LCAT1 is an oncogenic LncRNA by stabilizing the IGF2BP2-CDC6 axis

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Supplementary data

Supplementary Table 1. List of reagents and antibodies used in this study

Name	Manufacturer	Cat. no.
Cycloheximide	MedChem Express	HY-12320
bafilomycin A1	MedChem Express	HY-100558
NH_4Cl	Sangon Biotech	A100621
MG-132	Selleck Chemicals	S2619
Rapamycin	Selleck Chemicals	S1035
AZD8055	Selleck Chemicals	S1555
Anti-FLAG® M2 affinity gel	Sigma-Aldrich	A2220
EBSS	Thermo Fisher Scientific	14155063
3-methyladenine	Sigma-Aldrich	M9281
GAPDH	Proteintech Group	60004-1-Ig
CDC6	Cell Signaling Technology	3387
IGF2BP2	Santa Cruz Biotechnology	sc-377014
METTL3	Proteintech Group	15073-1-AP
ULK1	Cell Signaling Technology	8054
m6A	Synaptic Systems	202011

Supplementary Table 2. Sequences of siRNAs used in this study.

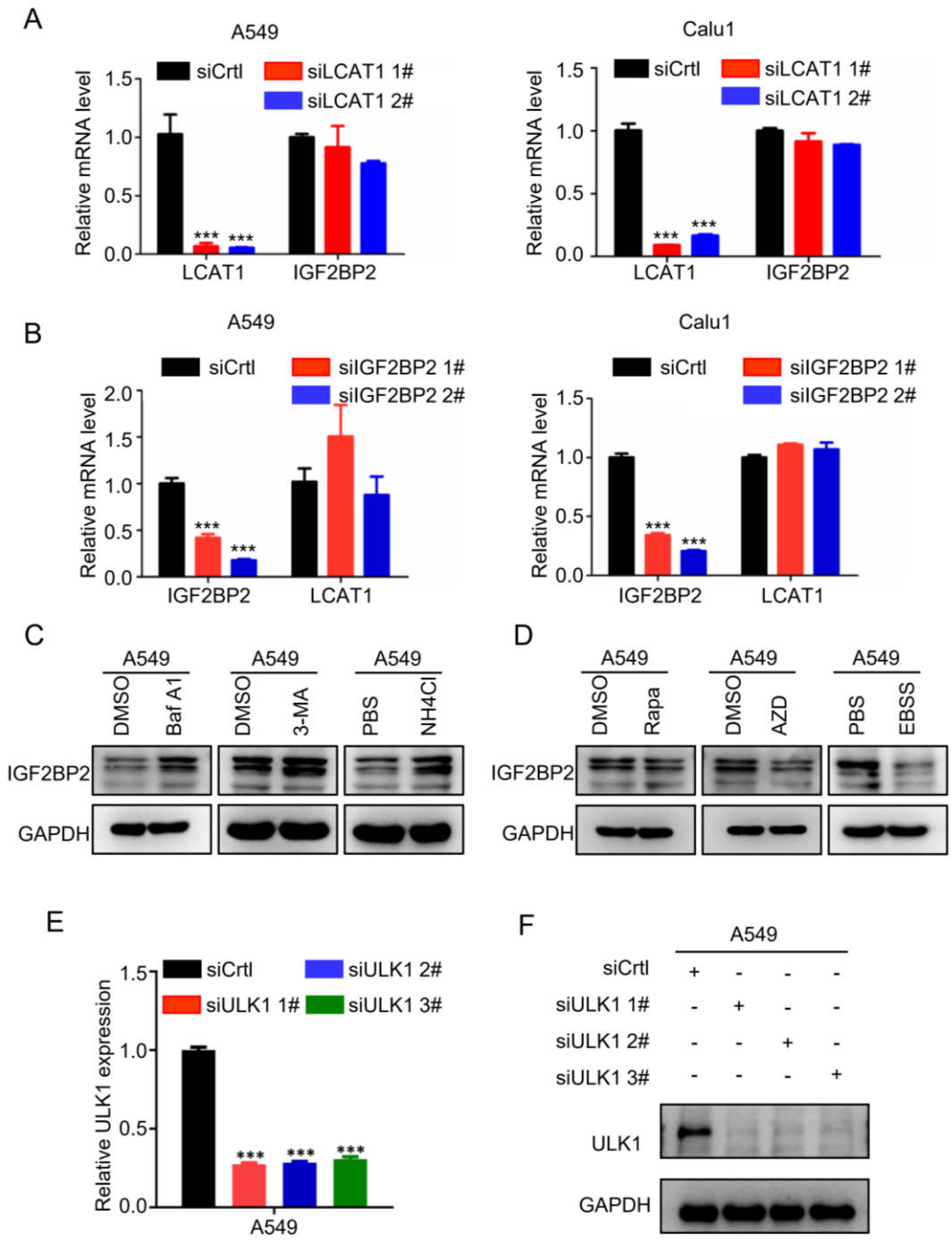
Oligo name	**Sequence**
LCAT1 siRNA 1#	AAUCUCCCAUUGACUGAGC
LCAT1 siRNA 2#	AAGUUGGCUGUAACUCUGG
IGF2BP2 siRNA 1#	UAAGUUCUGCAGUUCGUUC
IGF2BP2 siRNA 2#	AAUUUCCCUGAUCUUGCGC
IGF2BP2 siRNA 3#	UGUUAUCUUGGUCCCUGUU
CDC6 siRNA 1#	AAAGGUAAAGGCUUCCAG
CDC6 siRNA 2#	AUGUGAAUAAGACCAACCC
CDC6 siRNA 3#	AUCUUGUGCUCCUUCUUGG
ULK1 siRNA 1#	AGAAUUAGCCAUUUCCUGGAA
ULK1 siRNA 2#	UAGUGCUGGGACAUGAUGACC
ULK1 siRNA 3#	UUAAGGAGCAGGUCAGUGAGG

Supplementary Table 3. Quality control metrics of RNA-seq libraries

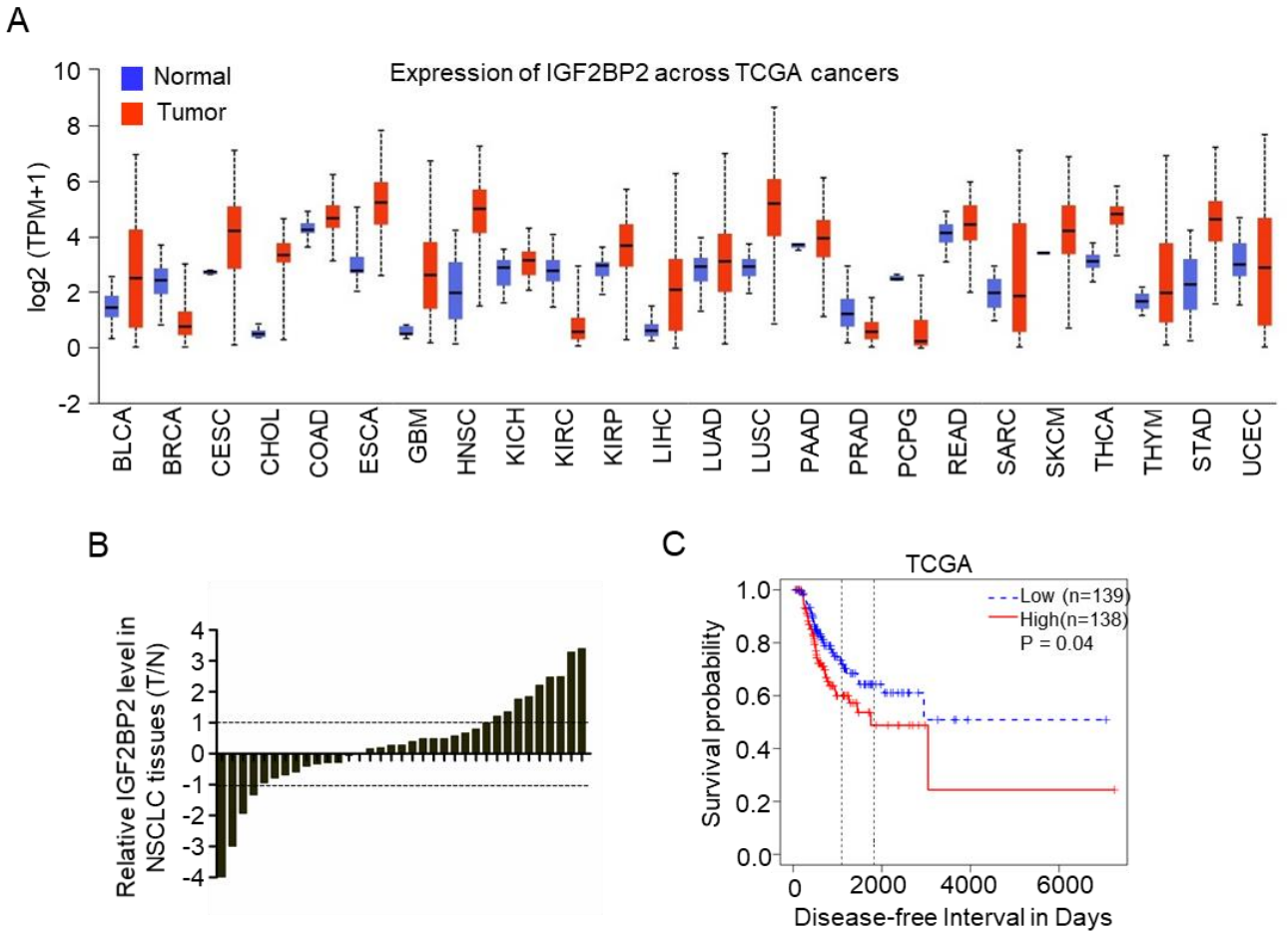
Sample ID	Yield (Gb)	#Reads	%of>=Q30 Bases (PF)	Mean Quality Score (PF)	%Of Mapping Rate
siCrtl1	8.77	62,804,374	96.26	37.77	89.80
siCrtl2	9.41	67,376,556	96.47	37.85	90.50
siCrtl3	8.99	64,354,262	96.45	37.84	90.70
siIGF2BP2 1#-1	9.48	67,862,530	96.67	37.92	88.90
siIGF2BP2 1#-2	9.11	65,210,096	96.45	37.84	87.00
siIGF2BP2 1#-3	8.62	61,689,044	96.68	37.90	87.50
siIGF2BP2 2#-1	9.22	65,987,158	96.63	37.90	89.20
siIGF2BP2 2#-2	9.80	70,165,158	96.55	37.86	88.20
siIGF2BP2 2#-3	9.18	65,722,210	96.41	37.82	88.20

Supplementary Table 4. Proteins identified by mass spectrometry analysis of RNA pull-down fractions.

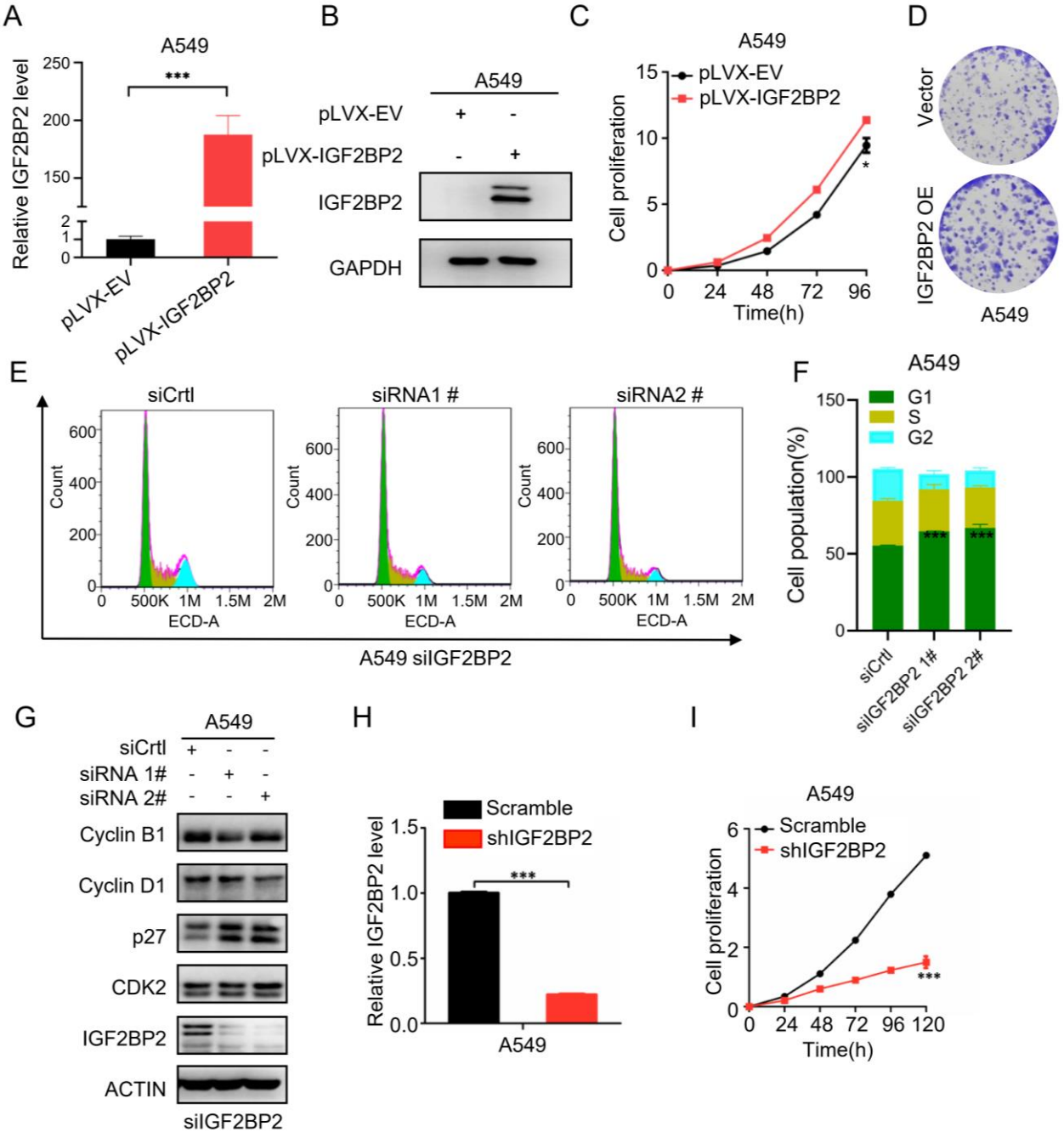
Protein name	Unique peptides		Mol. Weight [kDa]
	Antisense	Sense	
G3BP1	5	12	52.164
NONO	8	12	54.231
HNRPK	5	10	50.976
U2AF2	3	6	53.5
KHDR1	1	3	48.227
IGF2BP2	**0**	**4**	**66.121**
LBR	0	3	70.702
FA98A	0	2	55.4
PNKP	0	2	57.076



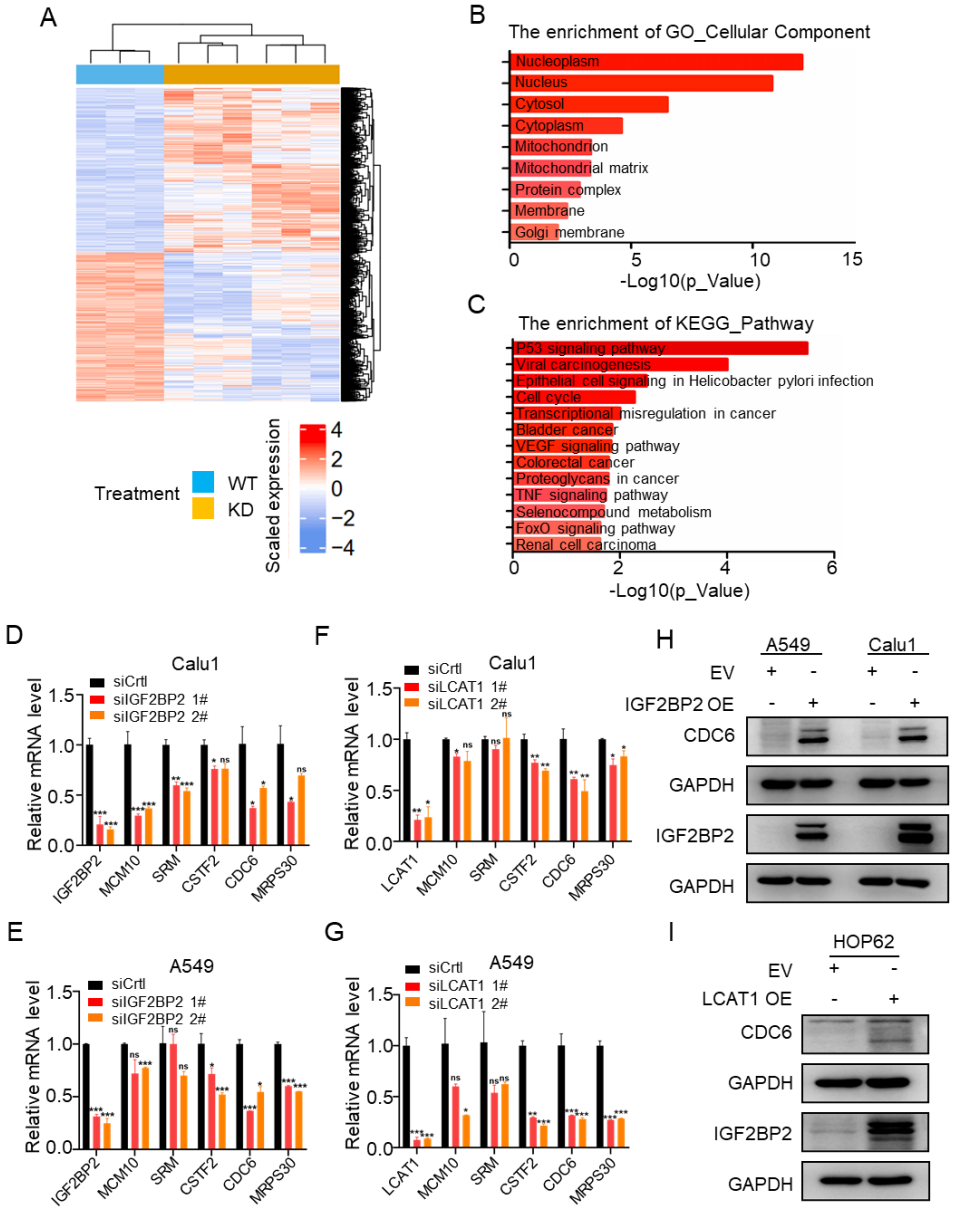
Supplementary Fig. 1 (**A**) LCAT1 and IGF2BP2 expression was measured by qPCR in LCAT1 knockdown cells. (**B**) LCAT1 and IGF2BP2 expression was measured by qPCR in IGF2BP2 knockdown cells. (**C**) Immunoblotting of IGF2BP2 in lung cancer cells with or without treatment of Bafilomycin A1, 3-MA or NH4Cl. Cell lysates were analyzed by western blotting with GAPDH as a loading control. (**D**) Immunoblotting of IGF2BP2 in lung cancer cells with or without treatment of Rapamycin, AZD8055 or EBSS. (**E, F**) qPCR and immunoblotting were employed to detect the ULK1 expression in lung cancer cells transfected with siRNA or negative controls. Data are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.



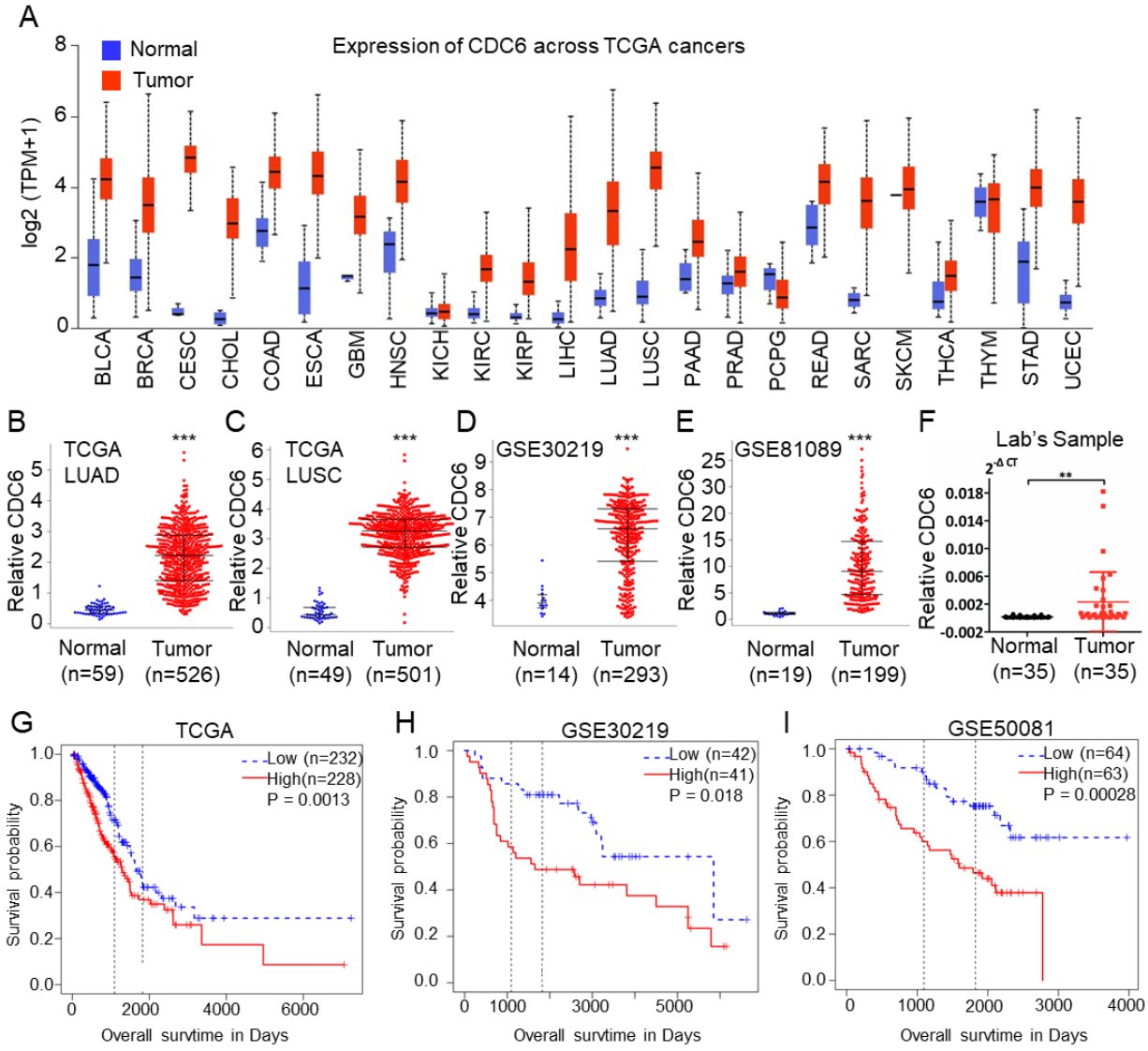
Supplementary Fig. 2 (**A**) The mRNA expression of IGF2BP2 in different cancer types of the TCGA. (**B**) qPCR was performed to detect IGF2BP2 expression in 35 new lung cancer tissues and matched adjacent non-tumor tissues. (**C**) Kaplan-Meier curves of disease-free survival time based on IGF2BP2 expression of lung cancer patients of the TCGA.



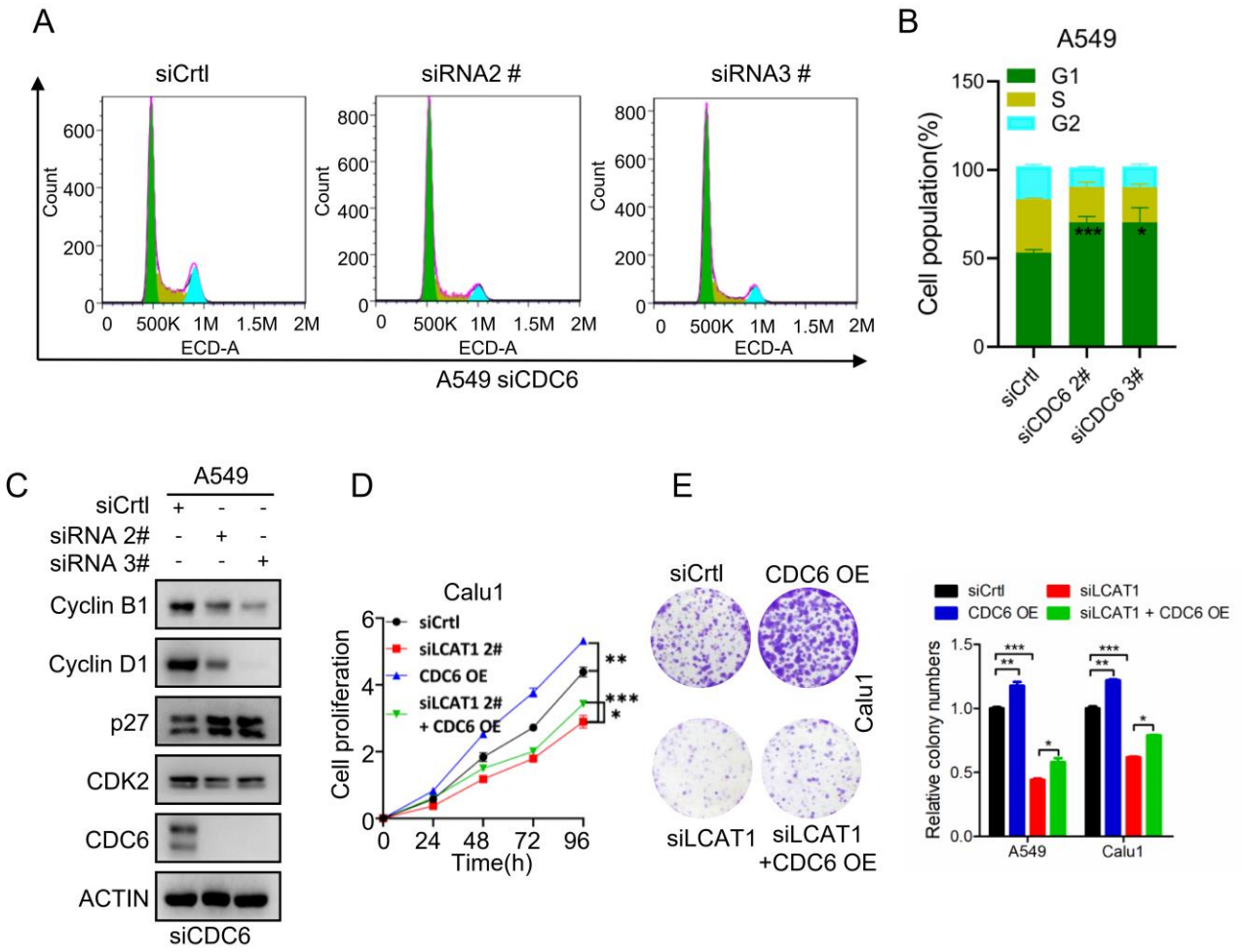
Supplementary Fig. 3 (**A, B**) IGF2BP2 mRNA and protein were measured in IGF2BP2 overexpressing cells. (**C, D**) CCK8 assay and colony formation assay were used to measure the growth of A549 cells after overexpression of IGF2BP2. (**E, F**) PI staining and flow cytometry were used to examine the G0/G1 cell cycle transition in IGF3BP2-knockdown A549 cells. (G) Western blots were conducted to detect cell cycle-related proteins. (**H**) qPCR was performed to detect IGF2BP2 expression in shCrtl or shIGF2BP2 cells. (**I**) CCK8 assay was used to evaluate the influence of IGF2BP2 knockdown on cell proliferation. Student's two-sided t tests were used. *P < 0.05, ** P < 0.01, ***P < 0.001.



Supplementary Fig. 4 (**A**) Heatmap of genes altered in Calu1 cells with IGF2BP2 knockdown (two siRNAs). (**B**) Gene ontology (GO) analysis of genes altered in Calu1 cells with IGF2BP2 knockdown. (**C**) KEGG pathway analysis of genes altered in Calu1 cells with IGF2BP2 knockdown. (**D, E**) Relative mRNA expression of IGF2BP2 and LCAT1/IGF2BP2 target genes, including MCM10, SRM, CSTF2, CDC6, MRPS30 in Calu1 and A549 cells, were examined by qPCR upon IGF2BP2 knockdown. (**F, G**) Relative mRNA expression of LCAT1 and LCAT1/IGF2BP2 target genes were examined by qPCR upon LCAT1 knockdown. (**H**) Immunoblotting of CDC6 in IGF2BP2 overexpressing cells. (**I**) CDC6 expression was detected in LCAT1 overexpressed cells. Statistics were performed using Student's two-sided t test. *P < 0.05, ** P < 0.01, ***P < 0.001.



Supplementary Fig. 5 (**A**) The mRNA expression of CDC6 in different cancer types of the TCGA. (**B-E**) The relative expression level of CDC6 in TCGA-LUAD, TCGA-LUSC, GSE30219 and GSE81089, respectively. (**F**) qPCR was performed to detect CDC6 expression in 35 fresh NSCLC tissues and matched adjacent non-tumor tissues. (**G-I**) Kaplan-Meier curves of overall survival of lung cancer patients based on CDC6 mRNA expression. Data are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.



Supplementary Fig. 6 (**A, B**) PI staining and flow cytometry were used to examine the G0/G1 cell cycle transition was examined in CDC6-knockdwon A549 cells. (**C**) Western blots were conducted to detect cell cycle-related proteins. (**D, E**) Growth curve and colony formation assays were performed in Calu1 cells co-transfected with siLCAT1 and CDC6 overexpression vectors. *P < 0.05, ** P < 0.01, ***P < 0.001.