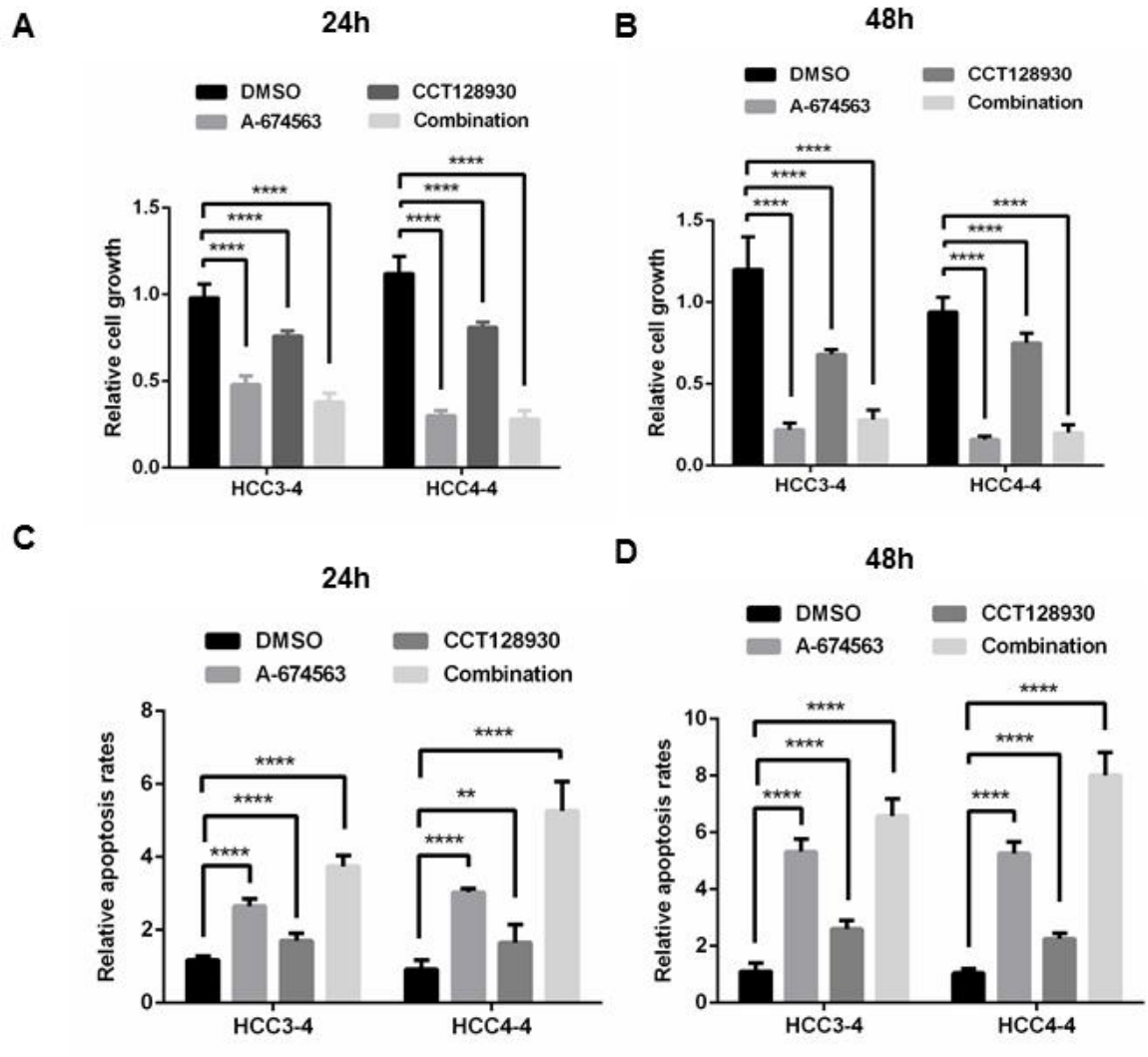
Supplementary Figure 4. Inhibition of different isoforms of Akt reduces HCC3-4 and HCC4-4 cell growth.

(A, B) Relative growth of HCC3-4 and HCC4-4 cell lines treated with control RNA or siRNAs targeting Akt1 or Akt2 or both compared to control group. ****P*<0.001, *****P*<0.0001. (C) (D) Western blotting was performed in HCC3-4 and HCC4-4 cell lines treated with control RNA or siRNAs targeting Akt1 or Akt2 or both.



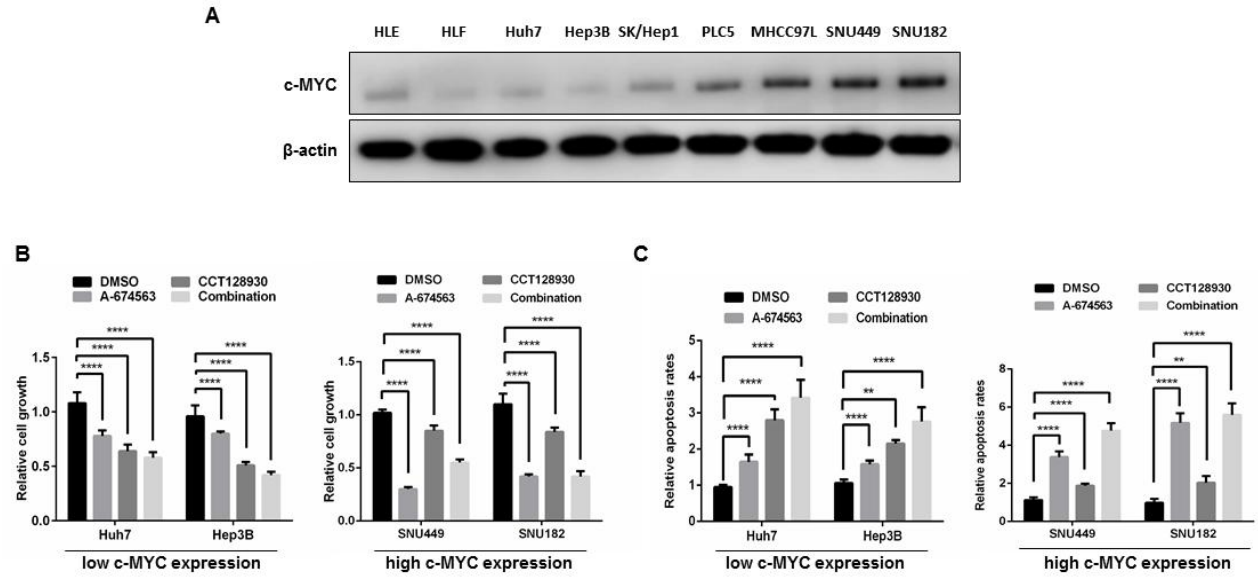
Supplementary Figure 5. Effect on cellular proliferation and apoptosis of inhibition of different Akt isoforms.

(A) BrdU Cell Proliferation Assay was performed in HCC3-4 and HCC4-4 cell lines treated with control RNA or siRNAs targeting Akt1 or Akt2 or both for 24 hours; and (B) for 48 hours. (C) Relative apoptosis rates in HCC3-4 and HCC4-4 cell lines treated with control RNA or siRNAs targeting Akt1 or Akt2 or both for 24 hours and (D) for 48 hours. ****P<0.0001. Abbreviation: BrdU, 5-bromodeoxyuridine.



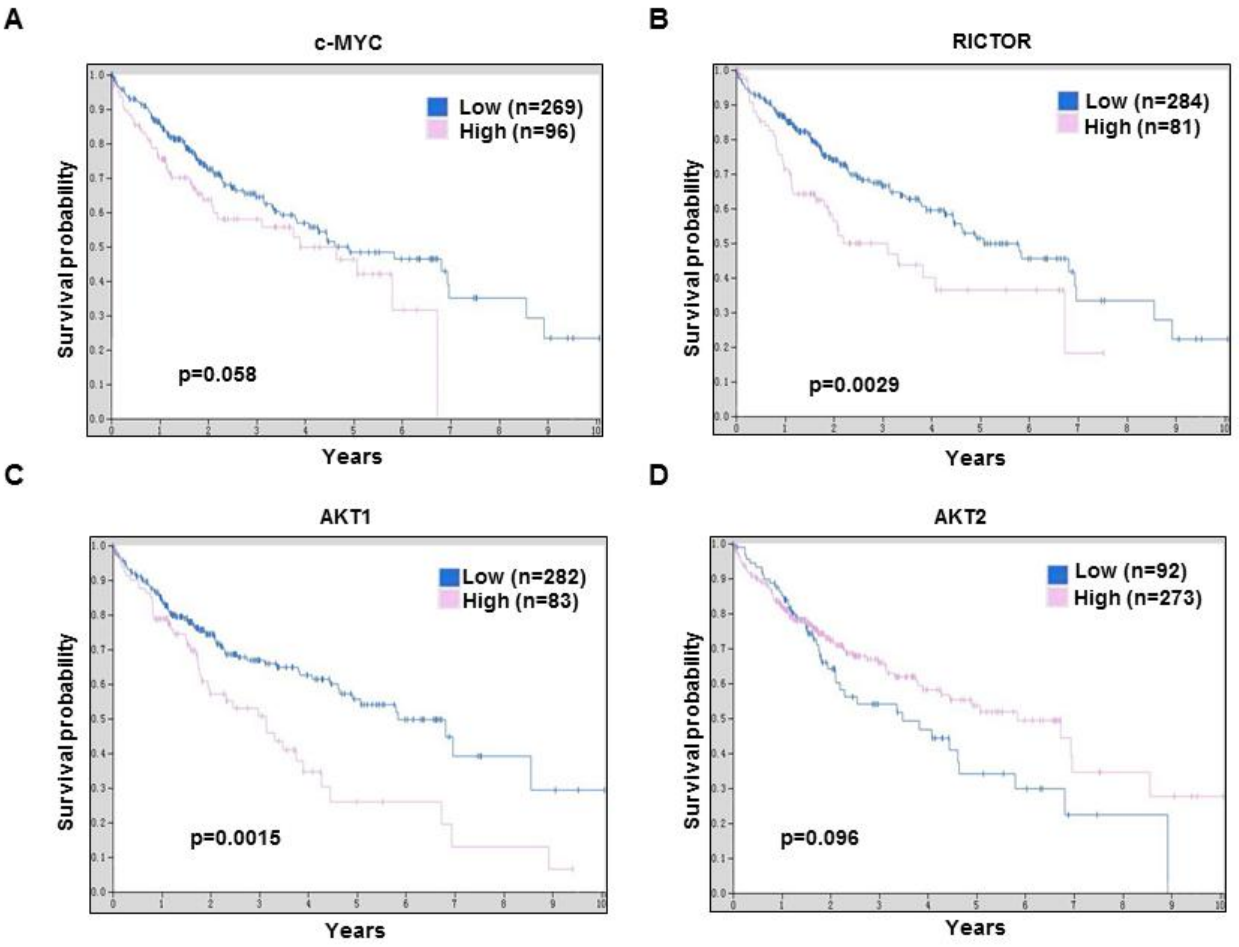
Supplementary Figure 6. Effects on proliferation and apoptosis of HCC3-4 and HCC4-4 cell lines following the administration of AKT1 or AKT2 selective inhibitors.

(A) BrdU Cell Proliferation Assay was performed in HCC3-4 and HCC4-4 cell lines treated with the AKT1 selective inhibitor, A-674563, or the AKT2 specific inhibitor, CCT128930, or both for 24 hours; and (B) for 48 hours. (C) Relative apoptosis rates in HCC3-4 and HCC4-4 cell lines treated with the AKT1 selective inhibitor, A-674563, or the AKT2 specific inhibitor, CCT128930 or both for 48 hours; and (D) for 48 hours. ***P*<0.01, *****P*<0.0001. Abbreviation: BrdU, 5-bromodeoxyuridine. DMSO, dimethyl sulfoxide.

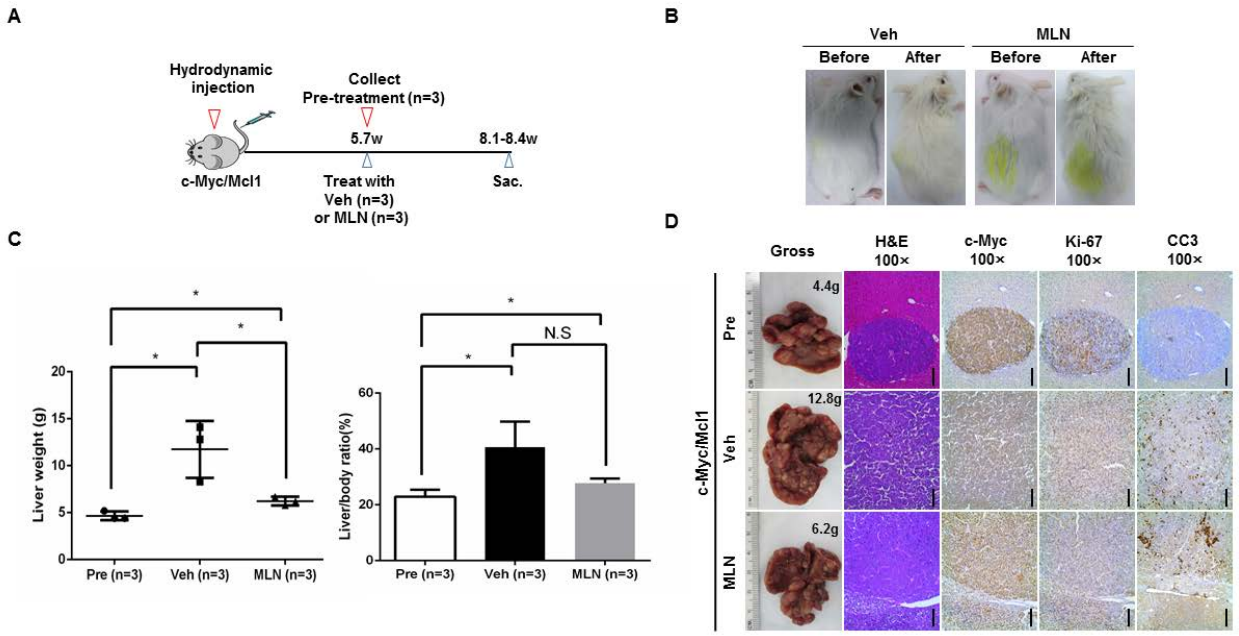


Supplementary Figure 7. Expression of c-MYC is associated with the response to AKT1 and AKT2 selective inhibitors in human HCC cell lines.

(A) Protein levels of c-MYC in HLE, HLF, Huh7, Hep3B, SK/Hep1, PLC5, MHCC97L, SNU449 and SNU182 human HCC cell lines, as detected by Western blot analysis. (B) BrdU Cell Proliferation Assay was performed in c-MYC high and low expressing cell lines treated with the AKT1 selective inhibitor, A-674563, or the AKT2 specific inhibitor, CCT128930, or both for 48 hours. (C) Cell death was detected in c-MYC high and low expression cell lines treated with the AKT1 selective inhibitor, A-674563, or the AKT2 specific inhibitor, CCT128930, or both for 48 hours.****P<0.0001. Abbreviation: BrdU, 5-bromodeoxyuridine. DMSO, dimethyl sulfoxide.

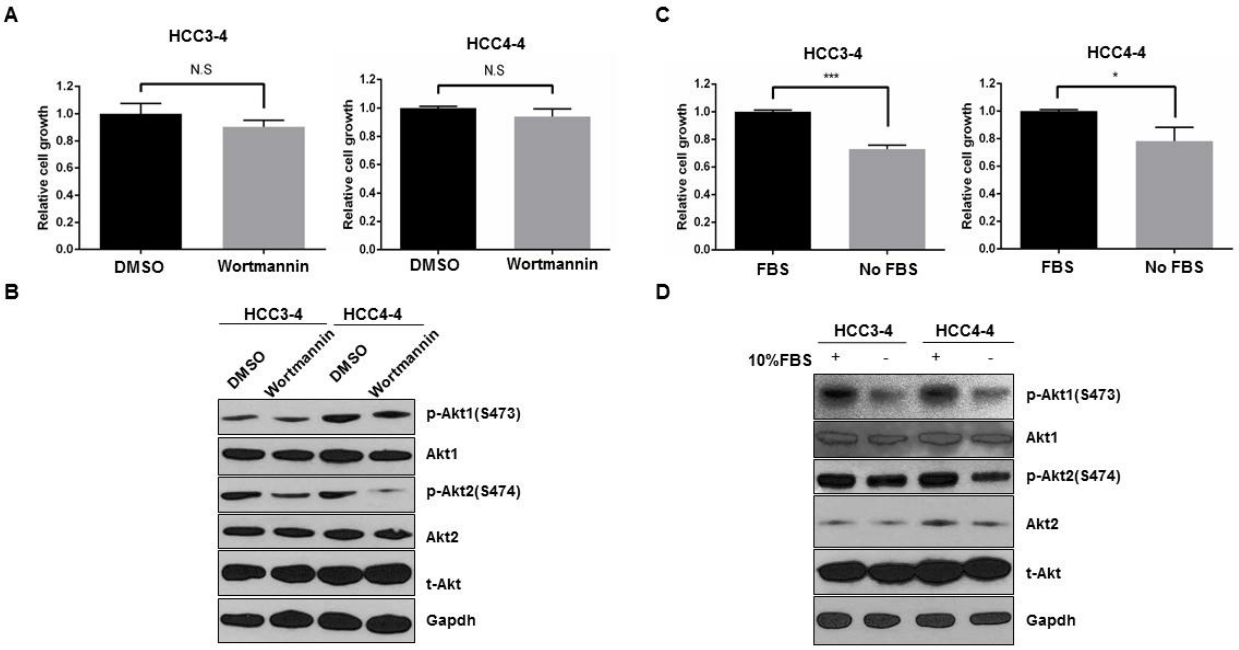


Supplementary Figure 8. Kaplan-Meier survival analysis of HCC in TCGA HCC dataset with high and low mRNA expression of c-MYC (A), RICTOR (B), AKT1 (C) and AKT2 (D).



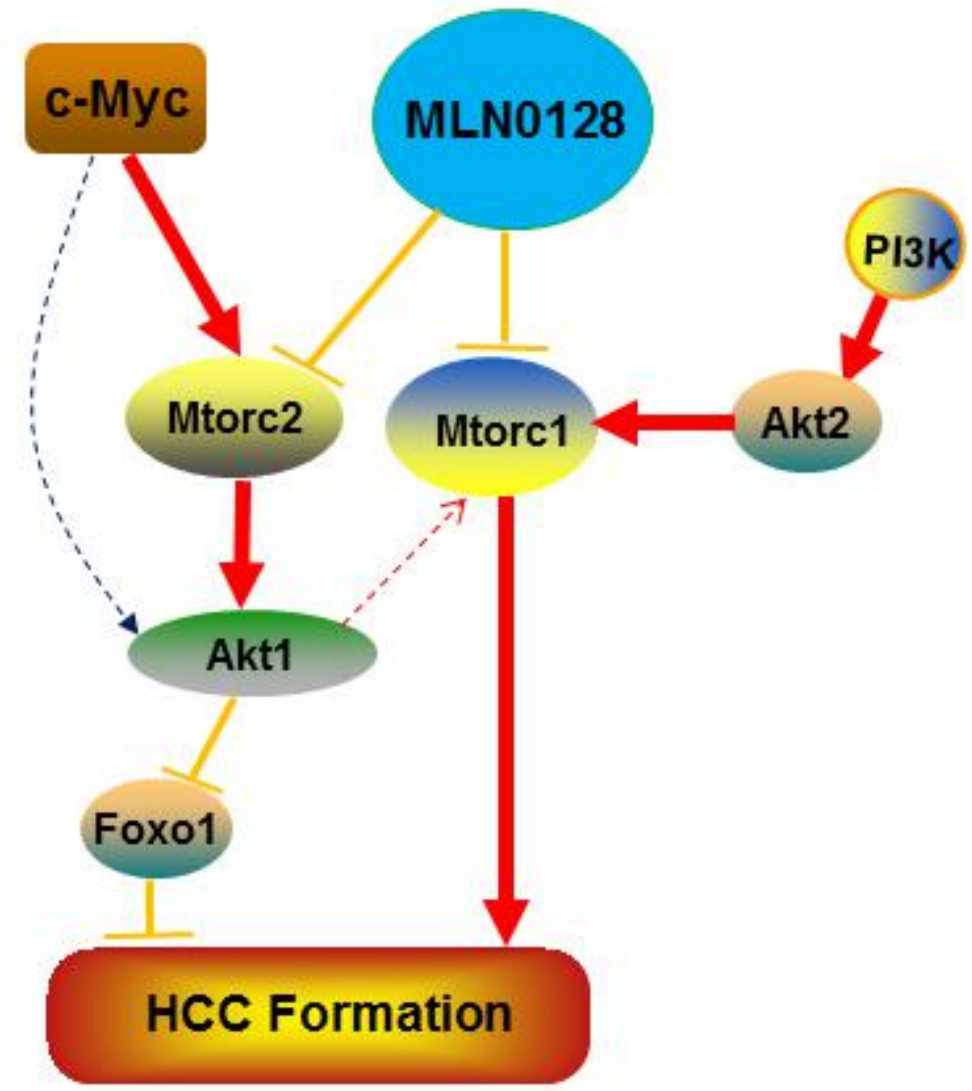
Supplementary Figure 9. Reduced efficacy of the dual mTORC1/2 inhibitor MLN0128 in HCC lesions developed in c-Myc/Mcl1 mice.

(A) Study design. (B) Gross images in vehicle-treated or MLN0128-treated FVB mice injected by c-Myc/Mcl1. (C) Liver weight and liver/body ratio of c-Myc/Mcl1 HCC mice treated with vehicle or MLN0128 for 2.7 weeks. Data are presented as mean ± standard error of the mean. **P*<0.05. (D) Gross, hematoxylin and eosin, c-Myc, Ki-67, and CC3 staining images in vehicle-treated or MLN0128-treated mice livers. Magnifications: ×100, scale bar=200 μm. Abbreviations: N.S, no significance; MLN, MLN0128; Veh, vehicle; HE, hematoxylin and eosin; CC3, cleaved caspase-3.



Supplementary Figure 10. c-Myc induced p-Akt1 activation might be triggered by extracellular signals, but independent of PI3K.

(A) Relative growth of HCC3-4 and HCC4-4 cell lines treated with DMSO or wortmannin for 48hours. (B) Representative Western blot results showing HCC3-4 and HCC4-4 cell lines treated with DMSO or wortmannin. (C) Relative growth of HCC3-4 and HCC4-4 cell lines treated with Dulbecco's modified Eagle medium with or without 10% FBS. ***P<0.001, *P<0.05. (D) Western blot analysis results showing HCC3-4 and HCC4-4 cell lines treated with medium containing or without 10% FBS for 24hours. Abbreviations: N.S, no significance; DMSO, dimethyl sulfoxide.



Supplementary Figure 11. Schematic summary of the main findings of the manuscript.

Briefly, c-Myc induces the activation of the mTORC2/Akt1 axis, resulting in inhibition of the Foxo1 tumor suppressor. On the other hand, phosphoinositide 3-kinase (PI3K) triggers the activation of Akt2, which in turn activates mTORC1. Thus, both mTORC1 and mTORC2 contribute to HCC formation and can be specifically targeted by the dual inhibitor MLN0128.

Supplementary Table 1. The mixture of plasmids used for mice injection.

Figure	Mice strain	group	Plasmid mixture
2	*Rictor*$^{f/f}$	Control	10μg c-Myc + 10μg Mcl1 + 20μg pCMV + 2μg SB
		Experiment	10μg c-Myc + 10μg Mcl1 + 20μg Cre + 2μg SB
3	*AKT1*$^{f/f}$	Control	10μg c-Myc + 10μg Mcl1 + 20μg pCMV + 2μg SB
		Experiment	10μg c-Myc + 10μg Mcl1 + 20μg Cre + 2μg SB
4	*AKT2*$^{f/f}$	Control	10μg c-Myc + 10μg Mcl1 + 20μg pCMV + 2μg SB
		Experiment	10μg c-Myc + 10μg Mcl1 + 20μg Cre + 2μg SB
6.7	FVB	Control	10μg c-Myc + 0.5μg SB
		Experiment	10μg c-Myc + 0.5μg SB
Sup. Fig. 3	*SGK3*$^{+/+}$	Control	10μg c-Myc + 10μg Mcl1 + 1μg SB
	SGK3$^{-/-}$	Experiment	10μg c-Myc + 10μg Mcl1 + 1μg SB
Sup. Fig. 9	FVB	Control	10μg c-Myc + 10μg Mcl1 + 1μg SB
		Experiment	10μg c-Myc + 10μg Mcl1 + 1μg SB

Supplementary Table 2. Clinicopathological features of HCC Patients.

Variables	Features	
	HCCP[a]	HCCB[b]
No. of patients	56	52
Male	42	38
Female	14	14
Age (Mean ± SD)	68.2 ± 11.8	66.8 ± 10.2
Etiology		
HBV	25	21
HCV	15	12
Ethanol	15	16
Wilson's disease	1	2
Hemochromatosis	0	1
Cirrhosis		
+	46	40
-	10	12
Tumor size		
> 5 cm	32	30
< 5 cm	24	22
Edmondson and Steiner grade		
II	22	19
III	18	22
IV	16	11
Alpha-fetoprotein secretion		
> 300 ng/ml of serum	36	40
< 300 ng/ml of serum	20	12
Survival after partial liver resection (months)（Means ± SD）	18.4 ± 10.8	56.2 ± 14.4

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

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

Supplementary Table 3. *Rictor*$^{fl/fl}$ mice injected with c-Myc/MCL1/pCMV or c-Myc/MCL1/Cre plasmids sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	5.1	25.3	8.2	Big tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	6.7	35.5	8.4	Big tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	7.0	27.2	7.9	Big tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	8.6	17.5	2.7	Tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	9.6	24.3	5.7	Big tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	11.1	28.5	3.1	Tumor
c-Myc-MCL1-pCMV *Rictor*$^{f/f}$	12.1	34.3	6.9	Big tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	5.0	26.5	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	6.0	27.6	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	7.0	25.2	1.2	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	8.0	27.5	1.3	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	8.6	31.8	1.5	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	8.6	27.7	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	11.3	32.2	1.2	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	12.0	27.9	1.1	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	12.0	28.8	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	13.3	26.5	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	13.9	30.6	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	13.9	31.3	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	18.9	35.9	1.6	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	18.9	32.9	1.4	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	19.1	31.9	1.3	No tumor
c-Myc-MCL1-Cre *Rictor*$^{f/f}$	19.1	32.8	1.5	No tumor

Supplementary Table 4. *Akt1*$^{fl/fl}$ mice injected with c-Myc/MCL1/pCMV or c-Myc/MCL1/Cre plasmids sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	4.0	24.2	8.4	Big tumor
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	6.1	20.3	5.8	Big tumor
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	7.6	20.8	4.5	Big tumor
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	7.7	22.4	4.9	Big tumor
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	10.1	29.1	4.7	Big tumor
c-Myc-MCL1-pCMV *Akt1*$^{f/f}$	11.0	27.9	4.9	Big tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	4.0	20.7	1.0	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	5.1	23.1	1.1	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	6.0	24.6	1.4	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	15.3	31.9	1.4	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	16.0	22.2	1.2	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	16.9	31.7	1.5	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	17.0	32.8	1.8	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	19.0	30.1	1.6	No tumor
c-Myc-MCL1-Cre *Akt1*$^{f/f}$	19.0	28.7	1.4	No tumor

Supplementary Table 5. *Akt2*$^{fl/fl}$ mice injected with c-Myc/MCL1/pCMV or c-Myc/MCL1/Cre plasmids sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-Myc-MCL1-pCMV *Akt2*$^{f/f}$	6.0	28.8	8.3	Big tumor
c-Myc-MCL1-pCMV *Akt2*$^{f/f}$	10.7	31.0	7.9	Big tumor
c-Myc-MCL1-pCMV *Akt2*$^{f/f}$	13.0	28.9	3.6	Big tumor
c-Myc-MCL1-pCMV *Akt2*$^{f/f}$	11.5	25.5	3.2	Big tumor
c-Myc-MCL1-Cre *Akt2*$^{f/f}$	9.0	21.9	1.1	No tumor
c-Myc-MCL1-Cre *Akt2*$^{f/f}$	15.0	27.6	3.6	Tumor
c-Myc-MCL1-Cre *Akt2*$^{f/f}$	16.0	27.8	2.4	Tumor
c-Myc-MCL1-Cre *Akt2*$^{f/f}$	16.0	29.4	3.9	Tumor
c-Myc-MCL1-Cre *Akt2*$^{f/f}$	16.0	22.1	1.1	Tiny tumor

Supplementary Table 6. *Sgk3*$^{-/-}$ and *Sgk3*$^{+/+}$ mice injected with the c-Myc/MCL1 plasmids sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-Myc-MCL1 *Sgk3*$^{+/+}$	4.3	28.9	10.4	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	4.4	26.8	6.8	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	4.6	26.3	7.5	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	5.3	17.8	3.6	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	5.6	29.1	12.5	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	5.6	25.5	6.1	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	5.7	20.1	5.3	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	6.0	28.8	5.6	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	6.7	24.2	6.4	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	6.7	20.3	3.8	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	7.3	28.6	6.6	Big tumor
c-Myc-MCL1 *Sgk3*$^{+/+}$	7.3	31.5	5.5	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	4.0	22.0	1.7	Small tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	4.3	28.3	10.7	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	5.6	29.7	10.6	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	6.1	23.0	4.5	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	6.4	23.7	5.3	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	7.3	33.1	8.8	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	8.3	29.7	10.0	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	8.3	17.4	1.4	Small tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	8.4	31.3	6.9	Big tumor
c-Myc-MCL1 *Sgk3*$^{-/-}$	8.4	24.2	1.7	Small tumor

Supplementary Table 7. Vehicle-treated, Rapamycin treated and MLN0128-treated for 3 weeks FVB/N mice injected with the c-Myc plasmid sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-myc FVB/N	3.6	25.2	3.6	Big tumor
c-myc FVB/N	4.9	25.0	4.0	Big tumor
c-myc FVB/N	4.9	27.1	4.0	Big tumor
c-myc FVB/N	7.3	27.9	5.3	Big tumor
c-myc FVB/N	6.3	26.2	4.1	Big tumor
c-myc FVB/N	7.0	24.4	4.7	Big tumor
c-myc FVB/N	6.6	24.5	5.3	Big tumor
c-myc FVB/N	5.6	23.9	3.6	Big tumor
c-myc FVB/N	7.3	24.4	5.7	Big tumor
c-myc FVB/N vehicle	8.6	31.8	13.7	Big tumor
c-myc FVB/N vehicle	8.8	32.0	12.9	Big tumor
c-myc FVB/N vehicle	7.4	25.9	10.8	Big tumor
c-myc FVB/N vehicle	6.6	28.0	10.4	Big tumor
c-myc FVB/N vehicle	7.3	28.6	9.7	Big tumor
c-myc FVB/N vehicle	7.3	28.3	11.3	Big tumor
c-myc FVB/N vehicle	7.3	30.4	12.2	Big tumor
c-myc FVB/N vehicle	8.4	29.3	9.6	Big tumor
c-myc FVB/N vehicle	8.1	32.7	12.1	Big tumor
c-myc FVB/N MLN0128	9.1	26.6	7.3	Big tumor
c-myc FVB/N MLN0128	9.6	25.1	1.2	small tumor
c-myc FVB/N MLN0128	9.6	24.5	1.2	small tumor
c-myc FVB/N MLN0128	9.6	23.4	1.2	small tumor
c-myc FVB/N MLN0128	9.6	23.4	1.1	small tumor
c-myc FVB/N MLN0128	9.6	23.0	1.2	small tumor
c-myc FVB/N MLN0128	10.0	23.2	2.8	Tumor

c-myc FVB/N MLN0128	10.0	22.7	1.1	small tumor
c-myc FVB/N MLN0128	10.0	22.3	1.5	small tumor
c-myc FVB/N MLN0128	10.0	25.4	1.4	small tumor
c-myc FVB/N MLN0128	10.0	22.0	1.2	small tumor
c-myc FVB/N Rapamycin	8.8	23.7	7.9	Big tumor
c-myc FVB/N Rapamycin	8.6	27.8	8.6	Big tumor
c-myc FVB/N Rapamycin	8.7	25.5	7.9	Big tumor
c-myc FVB/N Rapamycin	8.6	21.6	8.0	Big tumor
c-myc FVB/N Rapamycin	7.4	27.7	10.7	Big tumor
c-myc FVB/N Rapamycin	8.6	23.6	7.8	Big tumor
c-myc FVB/N Rapamycin	6.6	23.8	8.5	Big tumor
c-myc FVB/N Rapamycin	7.3	26.9	9.2	Big tumor
c-myc FVB/N Rapamycin	7.3	23.7	7.7	Big tumor
c-myc FVB/N Rapamycin	7.3	21.3	3.0	Big tumor

Supplementary Table 8. Vehicle-treated, Rapamycin treated and MLN0128-treated for 3 days FVB/N mice injected with the c-Myc plasmid sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-myc FVB/N vehicle	7.6	25.3	5.3	Big tumor
c-myc FVB/N vehicle	7.6	25.8	4.1	Big tumor
c-myc FVB/N vehicle	7.6	24.7	5.7	Big tumor
c-myc FVB/N MLN0128	7.3	24.7	3.1	Big tumor
c-myc FVB/N MLN0128	7.6	24.3	3.6	Big tumor
c-myc FVB/N MLN0128	7.6	24.8	3.8	Big tumor
c-myc FVB/N Rapamycin	7.6	24.8	4.2	Big tumor
c-myc FVB/N Rapamycin	7.6	23.3	3.9	Big tumor
c-myc FVB/N Rapamycin	7.6	23.9	4.5	Big tumor

Supplementary Table 9. Vehicle-treated and MLN0128-treated for 2.7 weeks FVB/N mice injected with the c-Myc/MCL1 plasmids sacrifice list.

Label	Weeks post injection	Body weight(g)	Liver weight(g)	Liver phenotype
c-Myc-MCL1 FVB	5.7	22.0	4.4	Big tumor
c-Myc-MCL1 FVB	5.7	21.1	5.2	Big tumor
c-Myc-MCL1 FVB	5.7	18.3	4.4	Big tumor
c-Myc-MCL1 FVB vehicle	8.4	46.8	14.1	Big tumor
c-Myc-MCL1 FVB vehicle	8.4	18.2	8.3	Big tumor
c-Myc-MCL1 FVB vehicle	8.1	27.8	12.8	Big tumor
c-Myc-MCL1 FVB MLN0128	8.4	22.4	5.8	Big tumor
c-Myc-MCL1 FVB MLN0128	8.4	21.9	6.2	Big tumor
c-Myc-MCL1 FVB MLN0128	8.4	23.1	6.7	Big tumor

Supplementary References

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