

Table S1 Recurrent stage-I non-small cell lung cancer cohort clinical data. (Sex: F-Female, M-Male; Rec Status: 0 - Non-Recurrence, 1 - Recurrence)

Patient ID	Sex	Age	Smoking history	Family history	Radiomics features	Subtypes	Pleural invasion	Vessel violated	Surgical method	Size (cm)	TNM	Stage	T stage	T stage	Rec status	Rec regions	Time of surgery	Last follow-up	Rec days
P1	M	70	No	No	Solid	MIA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	0	None	2018	2020/11/30	602
P2	F	53	No	No	Solid	APA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	0	None	2017	2020/12/2	848
P3	M	61	No	No	Solid	PPA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	0	None	2017	2020/11/30	1079
P4	M	70	Yes	No	Solid	APA	No	No	Lobe Wedge	2~3	T1c	IA3	T1c	T1	0	None	2017	2020/7/30	1079
P5	F	65	No	No	Sem-Solid	PPA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	0	None	2017	2019/2/12	1078
P6	F	41	No	No	Sem-Solid	PPA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	0	None	2017	2020/11/30	1047
P7	M	50	Yes	No	Solid	PPA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	0	None	2017	2020/11/30	813
P8	F	65	No	No	Solid	MPA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	0	None	2017	2020/11/30	785
P9	M	52	Yes	No	Solid	APA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	0	None	2017		776
P10	M	82	No	No	Solid	APA	No	No	Wedge	1~2	T1b	IA2	T1b	T1	0	None	2018	2020/11/17	710
P11	F	47	No	Yes	Solid	APA	NA	No	Lobe	2~3	T1c	IA3	T1c	T1	1	Brain	2017	2020/9/24	1290
P12	M	74	No	No	Sem-Solid	PPA	Yes	No	Lobe	1~2	T1b	IA2	T1b	T1	1	Lung	2017	2020/10/23	1158
P13	F	66	No	Yes	Solid	APA	Yes	No	Lobe	1~2	T2a	IB	T2a	T2	1	Brain	2018	2020/11/23	143
P14	M	67	Yes	No	Solid	SPA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	1	Multi	2018		49
P15	F	70	No	No	Solid	PPA	Yes	No	Lobe Wedge	3~4	T2a	IB	T2a	T2	1	Lung	2018	2020/10/22	142
P16	M	74	No	No	Solid	APA	No	No	Lobe	1~2	T1b	IA2	T1b	T1	1	Bone	2018	2020/11/17	344
P17	M	71	Yes	No	Solid	APA	No	No	Lobe Wedge	3~4	T2a	IB	T2a	T2	1	Lung	2017	2020/9/10	384
P18	M	73	No	No	Sem-Solid	PPA	No	Yes	Lobe Wedge	2~3	T1c	IA3	T1C	T1	1	Multi	2017	2020/11/28	751
P19	M	63	Yes	No	Solid	SPA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	1	Multi	2017		318
P20	M	61	Yes	No	Solid	APA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	1	Multi	2017		518
P21	M	56	No	No	Solid	APA	Yes	No	Lobe	3~4	T2a	IB	T2a	T2	1	Lung	2017	2020/12/1	986
P22	F	52	No	No	Solid	APA	No	No	Lobe	1~2	T1b	IA2	T1b	T1	1	Multi	2017	2020/11/30	515
P23	F	42	No	No	Solid	APA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	1	Bone	2017	2020/11/11	540
P24	F	49	No	No	Solid	PPA	No	No	Lobe	2~3	T1c	IA3	T1c	T1	1	Bone	2018	2020/11/19	273
P25	F	55	No	No	Solid	APA	Yes	No	Lobe	2~3	T2a	IB	T2a	T2	1	Lung	2018	2020/11/30	434
P26	F	70	No	Yes	Solid	APA	Yes	No	Lobe	2~3	T2a	IB	T2a	T2	1	Bone	2018	2020/8/18	112
P27	F	70	No	No	Sem-Solid	APA	No	No	Segment	1~2	T1b	IA2	T1b	T1	1	Adrenal	2018	2020/12/1	141
P28	M	69	Yes	No	Solid	SPA	Yes	No	Lobe	2~3	T1c	IA3	T1c	T1	1	Bone	2018		131

MIA: Microinvasive Adenocarcinoma; APA: Acinar Predominant Adenocarcinoma; PPA: Papillary Predominant Adenocarcinoma; MPA: Micropapillary Predominant Adenocarcinoma; SPA: Solid Predominant Adenocarcinoma.

Table S2 Quantitative reverse transcription polymerase chain reaction (qRT-PCR) primer sequence of functional experiment

qRT-PCR target	qRT-PCR primer sequence
PRAME-F	GCCCATGACAAAGAAGCGAA
PRAME-R	GTCCAGCTGCACCATTTTCA
E-cadherin-F	AGAATGACAACAAGCCCGAATTC
E-cadherin-R	ATCTTGGCTGAGGATGGTGTAAAG
N-cadherin-F	TATGGATGAAACGCCGGGATAAA
N-cadherin-R	GCTCAAGTCATAGTCCTGGTCTT
Vimentin-F	TGACATTGAGATTGCCACCTACA
Vimentin-R	CCAGATTAGTTTCCCTCAGGTTCA
Snai1-F	ATCCAGAGTTTACCTTCCAGCAG
Snai1-R	CTTCCCACTGTCCTCATCTGAC
GAPDH-F	GGAGTCCACTGGCGTCTTCA
GAPDH-R	GTCATGAGTCCTTCCACGATACC

Table S3 Antibody details of functional experiment

Protein Target	Antibody	Brand	RRID	Cat.number	Species	kDa	Dilution
PI3K	PI3 Kinase p85 α (6G10) Mouse mAb	CST	AB_2798288	#13666	Mouse	85	1:1000
Phospho-PI3K	Phospho-PI3 Kinase p85 (Tyr458)/p55 (Tyr199) Antibody	CST	AB_659940	#4228	Rabbit	60,85	1:1000
Akt	Akt (pan) (C67E7) Rabbit mAb	CST	AB_915783	#4691	Rabbit	60	1:1000
Phospho-Akt (Ser473)	Phospho-Akt (Ser473) (D9E) XP [®] Rabbit mAb	CST	AB_2315049	#4060	Rabbit	60	1:1000
PRAME	Anti-PRAME antibody [EPR20330]	Abcam	AB_3677388	ab219650	Rabbit	57	1:1000
E-Cadherin	E-Cadherin (4A2) Mouse mAb	CST	AB_2728770	#14472	Mouse	135	1:1000
N-Cadherin	N-cadherin Polyclonal antibody	Proteintech	AB_2813891	22018-1-AP	Rabbit	140	1:2000
Rap1	Rap1A/Rap1A (26B4) Rabbit mAb	CST	AB_2284915	#2399	Rabbit	21	1:1000
mTOR	mTOR (7C10) Rabbit mAb	CST	AB_2105622	#2983	Rabbit	289	1:1000
p-mTOR	Phospho-mTOR (Ser2448) (D9C2) XP [®] Rabbit mAb	CST	AB_10691552	#5536	Rabbit	289	1:1000
GAPDH	GAPDH (14C10) Rabbit mAb	CST	AB_561053	#2118	Rabbit	37	1:1000
Vimentin	Vimentin (D21H3) XP [®] Rabbit mAb	CST	AB_10695459	#5741	Rabbit	57	1:1000
Snai1	Snail (C15D3) Rabbit mAb	CST	AB_2255011	#3879	Rabbit	29	1:1000
Caspase-8	Caspase-8 (D35G2) Rabbit mAb	CST	AB_10545768	#4790	Rabbit	10,57	1:1000
Cleaved Caspase-8	Cleaved Caspase-8 (Asp374) (E6H8S) Rabbit mAb	CST	AB_3107200	#98134	Rabbit	18,41,43	1:1000
Bax	Bax (D2E11) Rabbit mAb	CST	AB_10557411	#5023	Rabbit	20	1:1000
Bcl-2	Bcl-2 (D17C4) Rabbit mAb	CST	AB_1903907	#3498	Rabbit	26	1:1000
Caspase-3	Caspase-3 Antibody	CST	AB_331439	#9662	Rabbit	17, 19, 35	1:1000
Flag-ZNF740	Anti-DDDDK(FLAG)-tag mAb	MBL	AB_11123930	M185-3L	Mouse	30	1:500
ZNF740	ZNF740 Polyclonal antibody	Proteintech	AB_2880065	25411-1-AP	Rabbit	22	1:500
Ki67	Ki-67 Polyclonal antibody	Proteintech	AB_2918145	28074-1-AP	Rabbit	/	1:10000
5-mC	5-Methylcytosine Monoclonal Antibody (GT4111)	Invitrogen	AB_2787107	MA5-31475	Mouse	/	/
Anti-mouse IgG	Anti-mouse IgG, HRP-linked Antibody	CST	AB_330924	#7076	/	/	1:3000
Anti-Rabbit IgG	Anti-rabbit IgG, HRP-linked Antibody	CST	AB_2099233	#7074	/	/	1:3000

Table S4 The target sequence and insertion sequence information of the sgRNA vector used for (de)methylation editing, as well as the RTA-AD sequence

Function	Plasmids	Cleavage site	Cleavage site	Target sequence	Insert sequence
Methylation(NC)	pU6-sgRNA-mcherry-Puro(dcas9)-NC	BsmBI	BsmBI	GGTTC TCCGAACGTGTCACGT	atccgGGTTC TCCGAACGTGTCACGT
Methylation(PRAME)	pU6-sgRNA-mcherry-Puro(dcas9)-PRAME	BsmBI	BsmBI	GACCAGCCTCGACTGACAGT	atccgGACCAGCCTCGACTGACAGT
Demethylation(NC)	pU6-sgRNA-Puro-ccdb-NC	EcoRI	PmeI	GAGAA TGGCATGAACCCGGG	CTTGAAAGTATTTCGATTTC TTGGCTTTATATATCTTGTGAAAG GACGAATTgGAGAATGGCATGAACCCGGGGTTTAAACGAAACA CCGCGGGCTCAATTTATAGAAACGTTTATAGAGCTAGGCCA
Demethylation(PRAME)	pU6-sgRNA-Puro-ccdb-PRAME	EcoRI	PmeI	GAGAA TGGCATGAACCCGGG	CTTGAAAGTATTTCGATTTC TTGGCTTTATATATCTTGTGAAAG GACGAATTgGAGAATGGCATGAACCCGGGGTTTAAACGAAACA CCGCGGGCTCAATTTATAGAAACGTTTATAGAGCTAGGCCA
RTA-AD sequence					
cgggattccagggaagggatgtttttccgaagcctgagccggctccgctattagtgcagtgtttgaggccgcgaggtgtgccagccaaaacgaatccggccatttcatcctccaggaagtcctggccaaccgcccact ccccccagcctcgaccaacaccaaccggtccagfacatgagccagtcgggtcactgaccccgccaccagtcctcagccactggatccagcgccgcagtgactcccaggccagtcacctgttgaggatccgatga agagacgagccaggctgtcaaagccctcgggagatggccgatactgtgattcccagaaggaaggctgcaatctgtggccaaatggacctttccatccgcccccaaggggccatctggatgagctgacaaccacatt gagtcctgaccgaggatctgaacctggactcaccctgaccccggaattgaacgagattctggataccttctgaacgacagtgacctctgcatgccaatgcatatcagcacaggactgtccatcttcacacatctctgttt					

Table S5 Tumor growth of subcutaneous tumor model

Group	Mouse/Tumor Volume (mm ³)	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19
Group 1										
A549	Mouse 1	89.839426	167.450814	320.612058	389.8440395	508.1755455	899.198901	1250.594611	1555.660412	1534.154948
	Mouse 2	161.44128	236.7181625	327.2397705	244.299726	596.580625	767.4815625	1067.913113	1528.639583	1935.253764
	Mouse 3	100.29505	183.206898	274.7631375	386.0779205	623.4290145	576.982062	684.193062	1000.337188	1866.638615
	Mouse 4	104.234208	186.120718	259.2059265	380.952384	392.697696	682.892469	801.328779	952.970416	1562.10285
	Mouse 5	151.409628	253.923292	174.716416	328.5949875	425.3345125	671.883296	787.997696	1134.319888	1683.38493
	Mouse 6	117.70659	180.8107905	271.717916	277.917475	662.997816	925.132914	1076.007114	1537.553872	1530.41816
	Mouse 7	90.774657	140.85493	185.414824	207.160632	364.730688	838.5529645	973.0663345	1359.921879	1967.227624
	Mouse 8	93.056472	137.530098	177.2173875	250.11423	486	611.853089	721.572599	1005.703073	1920.375
Group 2										
A549-PRAME- Knockdown	Mouse 9	123.7113425	166.617	129.634308	198.342837	109.988243	199.8046875	295.1384375	907.819812	575.911462
	Mouse 10	96.597558	117.7815875	189.3212165	108.644436	183.143772	298.395468	418.402868	512.401072	453.519616
	Mouse 11	113.242176	160.9728	186.5338	209.0911255	135.4434	208.35837	306.54682	497.096436	1156.179785
	Mouse 12	98.256726	135.1671705	106.700048	128.374868	158.328594	240.18376	347.78496	449.76384	620.268264
	Mouse 13	113.5779525	142.47554	200.655	51.494648	53.086914	205.9961655	300.7393155	408.3452435	520.206246
	Mouse 14	84.94137	113.89125	111.6590625	199.82592	288.238122	602.51625	788.87125	424.579025	1500.2496
	Mouse 15	44.696896	54.992896	61.2157	284.35035	313.1356905	71.29088	121.05808	800.6163	612.51088
	Mouse 16	141.864525	187.223781	155.832876	105.4623375	119.377476	327.07476	458.63696	565.1186535	592.56364

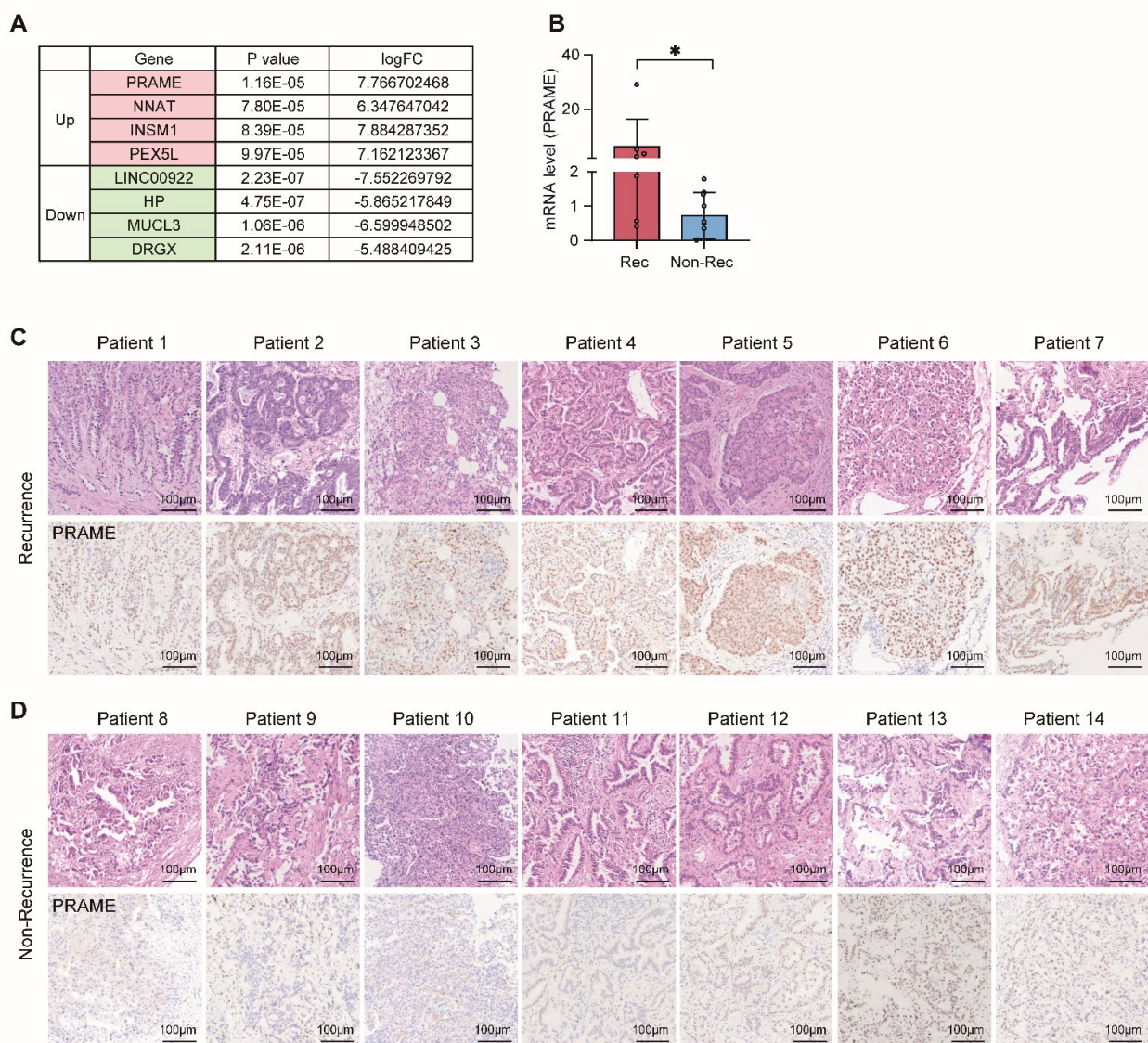


Figure S1 Analysis of *PRAME* expression characteristics in recurrent and non-recurrent early-stage LUAD samples. (A) Differentially expressed genes (DEGs) between recurrent and non-recurrent early-stage LUAD patients identified by DESeq2 analysis. (B) The mRNA level of *PRAME* in recurrent and non-recurrent early-stage LUAD patients was validated by qRT-PCR. (C,D) Representative Hematoxylin-eosin (H&E) and immunohistochemistry (IHC) staining of *PRAME* in 7 recurrent cases (C) and 7 non-recurrent cases (D). Scale bar: 100 μ m. Statistical significance was determined by unpaired two-tailed Student's t test (B). * $P < 0.05$.

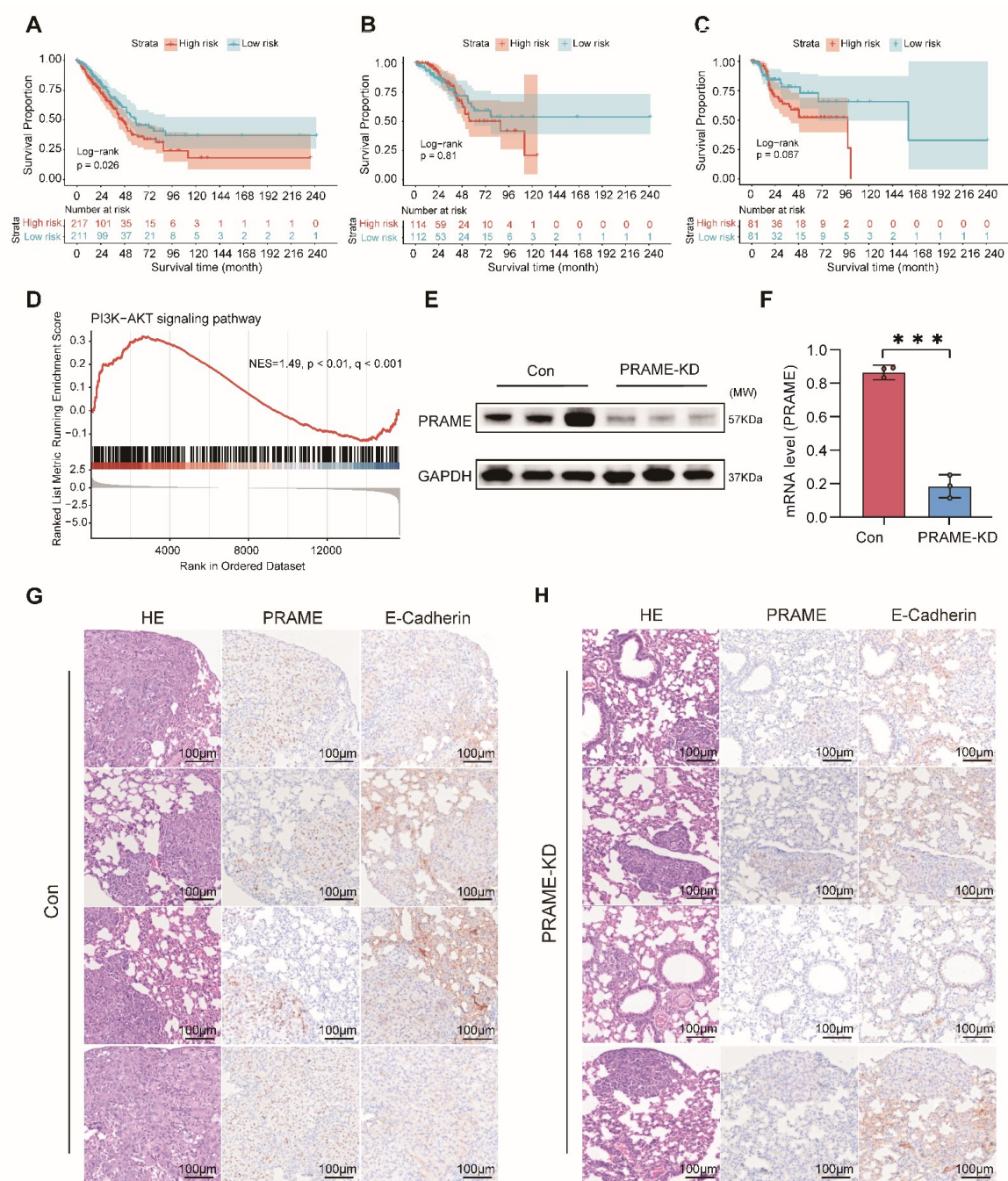


Figure S2 Risk-stratified survival analysis and functional validation of the *PRAME* gene. (A) The Kaplan-Meier plots of overall survival analysis results of all-stages samples in validation cohort (n=428). (B) The Kaplan-Meier plots of overall survival analysis results of early-stage (stage IA/IB) samples in validation cohort (n=226). (C) The Kaplan-Meier plots of disease-free survival (DFS) analysis results of early-stage samples in validation cohort (n=162). (D) The Gene Set Enrichment Analysis (GSEA) plots of PI3K-AKT signaling pathway between high *PRAME* expression group and low *PRAME* expression group in DFS samples at early-stage in validation cohort (NES: Normalized Enrichment Score). (E,F) *PRAME* expressions in the mice lung metastasis model tissues from control and *PRAME*-KD were tested by WB (E) and qRT-PCR (F), GAPDH served as a loading control (mean $\pm$ SD). (G,H) Representative H&E staining and IHC for *PRAME* and E-cadherin in lung metastasis model tissue sections from control (G) and *PRAME*-KD (H) groups. Scale bars: 100 μ m. Statistical significance was determined by unpaired two-tailed Student's t test (F). ***P<0.001.

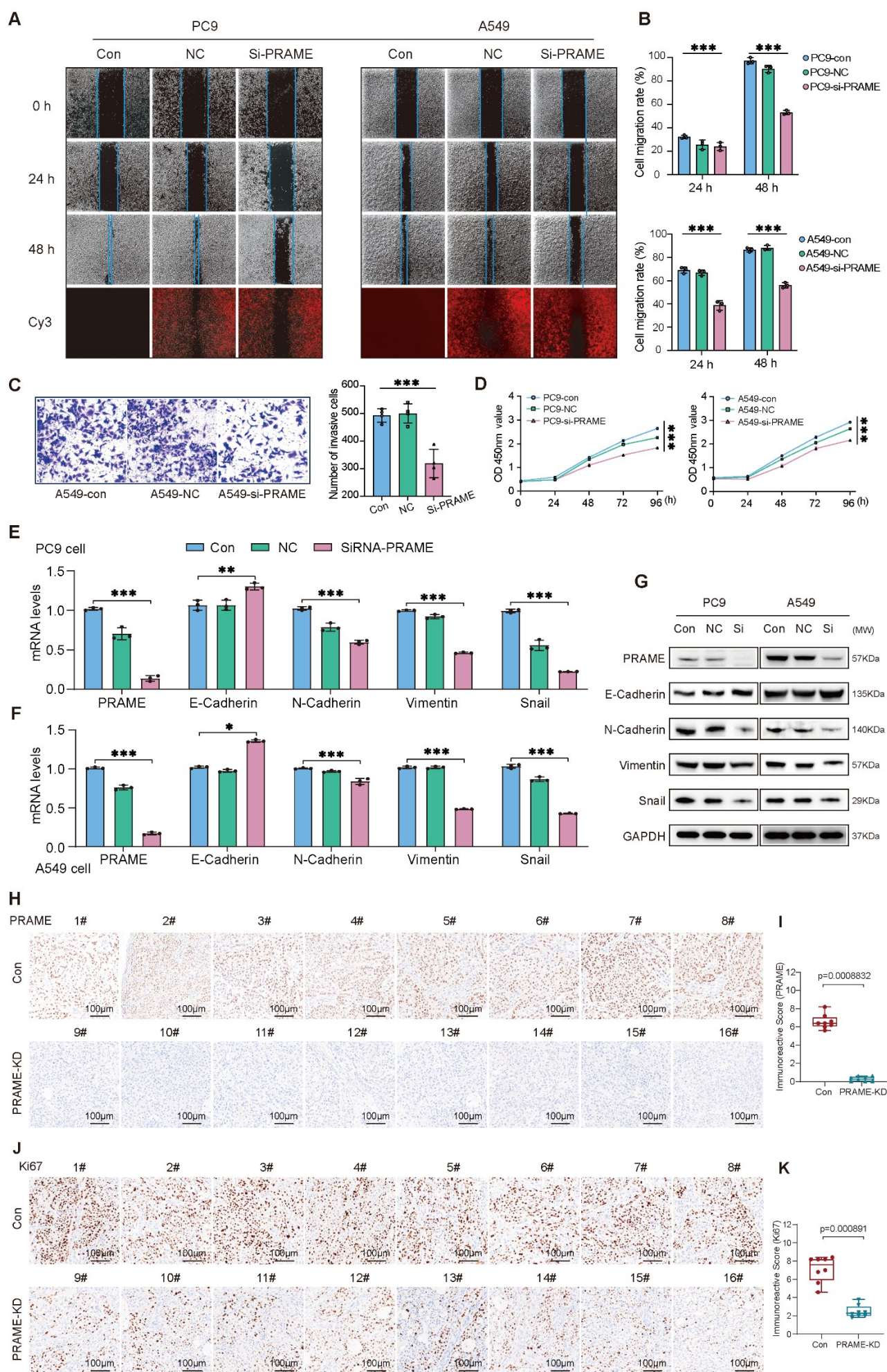


Figure S3 PRAME knockdown inhibits migration, invasion, proliferation and EMT in LUAD cells. (A, B) The migration ability in PC9 cells and A549 cells transfected with siRNA-PRAME via wound healing assay; Cy3 indicates siRNA-PRAME transfection efficiency. (C) Transwell invasion assay showing the invasion capacity in A549 cells upon PRAME knockdown. (D) The proliferation of PC9 and A549 cells after PRAME knockdown by siRNA via CCK-8 assays in 96 hours. (E-G) Expression of PRAME and EMT-related gene (E-cadherin, N-cadherin, Vimentin, Snail) of qRT-PCR analysis (E, F) and WB (G) in PC9 and A549 cells after PRAME knockdown (mean $\pm$ SD). (H,I) Representative IHC images show PRAME expression in subcutaneous xenograft tumor tissues from Con and PRAME-KD groups, with PRAME staining quantified by immunoreactivity score (IRS) (n=8 per group). (J,K) Representative IHC images show ki67 expression in subcutaneous xenograft tumor tissues from Con and PRAME-KD groups, with PRAME staining quantified by immunoreactivity score (IRS) (n=8 per group). Scale bar: 100 μ m. Statistical significance was determined by Kruskal-Wallis H test with post-hoc Dunn's test (B,C,E,F), one-way ANOVA with Bonferroni correction (D) and the Mann-Whitney U test (I, K). *P<0.05, **P<0.01, ***P<0.001

