

Figure S1 overexpression of SAM68 promoted the malignant phenotypes of LUAD cells in vitro. (A and B) NCI-H1975 cells were transfected with Flag-SAM68 plasmids, Flag (A) and SAM68 (B) were immuno-stained using anti-Flag and anti-SAM68 antibodies, respectively. (C and D) After SAM68 overexpression, *SAM68* mRNA (C) and protein levels (D) level were determined by qPCR and Western blot, respectively. (E~G) The effects of SAM68 overexpression on NCI-H1975 and A549 cells growth (E), colony formation (F), and migration and invasion (G) were detected. Data are represented as mean \pm

SEM. * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. Two-tailed t-tests were used C, F and G. Two-way ANOVA was used in E.

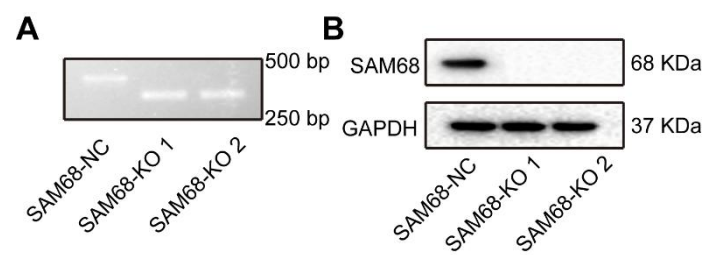


Figure S2 The SAM68 KO cell colonies were identified by RT-PCR (A) and Western blot (B).

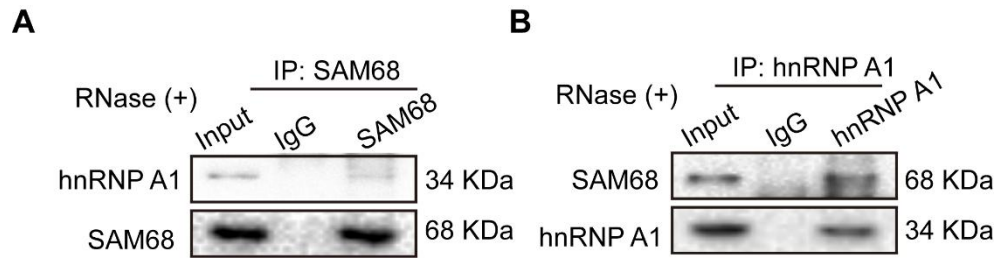


Figure S3 (A and B) Endogenous SAM68 and hnRNP A1 were coimmunoprecipitated using anti-SAM68 antibody or anti-hnRNP A1 antibody, in the presence of RNase A treatment.

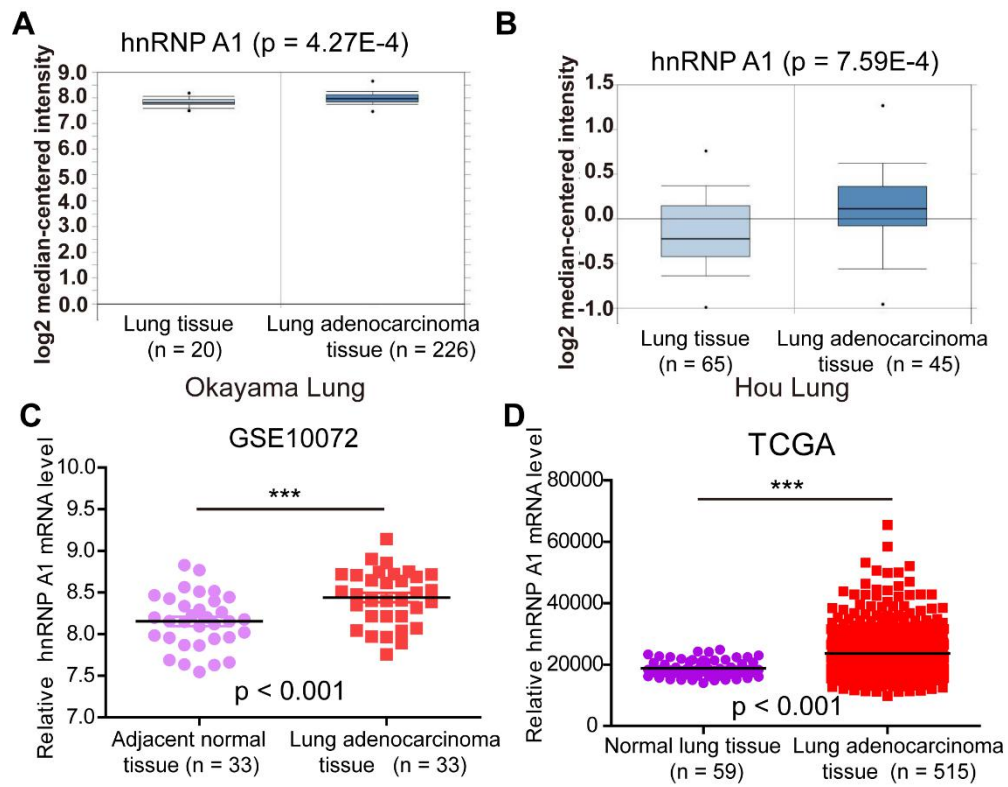


Figure S4 **hnRNP A1 is up-regulated in Lung adenocarcinoma (LUAD)** (A~D) *hnRNP A1* mRNA levels was up-regulated in LUAD compared to normal lung tissue based on the Oncomine (A and B), GEO (C) and TCGA (D) database.

splicing. (C) RNA-seq analysis showed that ZNF621 regulated ANAPC11 pre-mRNA splicing. (D) RT-PCR was used to detect the role of SAM68 in the regulation of ZNF621 exon1 alternative splicing. (E) RNA-seq analysis showed that SAM68 regulated ACADVL pre-mRNA splicing. (F) SAM68 promotes the inclusion of ACADVL intron3, exon 4 and intron 4 using RT-PCR. (G) RNA-seq analysis showed that SAM68 regulated OSBPL1A pre-mRNA splicing. (H) Q-PCR was used to detect the role of SAM68 in the regulation of OSBPL1A alternative splicing. (I) RNA-seq analysis showed that SAM68 regulated ANAPC11 pre-mRNA splicing. (J and K) SAM68 promotes the inclusion of ANAPC11 exon 2 using RT-PCR (J) and PCR fragment sequencing analysis(K).

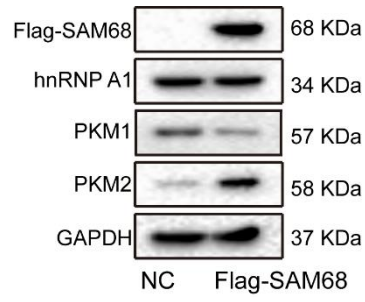


Figure S6 The Flag-SAM68 plasmid was transfected into NCI-H1975 cells, the indicated protein levels were detected.

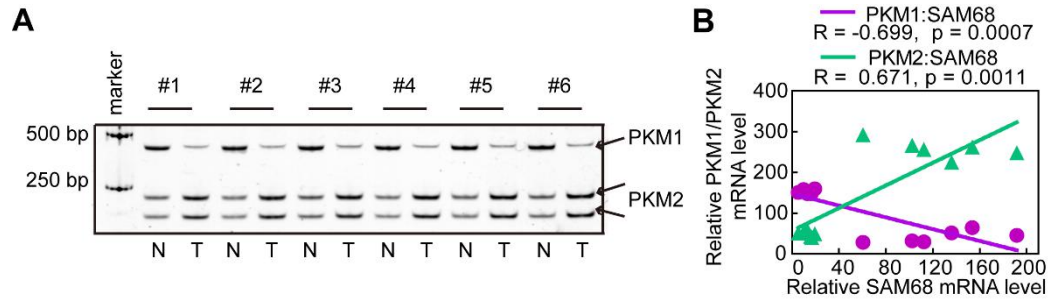


Figure S7 (A) *PKM* splicing was performed in the adjacent normal tissues and LUAD tissue samples with high *SAM68* expression (n=6). (B) The *PKM1* and *PKM2* mRNA levels were negatively and positively correlated with *SAM68* mRNA levels in six pairs of matched LUAD tissues and corresponding normal lung tissues, respectively.

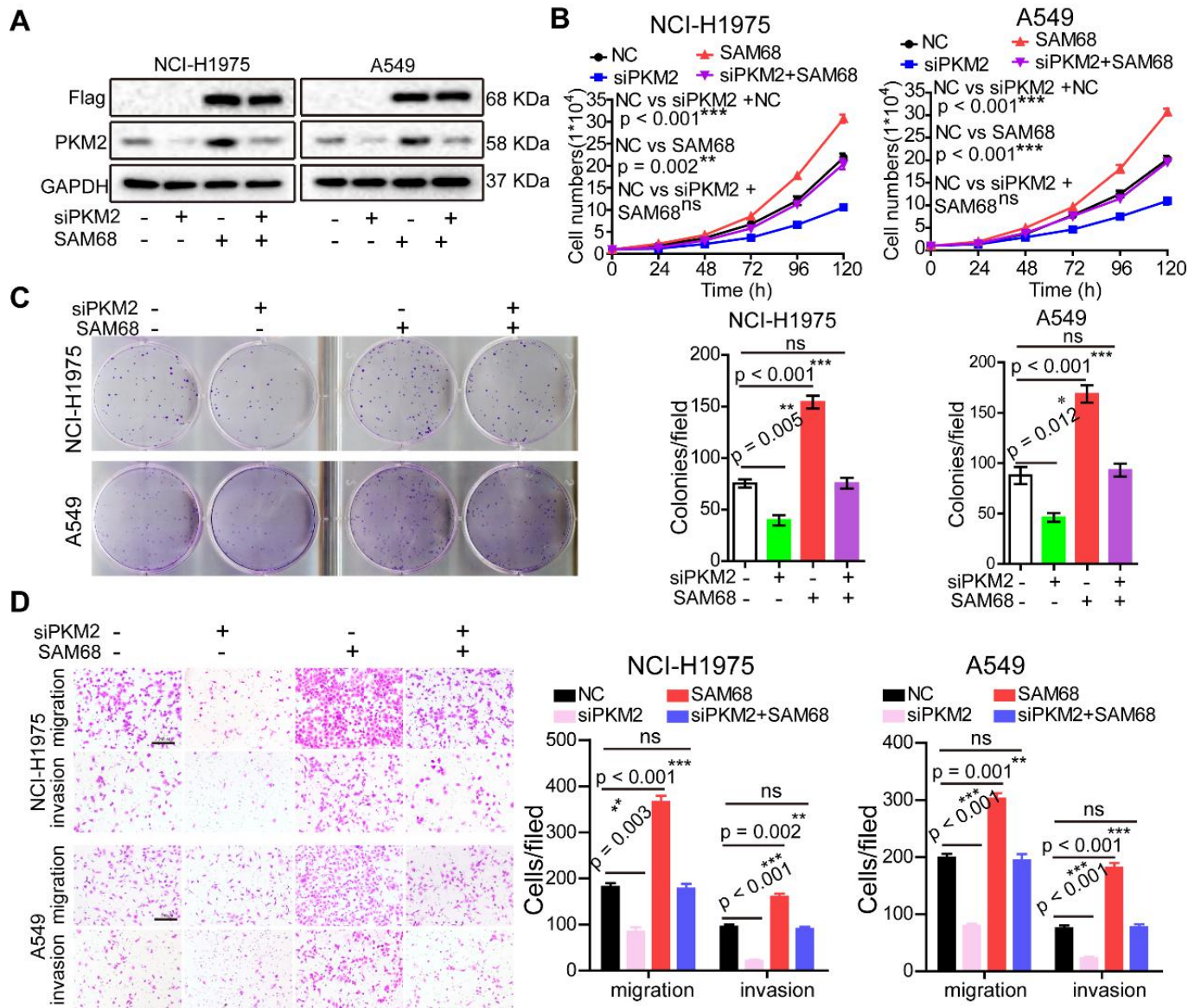


Figure S8. Silencing PKM2 antagonized the enhancement of malignant phenotypes induced by SAM68 overexpression. (A~B) The Flag-SAM68 plasmid and anti-PKM2 siRNAs were co-transfected into NCI-H1975 and A549 cells, the indicated protein levels (A), cell growth (B), colony formation (C), migration and invasion (D) were detected. Data are represented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. Two-way ANOVA was used in B, two-tailed t-tests were used in C and D.

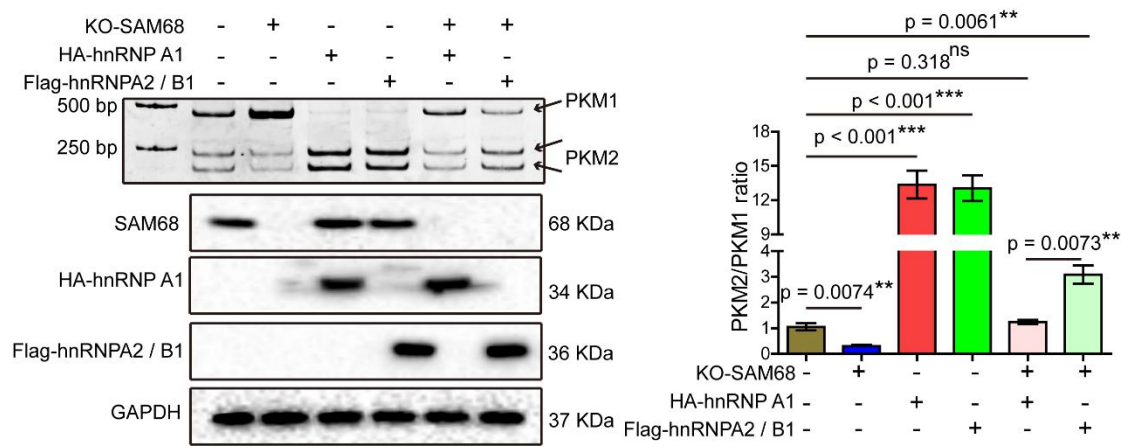


Figure S9. The hnRNPA1 or hnRNPA2/B1 plasmid was transfected into SAM68 KO NCI-H1975 cells, and the PKM splicing were detected.

Table S1. The antibodies, primers ,oligonucleotides and Cas9/sgRNAs used in this study are shown.

Antibodies		
Rabbit Polyclonal anti-SAM68	Proteintech	Cat# 10222-1-AP
Mouse Monoclonal anti- hnRNP A1	Santa Cruz Biotechnology	Cat# sc-32301
Rabbit Polyclonal anti-PKM1	Proteintech	Cat# 15821-1-AP
Rabbit Polyclonal anti-PKM2	Proteintech	Cat#15822-1-AP
Rabbit Polyclonal anti-Flag	abcam	Cat# ab1162
Rabbit Polyclonal anti-HA	abcam	Cat# ab9110
Goat Anti-Mouse IgG (H+L) HRP	Proteintech	Cat# SA00001-1
Goat anti-Rabbit IgG (H+L) HRP	Proteintech	Cat# SA00001-2
Rabbit Polyclonal anti-GAPDH	Proteintech	Cat# 10494-1-AP
Primers name		
SAM68 (RT-PCR)	Forward	TTAACGGCAGTAGGCACCAC
	Reverse	GGCTAGATTTCAAAACCCACGTA
GAPDH (RT-PCR)	Forward	TCTTCCAGGAGCGAGATCCCT
	Reverse	TGGTCATGAGTCCTTCCACGAT
SAM68 (q-PCR)	Forward	ATTAACGGCAGTAGGCACCAC
	Reverse	ACCTTAAGATTCAACCGCCAT
GAPDH (q-PCR)	Forward	CCTCTGACTTCAACAGCGACACC
	Reverse	ACCACCCTGTTGCTGTAGCCAA
PKM (RT-PCR)	Forward	CTGAAGGCAGTGATGTGGCC
	Reverse	ACCCGGAGGTCCACGTCCTC
SAM68 (gDNA-RT-PCR)	Forward	TGACTATTCTACAGTAGGCAT
	Reverse	CATTCTGCTCCTTAGGGTT
SAM68-Flag	Forward	ttaaacttaagcttggtaccATGCAGCGCCGGGACGAC

	Reverse	tcatcgctgcctttagtcATAACGTCCATATGGGTG CTCTC
hnRNP A1-HA	Forward	ttaaacttaagcttggtaccATGTCTAAGTCAGAGTCT CCTAAAGAGC
	Reverse	tctggaacatcgtatgggtaAAATCTTCTGCCACTGCC ATAGC
hnRNP A2/B1-HA	Forward	ttaaacttaagcttggtaccATGGAGAAAACCTTAGAA ACTGTTCT
	Reverse	tctggaacatcgtatgggtaGTATCGGCTCCTCCCACC A
hnRNP A1- MUT1- HA	Forward	ttaaacttaagcttggtaccATGTCTAAGTCAGAGTCTCC TAAAGAGC
	Reverse	tctggaacatcgtatgggtaTGGTTCCACAACCTCTCCA TCC
hnRNP A1- MUT2- HA	Forward	ttaaacttaagcttggtaccATGTCTAAGTCAGAGTCT CCTAAAGAGC
	Reverse	tctggaacatcgtatgggtaTTGCTTTGACAGGGCTTT TCTAA
hnRNP A1- MUT3- HA	Forward	ttaaacttaagcttggtaccATGTCTAAGTCAGAGTCT CCTAAAGAGC
	Reverse	tctggaacatcgtatgggtaACTGCCACCATATCCACC ACC
hnRNP A1- MUT4- HA	Forward	ttaaacttaagcttggtaccATGGAACCAAAGAGAGC TGTCTCC
	Reverse	tctggaacatcgtatgggtaAAATCTTCTGCCACTGCC ATAGC
hnRNP A1 ^{RAAMUT} - HA	Forward-1	ggtgggaatgacaacttcggtcgtgcagcaAACTTCAGTG GTCGTGGTGGC
	Reverse-1	gaagttgtcattcccaccgaaacctgctgcACGACCACCAC CAAAGTTTCC
	Forward-2	atttggtggcagccgtgcagcaGGTGGATATGGTGGCA GTGG

	Reverse-2	cacggctgccaccaaagtctgcACGACCACTGAAGTT TGCTGC
FN-1	Forward	GAAGAGCGAGCCCCTGATTGG
	Reverse	CACCTACATTCGGCGGGTATG
ZNF621	Forward	ACCAGCTCCTCGGCGTTCTG
	Reverse	TTGTTTGGAGCATGGCGGATG
ACADVL	Forward	TCCCATACCCGTCCGTGCTCA
	Reverse	CTCCACCATCTCCAGAGCGTCA
OSBPL1A-Exon 5-6	Forward	TCCGAGTCCGAAAGGT
	Reverse	GAGGATGCTCCAGATA
OSBPL1A-Exon 6-8	Forward	AAGTTTGCCTTCTCCTATGTT
	Reverse	TTTCCAGTCCGTTCCC
OSBPL1A-Exon 5-7	Forward	GAGAGCATTGGGCAGTAGAA
	Reverse	TTAGGCGCTGTAGGAA
OSBPL1A-Exon 7-8	Forward	GAGCCTCTGAGCTTCCTACAG
	Reverse	GTTTTCCAGTCCGTTCC
ANAPC11	Forward	GCTGTTGAGGGAGTCGG
	Reverse	ATGCCACAGTTCTCATCGTTG
Oligonucleotides		
siSAM68#1:	sense:5'-CCUGCACCAGAAACAUACGAAGAUU -3',	
siSAM68#2:	sense: 5'- GAGAGCAUCCAUAUGGACGUUAUUA -3',	
sihnRNP A1#1:	sense: 5'- GUGUAGUUGAACUGAUAGUTT -3',	
sihnRNP A1#2:	sense: 5'- GCUGUGUAAAGUUAGUCUATT -3',	
siPKM2:	sense: 5'- GCCAUCUACCACUUGCAAUTT -3',	
siNC:	sense: 5'-GCACAAGCUGGAGUACAACUACATT-3'	
EI9(50-68)	Biotin-AGGUAGGGCCCUAAGGGCA	
EI9(50-68, G3C)	Biotin-AGGUACGGCCCUAAGGGCA	

SAM68 Cas9/sgRNAs	
Site 1	5'-TACGCAGAACAA AGTTACGA-3'
Site 2	5'-TGACTCTGGCTGTAATAGCC-3'