

Supplementary Figure 1. Identification of Brain Metastasis-Associated Genes in Mouse Models

(A) Heatmap of the top 50 upregulated genes identified from differential expression analysis of the GSE131907 single-cell RNA sequencing dataset, comparing epithelial cells from brain metastases with those from normal lung and primary tumor tissues. (B) Heatmap of the top 50 differentially expressed genes ($|\log_2 \text{FC}| > 1$, $P < 0.05$) in transcriptome sequencing of the brain-metastatic H2030-BrM5 cell line compared to its parental H2030 line.

Supplementary Figure 2. SEC61G Promotes Enhances Proliferation in Lung Cancer Cells. CCK-8 assay showing that overexpression of SEC61G significantly increases the proliferation of H2030 and PC9 cells.

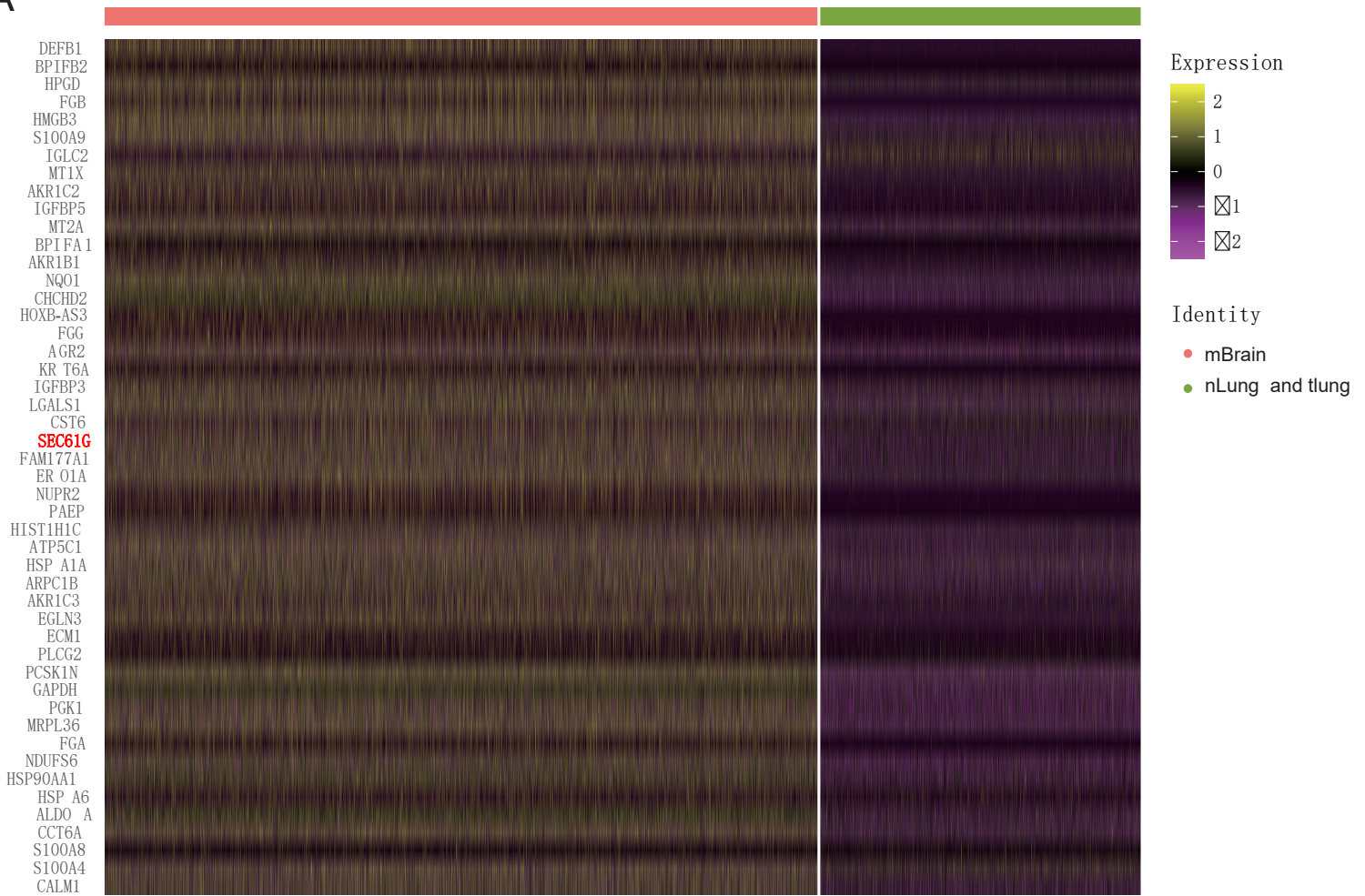
Supplementary Figure 3. SEC61G Expression Correlates with Ubiquitination Related Processes and Protein-Protein Interaction Networks in Lung Cancer Cells.

(A) Transcriptomic analysis of lung cancer cell lines also showed a significant inverse correlation between SEC61G expression and ubiquitination-related biological processes. (B) Protein-protein interaction networks and epigenetic regulatory analyses revealed that SEC61G, PGAM1, and UBE3C are closely linked in processes such as protein ubiquitination and tumor metabolic regulation (Supplementary Figure 2B).

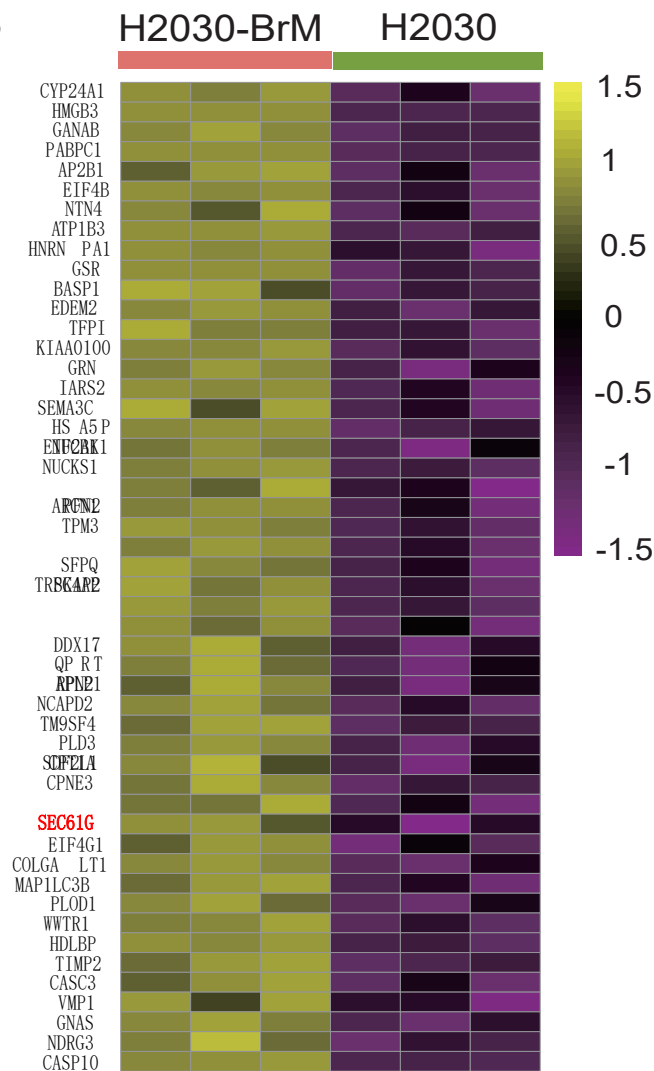
Supplementary Figure 4. Identification of Potential Lysine Ubiquitination Sites on PGAM1 Through Bioinformatic Analysis.

We analyzed predicted ubiquitination sites on PGAM1 (amino acids 100-200) from the PhosphoSitePlus database, identifying five potential lysine ubiquitination sites.

A

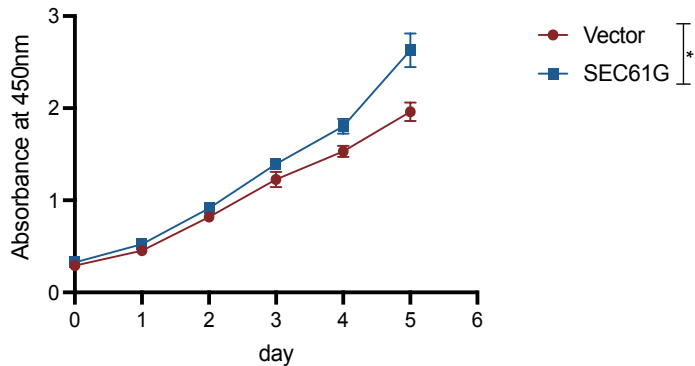


B



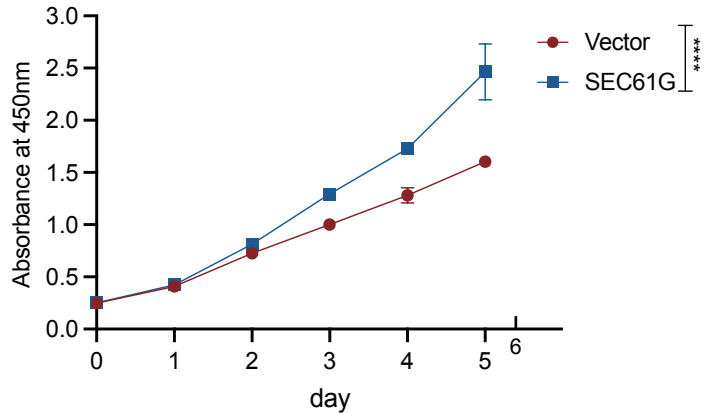
A

H2030



B

PC9



Statistics

Number of ubiquitination sites	5
Number of potential E3 recognizing domain	0
Number of potential E3 recognizing motif	1

Legends

	Known ubiquitination site
	Inferred E3 recognizing domain
	Inferred E3 recognizing motif

Notice: Click the lines for details of ubiquitination site and potential E3 recognizing domain/motif. ?

5 10 15 20 25 30 35 40 45

1 M A A Y K L V L I R H G E S A W N L E N R F S G W Y D A D L S P A G H E E A K R G G Q A L R

47 D A G Y E F D I C F T S V Q K R A I R T L W T V L D A I D Q M W L P V V R T W R L N E R H Y

93 G G L T G L N **K** A E T A A **K** H G E A Q V **K** I W R R S Y D V P P P P M E P D H P F Y S N I S **K**

P.Y.N.S

139 D R R Y A D L T E D Q L P S C E S L **K** D T I A R A L P F W N E E I V P Q I K E G K R V L I A

185 A H G N S L R G I V K H L E G L S E E A I M E L N L P T G I P I V Y E L D K N L K P I K P M

231 Q F L G D E E T V R K A M E A V A A Q G K A K K

Table S1. Primer Sequencing list.

Gene ID	Sequencing (5'3')
PGK1-F	CAAGGTTAAAGCCGAGCCAGCCAA
PGK1-R	GCCTTCTGTGGCAGATTGACTCC
HMOX1-F	AAGACTGCGTTCCTGCTCAAC
HMOX1-R	AAAGCCCTACAGCAACTGTCTG
HK2-F	GATTGTCCGTAACATTCTCATCGA
HK2-R	TGTCTTGAGCCGCTCTGAGAT
PFKL-F	CACAGGTGCCAACATCTTCCGCA
PFKL-R	TCATGTCGGTGCCGCAGAAGTCG
PGAM1-F	ATGATGTCCCACCACCTCCGAT
PGAM1-R	ATCCTTCAGACTCTCACAGGAG
ENO1-F	GCTCCGGGACAATGATAAGACTCG
ENO1-R	CTGTTCCATCCATCTCGATCATC
PKM2-F	CAAAGGACCTCAGCAGCCATGTC
PKM2-R	GGGAAGCTGGGCCAATGGTACAGA
LDHA-F	TGGAGATTCCAGTGTGCCTGTATGG
LDHA-R	CACCTCATAAGCACTCTCAACCACC
ALDOB-F	CACCATTCAAGGGCTTGATGGCCT
ALDOB-R	TTCCTGGATAGCGAGGCTGGAT
CXCL9-F	CCACCCGAACGTCTTATCTAATC
CXCL9-R	GTGGGTCACAGACTCTCAAAT
iNOS-F	GCAGAATGTGACCATCATGG
iNOS-R	ACAACCTTGGTGTTGAAGGC
IL1 β -F	TGATGTTCCATTAGACAGC
IL1 β -R	GAGGTGCTGATGTACCAGTT
TNF α -F	GTAGCCACGTCGTAGCAAA
TNF α -R	CCCTTCTCCAGCTGGGAGAC
CD86-F	TAGGGATAACCAGGCTCTAC
CD86-R	CGTGGGTGTCTTTTGCTGTA

CD206-F	GGACGTGGCTGTGGATAAAT
CD206-R	ACCCAGAAGACGCATGTAAAG
IGF1-F	CAGTTCGTGTGTGGACCAAG
IGF1-R	GTCTTGGGCATGTCAGTGTG
TGFβ1-F	TGAGTGGCTGTCTTTTGACG
TGFβ1-R	GGTTCATGTCATGGATGGTG
CCL2-F	TTCACTGGCAAGATGATCCC
CCL2-R	TGCTTGAGGTGGTTGTGGAA
CCR2-F	ATGCTGTCCACATCTCGTTCTCG
CCR2-R	TTATAAACCAGCCGAGACTTCCTGC

Table S2. Expression of SEC61G and Clinicopathological Characteristics in Lung Adenocarcinoma Tissue Microarray (n=79).

	SEC61G		P value
	High-expression (N=40)	Low-expression low (N=39)	
Sex			0.433
Male	22 (55.0%)	18 (46.2%).	
Female	18 (43.9%)	21 (55.3%)	
Age			0.0137
Mean ± SD	61.5 ± 9.28	55.9 ± 10.4	
Number of lymph node metastases			0.0132
Mean ± SD	2.75 ± 3.04	1.18 ± 1.99	
T stage			<0.001
T1a	2 (5.0%)	17 (43.6%)	
T1b	7 (17.5%)	13 (33.3%)	
T1c	11 (27.5%)	6 (15.4%)	
T2a	10 (25.0%)	1 (2.6%)	
T2b	4 (10.0%)	1 (2.6%)	
T3	4 (10.0%)	1 (2.6%)	
T4	2 (5.0%)	0 (0%)	
N stage			0.116
N0	13 (32.5%)	23 (59.0%)	
N1	15 (37.5%)	10 (25.6%)	
N2	9 (22.5%)	5 (12.8%)	
N3	4 (10.0%)	1 (2.6%)	
M stage			<0.001
M0	21 (52.5%)	35 (89.7%)	
M1	19 (47.5%)	4 (10.3%)	

Table S3. Univariate and Multivariate Cox Regression Analysis of Lung Adenocarcinoma Tissue Microarray (n=79)

Characteristic	Total	Univariate analysis		Multivariate analysis	
		P value	HR (95%CI)	P value	HR (95%CI)
SEC61G	79				
high	40		1.00 (Reference)		1.00 (Reference)
low	39	0.002	0.29 (0.13-0.63)	0.018	0.31 (0.12-0.82)
T stage					
T1a	19		1.00 (Reference)		1.00 (Reference)
T1c	20	0.165	2.17 (0.73-6.49)	0.406	0.55 (0.14-2.24)
T1b	17	0.384	1.67 (0.53-5.27)	0.226	2.24 (0.61-8.30)
T2a	5	0.010	4.91 (1.45-16.59)	0.927	1.08 (0.19-6.16)
T2b	11	0.894	0.86 (0.10-7.40)	0.425	0.33 (0.02-5.02)
T3	5	0.180	2.47 (0.66-9.22)	0.494	0.57 (0.11-2.87)
T4	2	0.045	5.45 (1.04-28.52)	0.153	0.17 (0.02-1.92)
N stage					
N0	36		1.00 (Reference)		1.00 (Reference)
N1	25	<.001	5.92 (2.15-16.32)	<.001	8.18 (2.55-26.28)
N2	14	<.001	6.98 (2.25-21.63)	0.111	2.87 (0.78-10.50)
N3	44	<.001	27.91 (20.58-36.92)	<.001	32.33(20.38-84.22)
M stage					
M0	46		1.00 (Reference)		1.00 (Reference)
M1	23	<.001	4.52 (2.20-9.31)	<.001	10.66(3.06-37.08)

Table S4. Correlation Between SEC61G Expression Levels and Clinicopathological Characteristics in TCGA LUAD

Characteristic	Low expression of	High expression of	P value
n	267	268	
Gender, n (%)			0.968
Female	142 (26.5%)	144 (26.9%)	
Male	125 (23.4%)	124 (23.2%)	
Age, n (%)			0.094
<=65	117 (22.7%)	138 (26.7%)	
>65	140 (27.1%)	121 (23.4%)	
T stage, n (%)			0.349
T1	97 (18.2%)	78 (14.7%)	
T2	136 (25.6%)	153 (28.8%)	
T3	23 (4.3%)	26 (4.9%)	
T4	9 (1.7%)	10 (1.9%)	
N stage, n (%)			0.023
N0	182 (35.1%)	166 (32%)	
N1-3	72 (13.9%)	99 (19.1%)	
M stage, n (%)			0.547
M0	172 (44.6%)	189 (49%)	
M1	14 (3.6%)	11 (2.8%)	
Stage, n (%)			0.144
Stage I	155 (29.4%)	139 (26.4%)	
Stage II	60 (11.4%)	63 (12.0%)	
Stage III	33 (6.3%)	51 (9.7%)	
Stage IV	15 (2.8%)	11 (2.1%)	
OS event, n (%)			< 0.001
Alive	196 (36.6%)	147 (27.5%)	
Dead	71 (13.3%)	121 (22.6%)	
DSS event, n (%)			0.006
Alive	205 (41.1%)	174 (34.9%)	
Dead	47 (9.4%)	73 (14.6%)	
PFI event, n (%)			0.007
Alive	170 (31.8%)	139 (26.0%)	
Dead	97 (18.1%)	129 (24.1%)	

Table S5. Univariate and Multivariate Cox Regression Analysis in TCGA-LUAD

Characteristics	Total(n)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95%	P value	Hazard ratio (95%	P value
T1 vs T2	282	1.521 (1.068-2.166)	0.020	1.521	0.069
T1 vs T3&T4	66	3.066 (1.950-4.823)	<0.001	2.574	0.001
N0 vs N1	94	2.382 (1.695-3.346)	<0.001	1.983	<0.001
N0 vs N2&N3	73	2.968 (2.040-4.318)	<0.001	2.437	<0.001
M0 vs M1	25	2.136 (1.248-3.653)	0.006	1.662	0.089
SEC61G	526	1.561 (1.295-1.881)	<0.001	1.338	0.018