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Supplemental information

lncRNA GAS6-AS1 inhibits progression and glucose metabolism reprogramming in LUAD via repressing E2F1-mediated transcription of GLUT1

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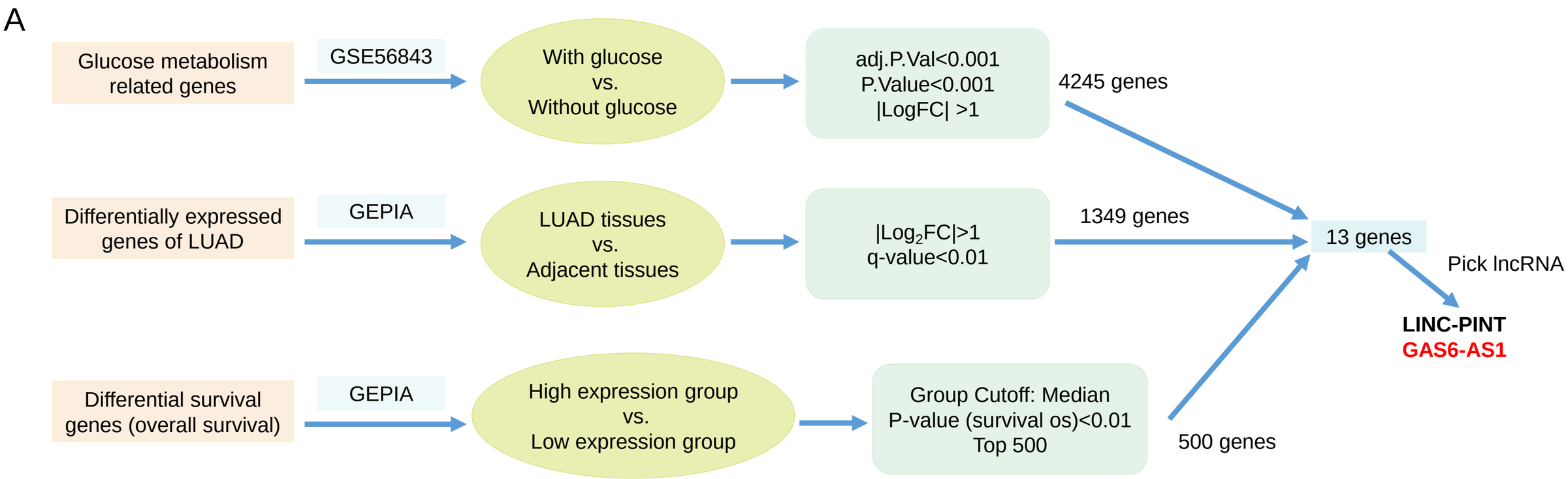
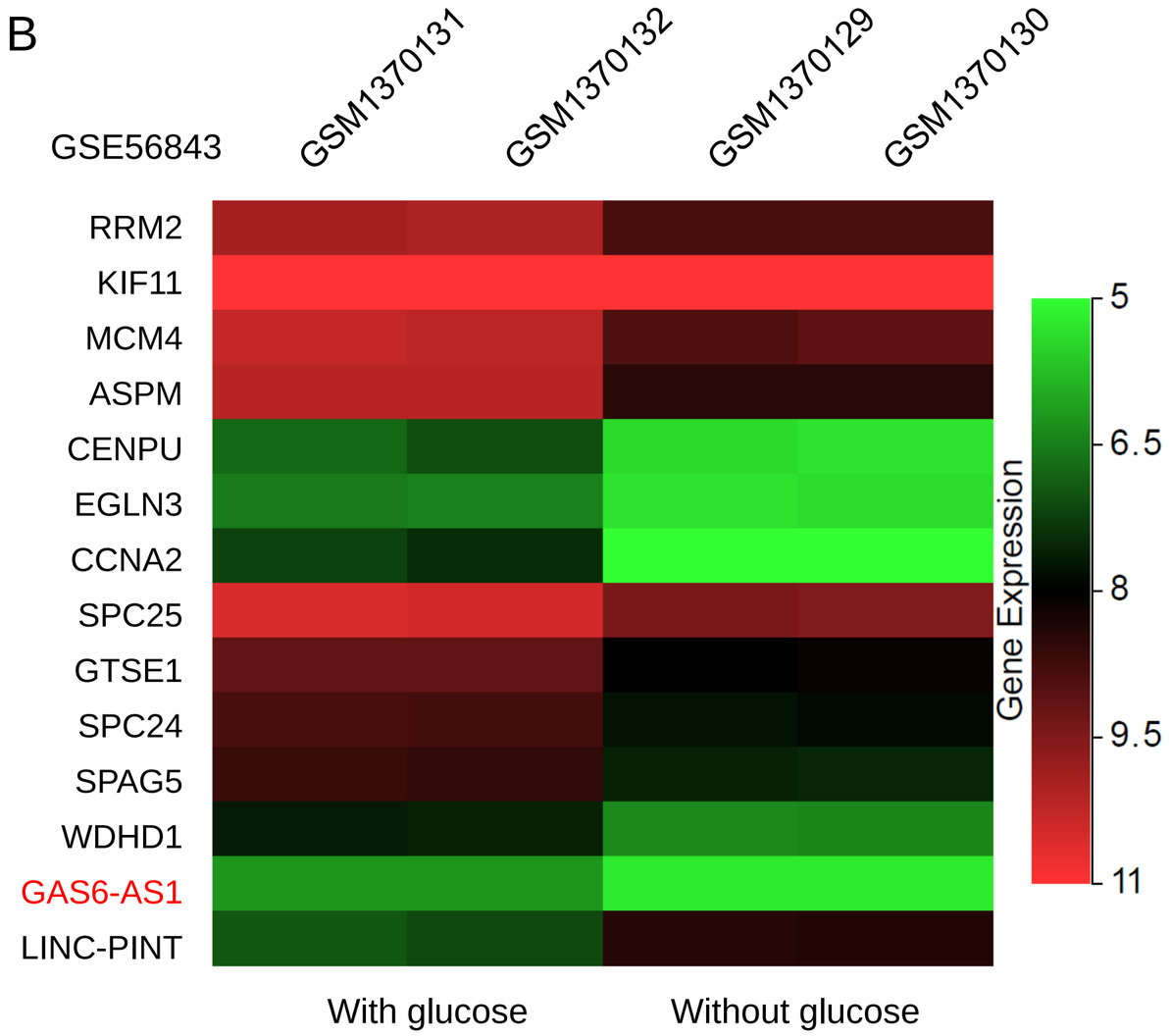
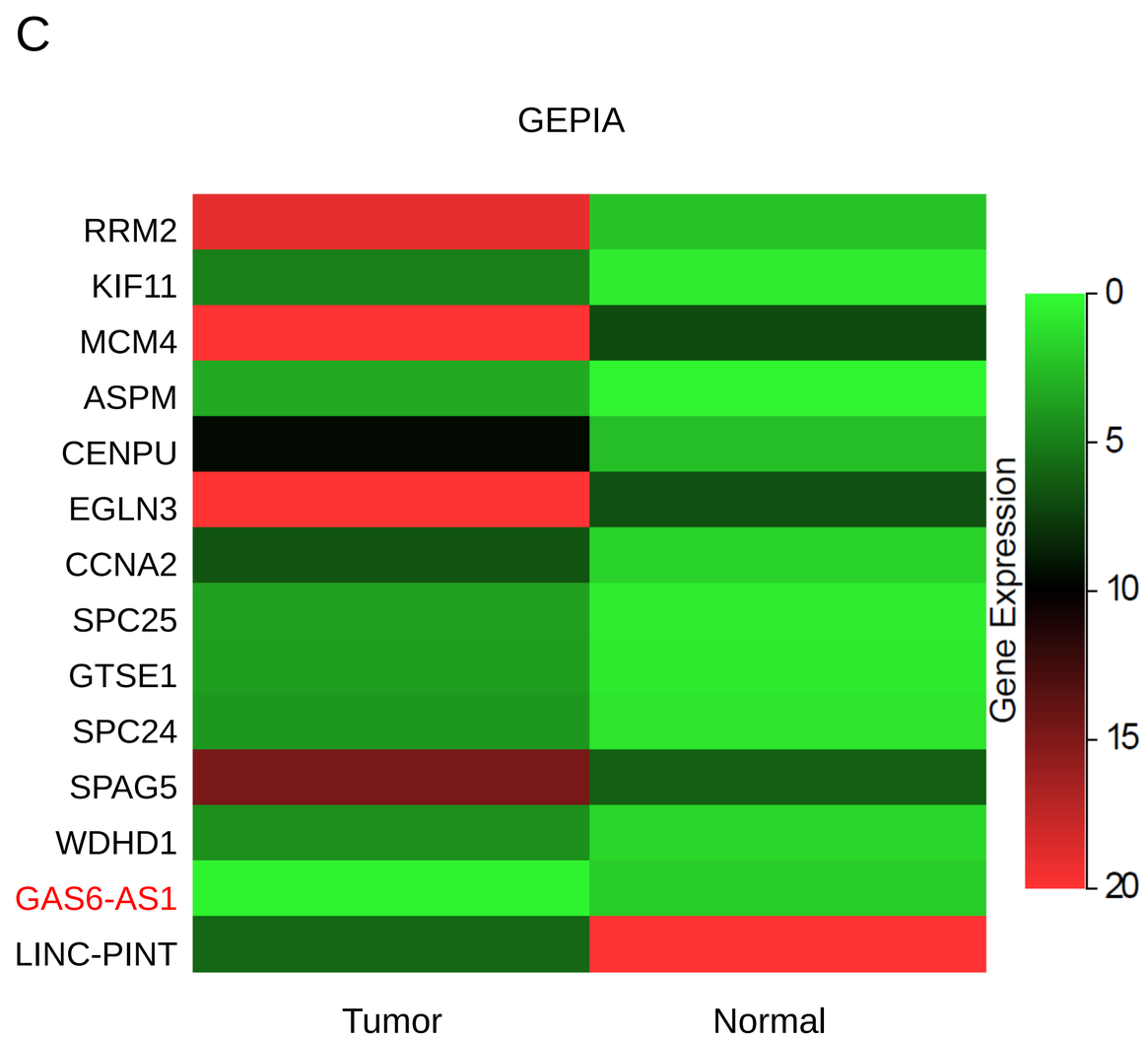
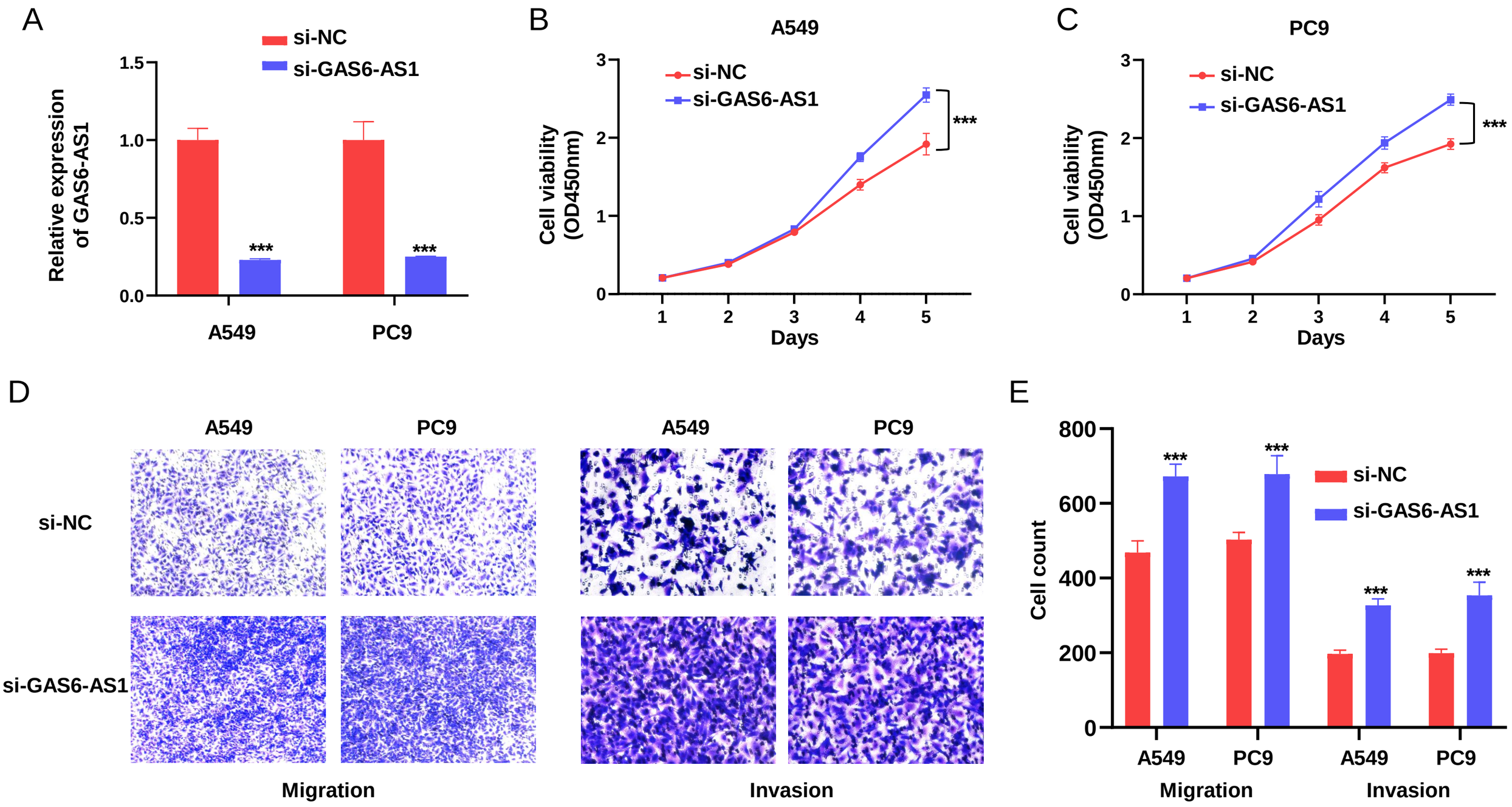


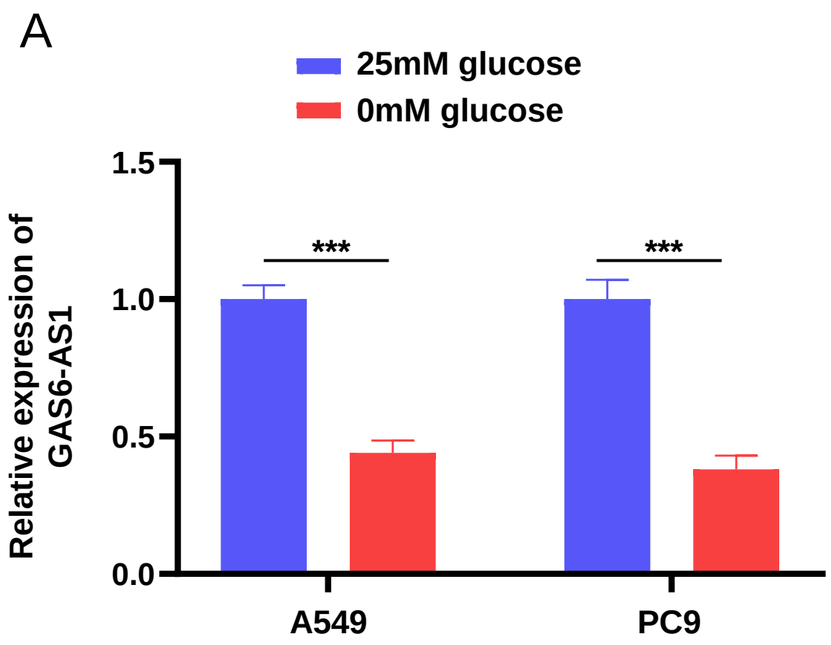


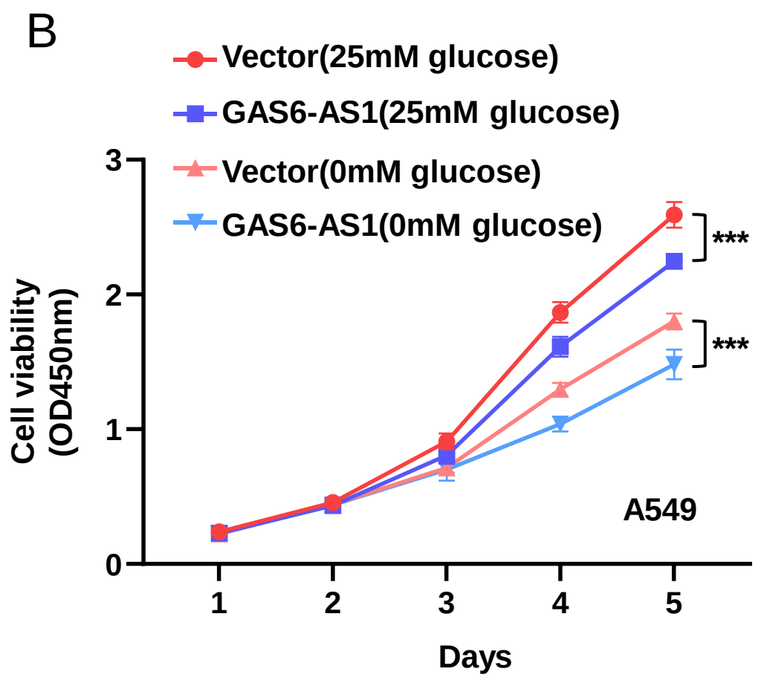


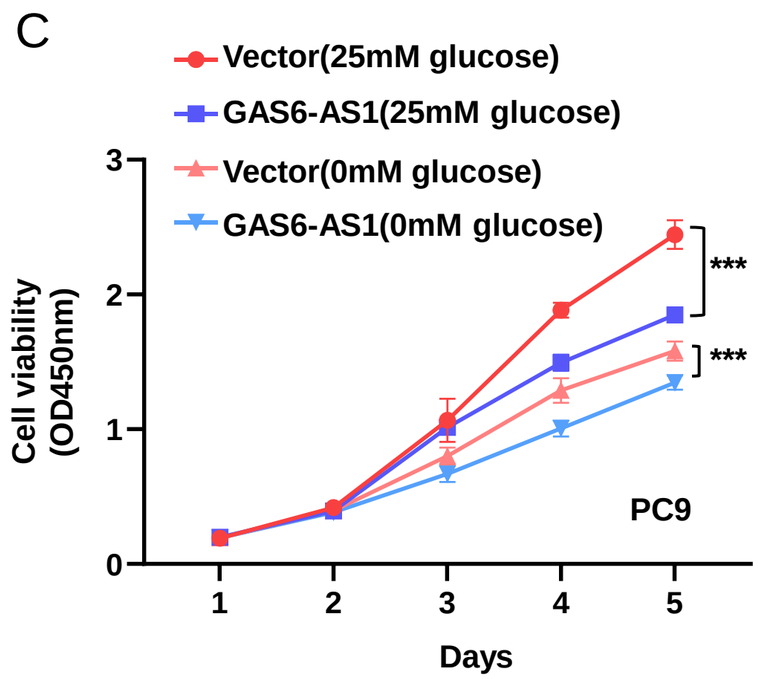
FigureS1. A. The flow chart for selecting lncRNAs that are abnormally expressed in glucose-free A549 cells and LUAD tissues, and meanwhile correlated with overall survival of LUAD patients. B. Heatmap of candidate genes that are abnormally expressed in A549 cells cultured with glucose and without glucose from GEO dataset (GSE56843). C. Heatmap of candidate genes that are abnormally expressed in LUAD tissues and normal tissues from GEPIA dataset.

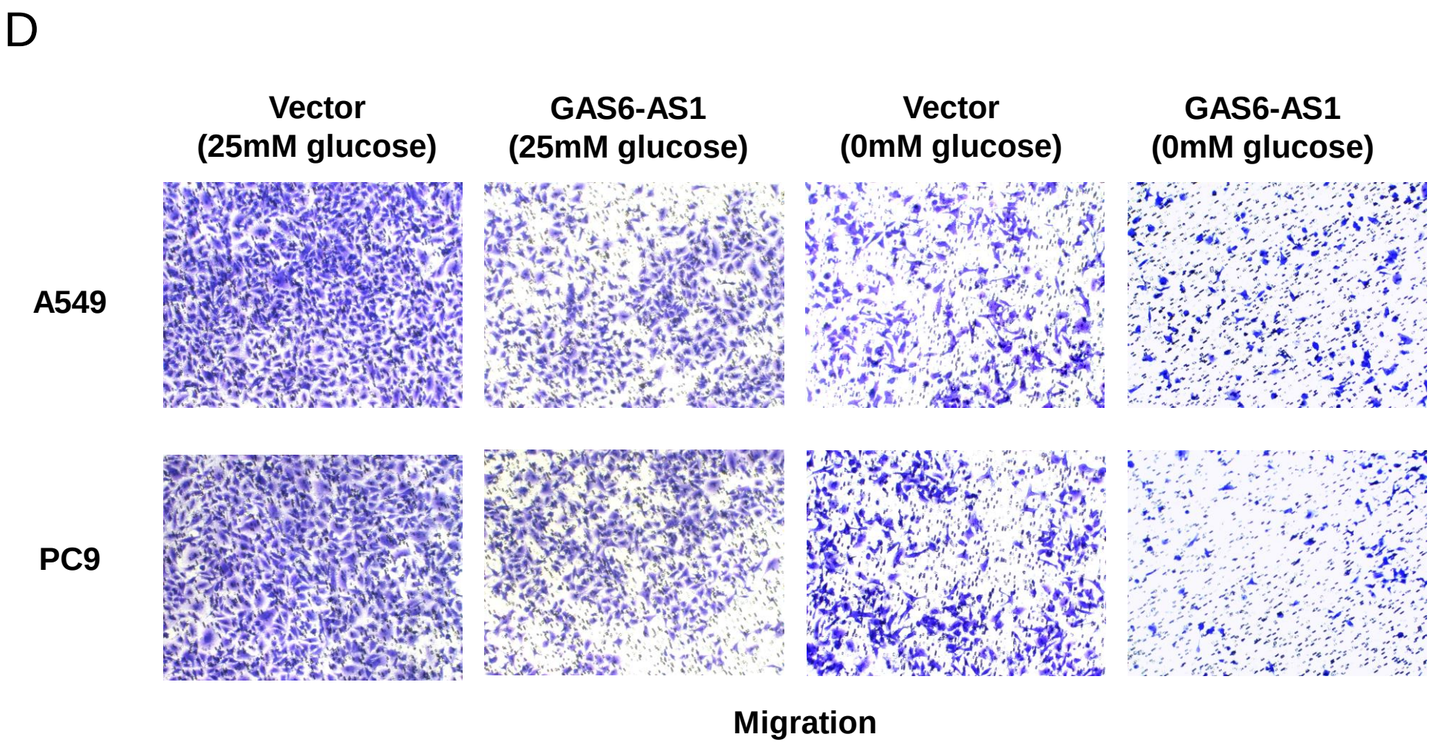


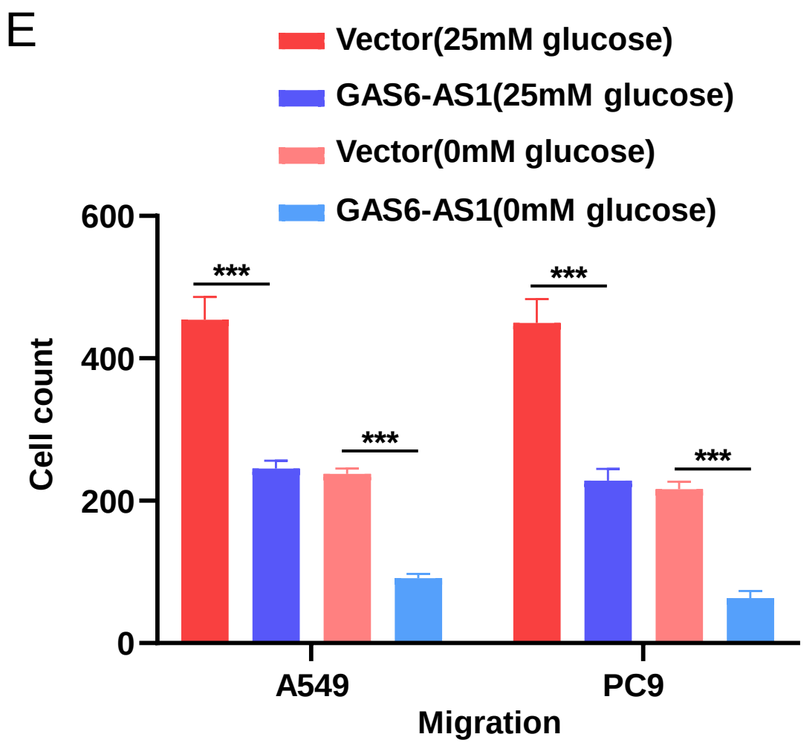
FigureS2. A. Transfection efficiency of siRNA targeting GAS6-AS1 was verified by qRT-PCR. B-C. CCK8 assays revealed that knockdown of GAS6-AS1 promoted proliferation of A549 and PC9 cells. D-E. Transwell and Matrigel assays indicated that knockdown of GAS6-AS1 promoted migration and invasion of A549 and PC9 cells.

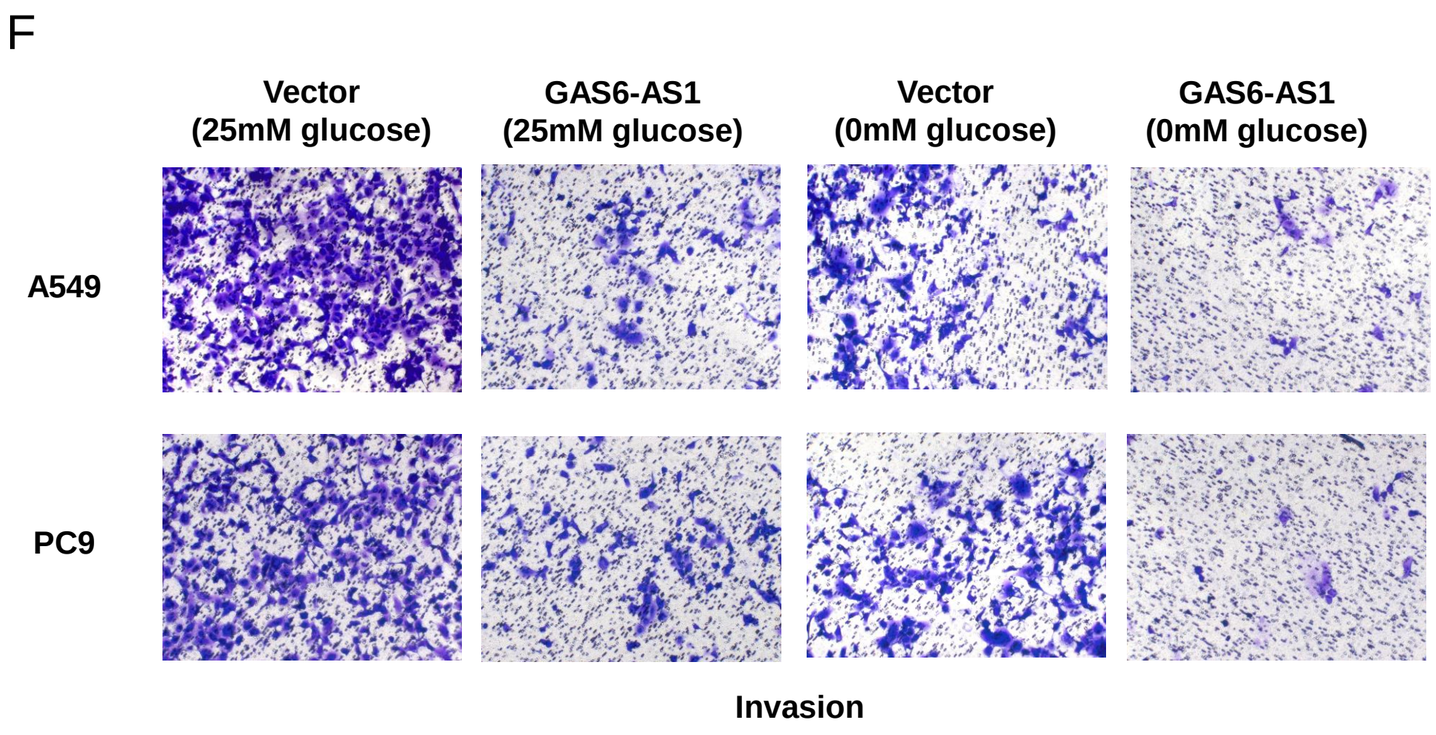


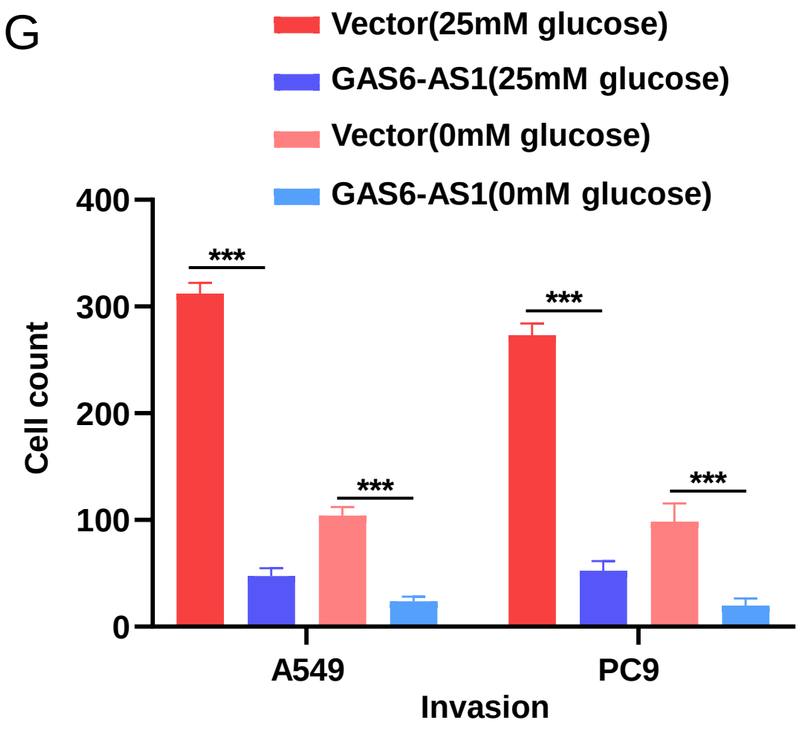




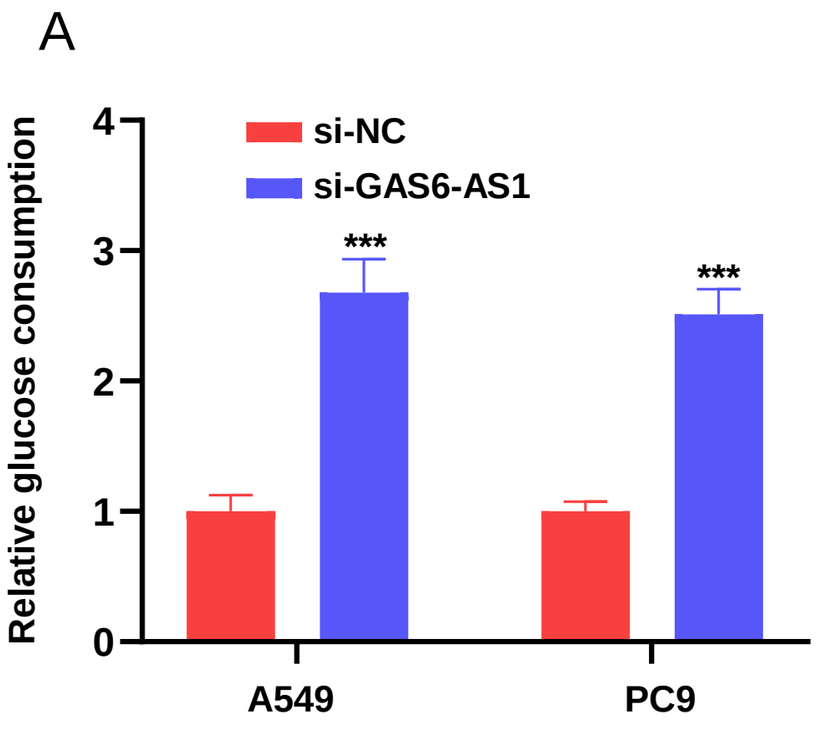


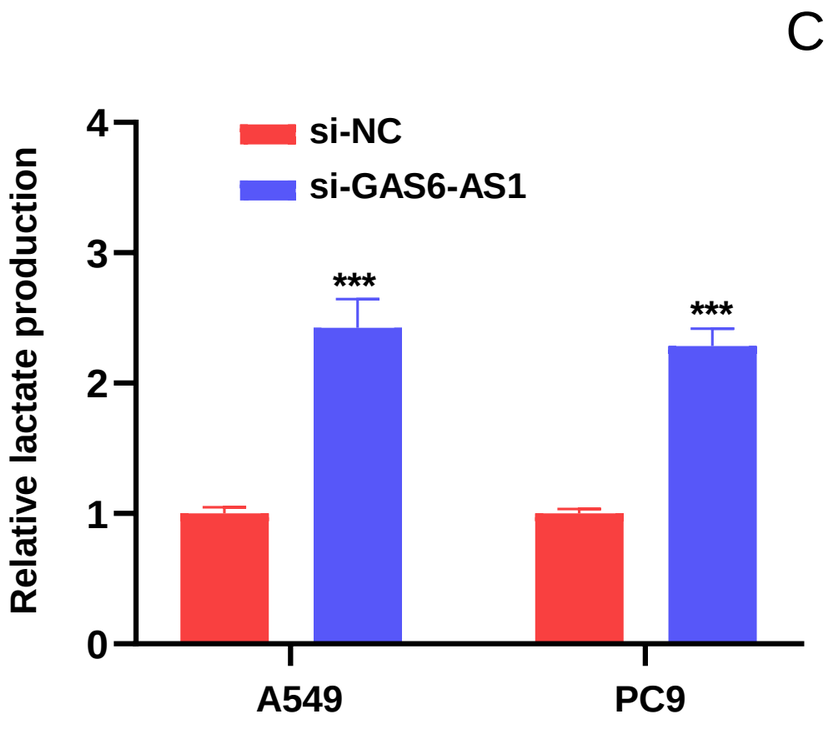


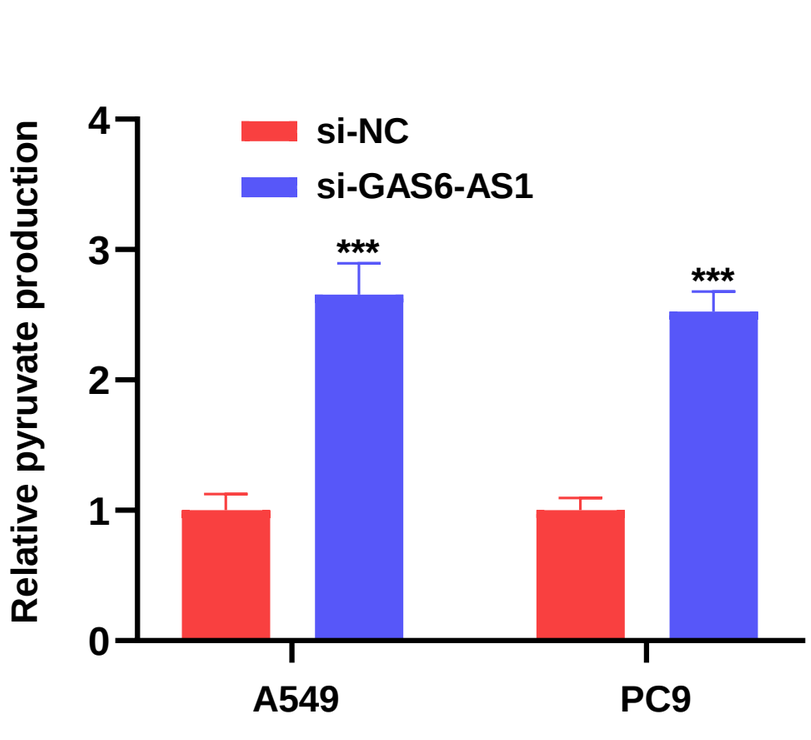


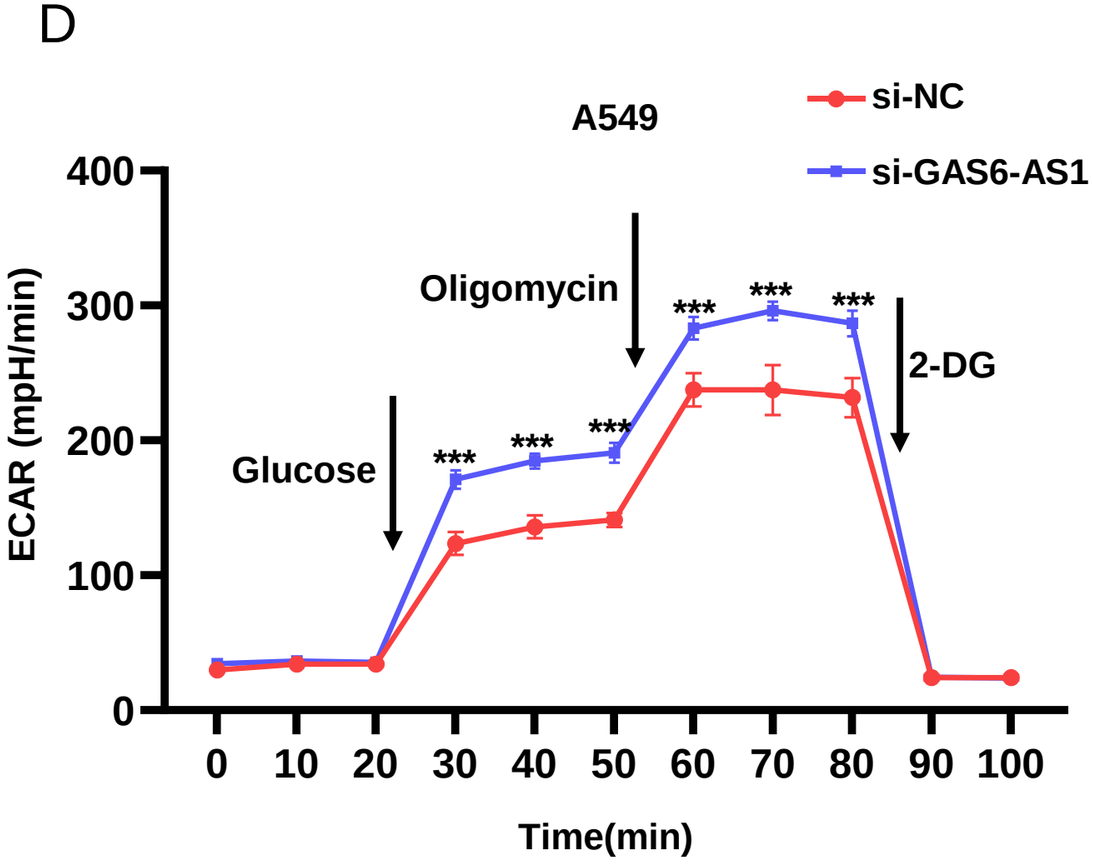


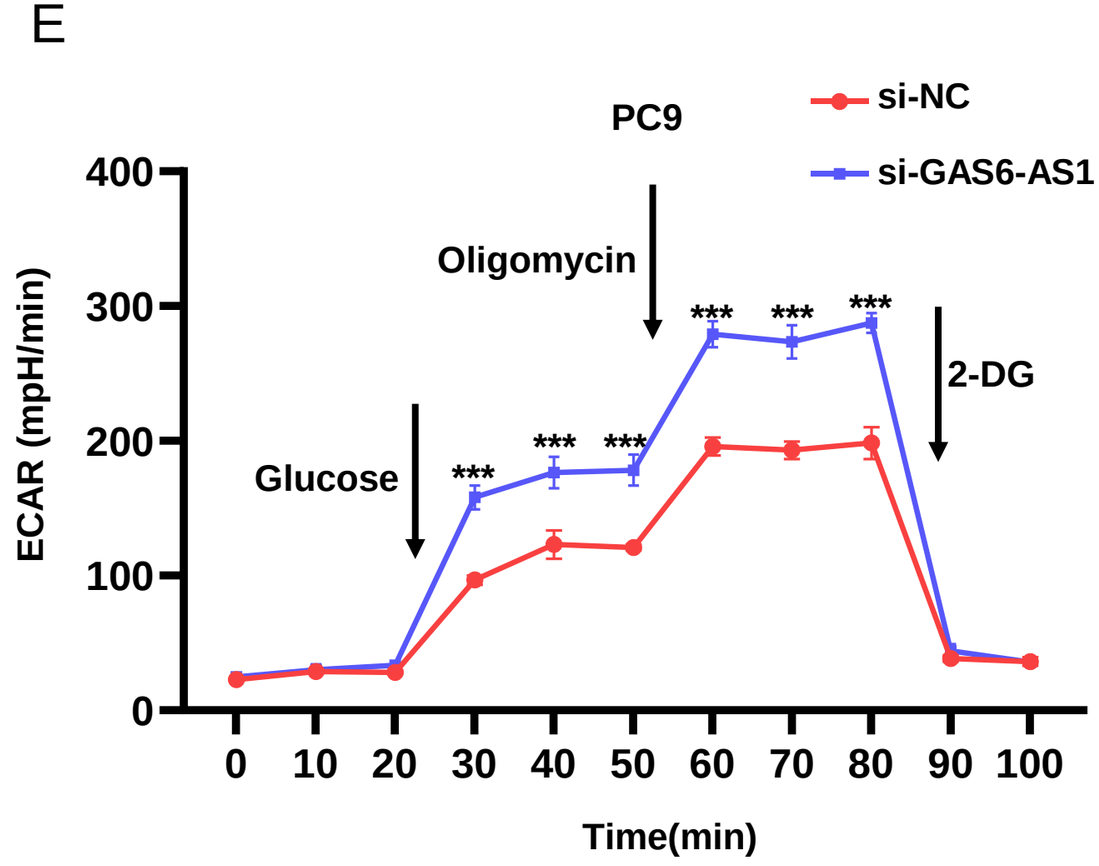
FigureS3. A. GAS6-AS1 was downregulated by glucose starvation (0mM) in A549 and PC9 cells. B-C. Ectopic expression of GAS6-AS1 inhibited proliferation of A549 and PC9 cells in both glucose-sufficient and glucose-free conditions. D-G. Ectopic expression of GAS6-AS1 inhibited migration and invasion of A549 and PC9 cells in both glucose-sufficient and glucose-free conditions.





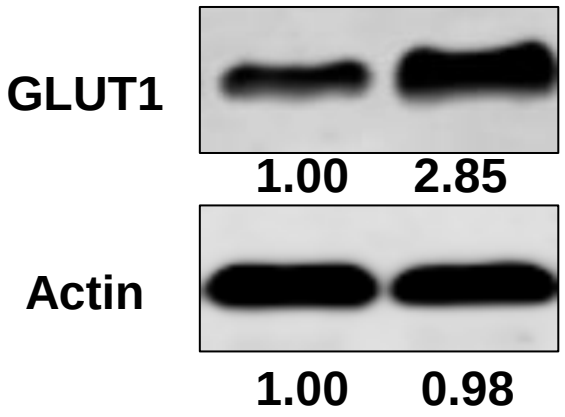






FigureS4. A-C. Knockdown of GAS6-AS1 promoted glucose consumption, lactate production and pyruvate production of A549 and PC9 cells. D-E. Seahorse analysis revealed that knockdown of GAS6-AS1 increased extracellular acidification rate (ECAR) of A549 and PC9 cells.

A



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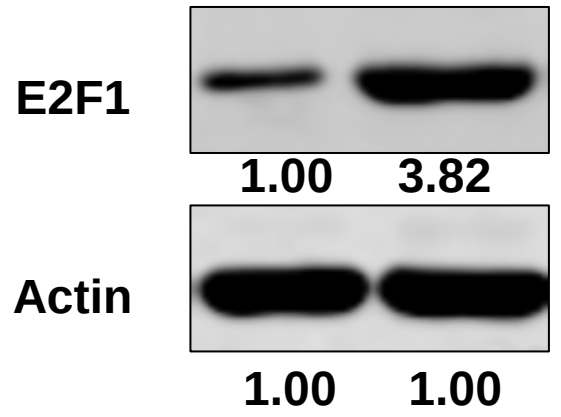

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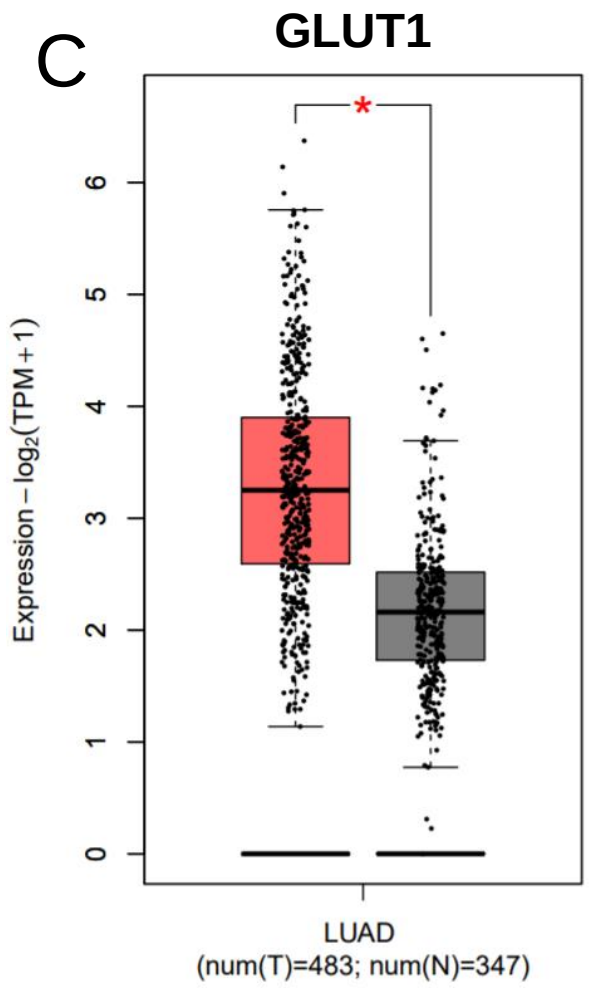
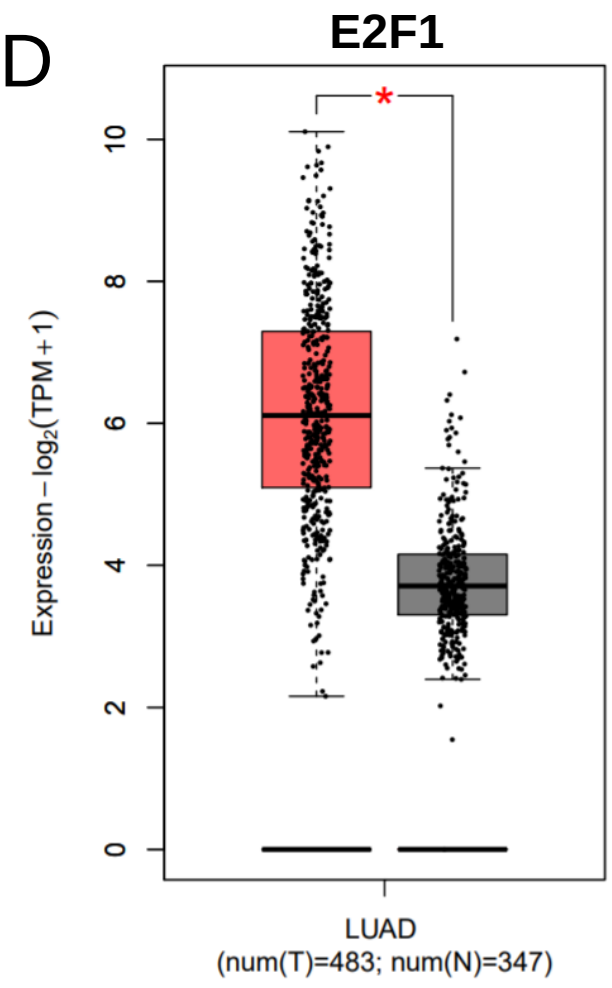
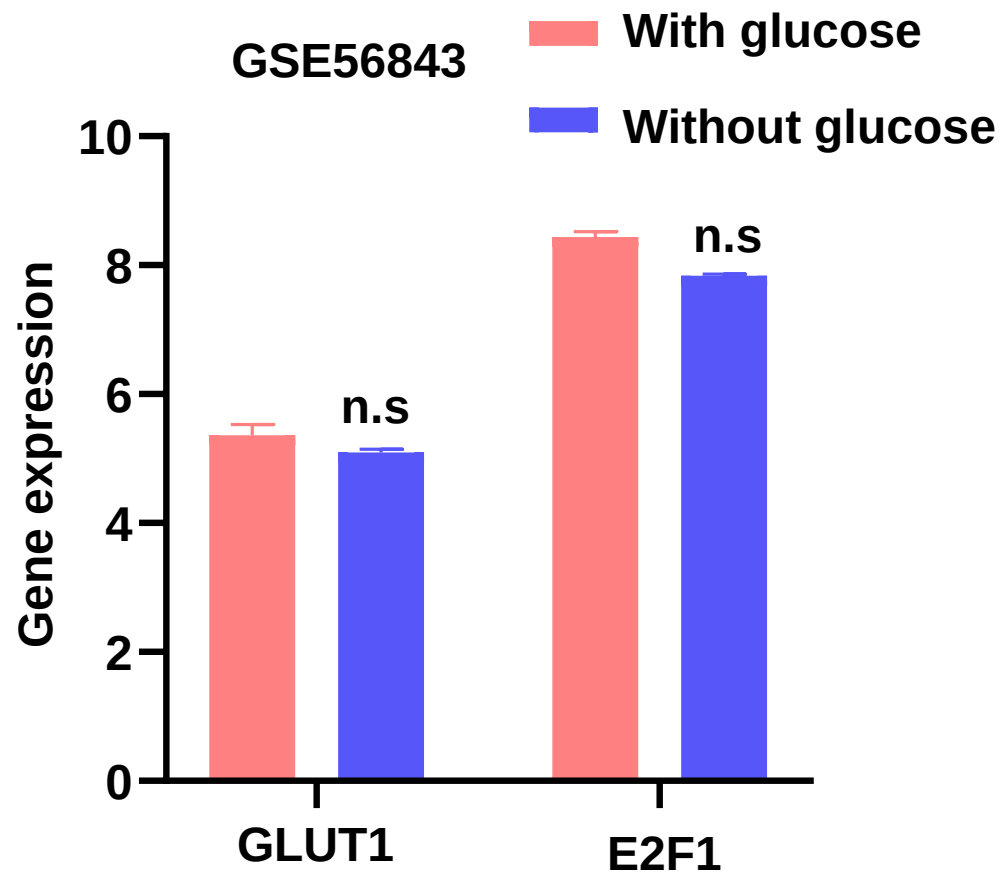


D



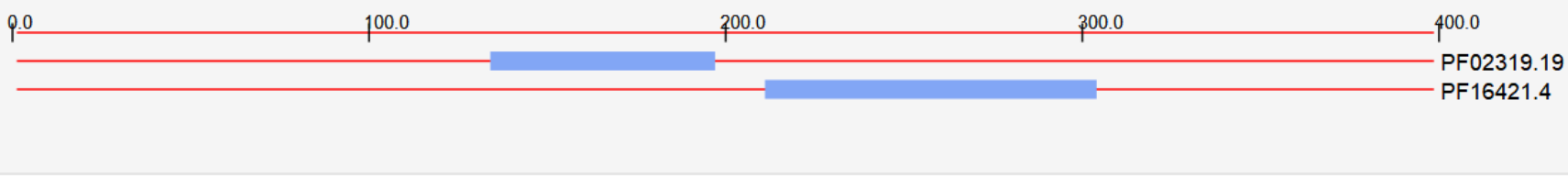
E



F

Protein Functional Domain

Protein ID	Start	End	Pfam ID	HMM Name	Type	HMM Start	HMM End	HMM Length	Score	Evalue
ENSP00000345571	206	299	PF16421.4	E2F_CC-MB	Domain	1	95	95	124	2.2e-36
ENSP00000345571	129	192	PF02319.19	E2F_TDP	Domain	2	71	71	80.3	7.2e-23



FigureS5. A. The western blot data for overexpression of GLUT1 in A549 cells. B. The western blot data for overexpression of E2F1 in A549 cells. C-D. GLUT1 and E2F1 were upregulated in LUAD tissues. E. The expression of GLUT1 and E2F1 were not altered under glucose-free conditions in LAUD cells. F. Protein function domain of E2F1 was presented.

A

Human (hg38): The binding site of E2F1 located within 1kb downstream of SLC2A1 gene in (sample HUMHG04230)

E2F1: binding site[1]	Regulatory range	within downstream 1kb
	Gene reference id	ENSG00000117394.19
	Official gene symbol	*SLC2A1*
	TSS	chr1:42959173
	Gene type	protein_coding
	E2F1 binding locus	chr1:42958143-42959037, Summit: 42958590
	Binding site distance	-583
	Motif locus	chr1:42958609-42958622[-], Summit: 42958616
	Motif distance	42958616

B

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End
MA0024.2	E2F1	12.048	0.949280746886	GLUT1	1221	1231
MA0024.2	E2F1	6.24431	0.861965549557	GLUT1	156	166
MA0024.2	E2F1	5.46131	0.850185526374	GLUT1	1150	1160
MA0024.2	E2F1	5.271	0.847322335162	GLUT1	974	984
MA0024.2	E2F1	5.09514	0.844676663351	GLUT1	1237	1247
MA0024.2	E2F1	5.06681	0.844250434081	GLUT1	71	81
MA0024.2	E2F1	5.06681	0.844250434081	GLUT1	1253	1263

FigureS6. A. The targeting of E2F1 with GLUT1 gene in ChIPBase. B. E2F1 potentially bound to the promoter region of GLUT1 in JASPAR.

TableS1. Correlation between GAS6-AS1 expression and clinicalpathological characteristics of LUAD.

Characteristics	low GAS6-AS1 expression group (n=40)	high GAS6-AS1 expression group (n=40)	χ^2	p-Value
Tumor diameter			**9.141**	**0.002**
>3cm	21	19		
⩽3cm	8	32		
Lymph node metastasis			**5.051**	**0.0246**
Yes	23	13		
No	17	27		
Tumor metastasis			**5.000**	**0.0253**
Yes	7	1		
No	33	39		
Tumor stage			**4.528**	**0.0333**
III-IV	18	9		
I-II	22	31		

TableS2: Gene clusters for GO enrichment analysis

TableS3: Primers used for qRT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
ACTIN	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
GAS6-AS1	GTGGGTACTGCATTCCTACCG	CTCTCCTCTGATGGCAGGAC
GLUT1	GGCCAAGAGTGTGCTAAAGAA	ACAGCGTTGATGCCAGACAG
GLUT2	GCTGCTCAACTAATCACCATGC	TGGTCCCAATTTTGAAAACCCC
GLUT3	GCTGGGCATCGTTGTTGGA	GCACTTTGTAGGATAGCAGGAAG
GLUT4	TGGGCGGCATGATTTCCTC	GCCAGGACATTGTTGACCAG
GLUT5	GAGGCTGACGCTTGTGCTT	CCACGTTGTACCCATACTGGA
HK2	TGCCACCAGACTAAACTAGACG	CCCGTGCCCACAATGAGAC
ALDOC	ATGCCTCACTCGTACCCAG	TTTCCACCCCAATTTGGCTCA
ENO1	AAAGCTGGTGCCGTTGAGAA	GGTTGTGGTAAACCTCTGCTC
PKM	AGGGCACTGGGCTGTTGTTC	TGAGTGGAGGGTGGGGACAG
LDH	TCCGGATCTCATTGCCACGC	GCCATGCCAACAGCACCAAC
U6	CTTCGGCAGCACATATACTAAAA	CGCTTCACGAATTTGCGTGTCAT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC
GLUT1 promoter	AACGCAGAGAGAACGAGCCG	GTGTGTCAGGGGTGTGTGGG