

Table S1. Enriched top 10 gene ontology annotations for biological process in transcriptome of S/G-deprived U87MG cells, compared to control

Biological processes	-log(p-value)
cellular response to starvation (GO:0009267)	6.40
regulation of autophagy (GO:0010506)	5.51
positive regulation of apoptotic process (GO:0043065)	5.13
regulation of apoptotic process (GO:0042981)	5.10
neutral amino acid transport (GO:0015804)	4.83
cellular response to amino acid starvation (GO:0034198)	4.70
serine family amino acid biosynthetic process (GO:0009070)	4.62
response to amino acid starvation (GO:1990928)	4.61
autophagy of mitochondrion (GO:0000422)	4.56
glycolipid transport (GO:0046836)	4.50

Table S2. Enriched top 10 gene ontology annotations for molecular function in transcriptome of S/G-deprived U87MG cells, compared to control

Molecular functions	-log(p-value)
neutral amino acid transmembrane transporter activity (GO:0015175)	5.81
collagen receptor activity (GO:0038064)	4.50
microtubule binding (GO:0008017)	4.37
insulin-like growth factor II binding (GO:0031995)	3.99
insulin-like growth factor binding (GO:0005520)	3.81
L-amino acid transmembrane transporter activity (GO:0015179)	3.76
L-glutamine transmembrane transporter activity (GO:0015186)	3.59
L-leucine transmembrane transporter activity (GO:0015190)	3.53
insulin-like growth factor I binding (GO:0031994)	3.30
L-aspartate transmembrane transporter activity (GO:0015183)	3.27

Table S3. Enriched top 10 gene ontology annotations for cellular component in transcriptome of S/G-deprived U87MG cells, compared to control

Cellular components	-log(p-value)
lysosome (GO:0005764)	8.90
lysosomal lumen (GO:0043202)	7.77
lytic vacuole (GO:0000323)	7.00
vacuolar lumen (GO:0005775)	6.19
microtubule (GO:0005874)	4.92
secondary lysosome (GO:0005767)	4.56
autolysosome (GO:0044754)	4.11
autophagosome (GO:0005776)	4.03
lytic vacuole membrane (GO:0098852)	3.93
lysosomal membrane (GO:0005765)	3.79

Table S4. Differentially expressed transcriptome of S/G-deprived U87MG cells, compared to control, in trimmed IPA network

Entrez gene name	Symbol	Entrez Gene ID for human	Location	Signal fold change ^a
aconitase 1	ACO1	48	Cytoplasm	1.30
androgen receptor	AR	367	Nucleus	1.44
aryl hydrocarbon receptor nuclear translocator 2	ARNT2	9915	Nucleus	1.47
activating transcription factor 4	ATF4	468	Nucleus	2.63
aurora kinase A	AURKA	6790	Nucleus	-1.31
basic helix-loop-helix family member e40	BHLHE40	8553	Nucleus	1.33
caveolin 1	CAV1	857	Plasma Membrane	-1.34
cyclin D1	CCND1	595	Nucleus	-1.35
CD274 molecule	CD274	29126	Plasma Membrane	-2.04

cyclin dependent kinase 1	CDK1	983	Nucleus	-1.32
cyclin dependent kinase 2	CDK2	1017	Nucleus	-1.43
DExD-box helicase 39A	DDX39A	10212	Nucleus	-1.42
histone deacetylase 5	HDAC5	10014	Nucleus	1.81
hypoxia inducible factor 3 subunit alpha	HIF3A	64344	Nucleus	1.53
hexokinase 2	HK2	3099	Cytoplasm	1.62
KLF transcription factor 6	KLF6	1316	Nucleus	-1.42
methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2,	MTHFD2	10797	Cytoplasm	2.39
methenyltetrahydrofolate cyclohydrolase				
MYB proto-oncogene, transcription factor	MYB	4602	Nucleus	-1.38
necdin, MAGE family member	NDN	4692	Nucleus	1.42
N-myc downstream regulated 1	NDRG1	10397	Nucleus	1.80
nuclear receptor subfamily 4 group A member 1	NR4A1	3164	Nucleus	1.60

6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	PFKFB2	5208	Cytoplasm	2.40
phosphoglycerate dehydrogenase	PHGDH	26227	Cytoplasm	3.26
Pim-2 proto-oncogene, serine/threonine kinase	PIM2	11040	Nucleus	-1.40
PPARG coactivator 1 alpha	PPARGC1A	10891	Nucleus	1.71
phosphoserine aminotransferase 1	PSAT1	29968	Cytoplasm	4.92
phosphoserine phosphatase	PSPH	5723	Cytoplasm	1.43
serine hydroxymethyltransferase 2	SHMT2	6472	Cytoplasm	1.69
solute carrier family 29 member 1 (Augustine blood group)	SLC29A1	2030	Plasma Membrane	-1.70
solute carrier family 2 member 1	SLC2A1	6513	Plasma Membrane	1.30
solute carrier family 2	SLC2A3	6515	Plasma	1.34

member 3			Membrane	
sequestosome 1	SQSTM1	8878	Cytoplasm	1.78
signal transducer and activator of transcription 2	STAT2	6773	Nucleus	1.75
tumor protein p53	TP53	7157	Nucleus	1.57
tribbles pseudokinase 3	TRIB3	57761	Nucleus	5.89
thioredoxin	TXN	7295	Cytoplasm	1.36
ubiquitin C	UBC	7316	Cytoplasm	1.31
exportin for tRNA	XPOT	11260	Nucleus	1.41

^aNormalized signal fold change in S/G-deprived U87MG cells group to corresponding signal of control group

Figure S1 (related to Figure 1). DEGs network analyses of transcriptome from U87MG cells in response to serine/glycine deprivation

(A) Analysis of DEGs network using IPA in S/G-deprived U87MG cells, compared to control. The analysis involved a fold change cut-off value of ± 1.3 . Red and green colors indicate genes that were upregulated and downregulated, respectively, compared to the control. Details for shape, which originate from Ingenuity Systems (<http://www.ingenuity.com>).

(B) Analysis of trimmed DEGs network with biological functions, including cell proliferation, cell survival, and glucose metabolism disorder, using IPA in S/G-deprived U87MG cells, compared to control. The analysis involved a fold change cut-off value of ± 1.3 . Red and green colors indicate genes that were upregulated and downregulated, respectively, compared to the control. Details for shape, which originate from Ingenuity Systems (<http://www.ingenuity.com>), are provided in Figure S1A.

(C) Analysis of trimmed DEGs network with biological functions, including cell proliferation, cell survival, L-serine biosynthetic process, glycolytic process, and glucose import, using IPA in S/G-deprived U87MG cells, compared to control. The analysis involved a fold change cut-off value of ± 1.3 . Red and green colors indicate genes that were upregulated and downregulated, respectively, compared to the control. Relationships between L-serine biosynthetic process and genes are highlighted with magenta. Details for shape, which originate from Ingenuity Systems (<http://www.ingenuity.com>), are provided in Figure S1A.

Figure S2 (related to Figure 2). Serine/glycine deprivation induces glucose uptake, glycolytic flux, and *de novo* serine biosynthesis

(A) Indicated cells were cultured with or without S/G(-) media for 24 h in the presence or

absence of exogenous serine (400 μ M) and/or glycine (400 μ M). Immunoblotting analyses were performed with the indicated antibodies.

(B) LN18 cells were cultured in complete media for 24 h in the presence or absence of serine transporter inhibitor (4-Fluoro-L-2-phenylglycine; 50 μ M) and/or glycine transporter inhibitor (Sarcosine; 50 μ M). Immunoblotting analyses were performed with the indicated antibodies.

Figure S3 (related to Figure 3). Serine synthesis pathway genes are overexpressed in gliomas and required for brain tumor growth

(A) GEO dataset (GSE4290) analysis of *PSAT1* and *PSPH* mRNA expression in normal (n = 23) and brain tumor (n = 157) tissues.

(B) LN18 cells with or without the depletion of SSP genes were cultured with or without S/G(-) media for the indicated periods of time, and harvested for cell counting.

(C) LN18 cells with or without the depletion of SSP genes were cultured with or without S/G(-) media for 4 d, and stained with Annexin V. Representative staining (top panel) and quantification of the staining (bottom panel) are shown. Scale bar, 20 μ m.

(D) U87MG cells with the depletion of SSP genes, and with or without the reconstituted expression of SSP genes, were cultured with S/G(-) media for the indicated periods of time, and harvested for cell counting.

The data represent the mean $\pm$ s.d. of three independent experiments **(B-D)**. * P < 0.05; ** P < 0.01; *** P < 0.001, based on the Student's t-test.

Figure S4 (related to Figure 4). HIF-1 α induces SSP gene expression in response to

serine/glycine deprivation

(A) Indicated cells were cultured with or without S/G(-) media for 24 h in the presence or absence of PX-478 (10 μ M). Western blot analyses were performed with the indicated antibodies.

(B) HRE luciferase activities were measured in the indicated cells cultured with or without S/G(-) media for 24 h in the presence or absence of PX-478 (10 μ M).

(C and D) Indicated cells with or without the depletion of HIF-1 α (left panel) were cultured with or without S/G(-) media for the indicated periods of time in the presence or absence of PX-478 (10 μ M). Quantitative real-time PCR (C) and immunoblotting (D) analyses were performed with the indicated primers and antibodies, respectively.

The data represent the mean $\pm$ s.d. of three independent experiments **(B, C)**. * P < 0.05; ** P < 0.01; *** P < 0.001, based on the Student's t-test.

Figure S5 (related to Figure 5). AMPK activation is required for the HIF-1 α -induced SSP gene expression in response to serine/glycine deprivation

(A) LN18 cells were cultured in complete media for 12 h in the presence or absence of serine transporter inhibitor (4-Fluoro-L-2-phenylglycine; 50 μ M) and/or glycine transporter inhibitor (Sarcosine; 50 μ M). Immunoblotting analyses were performed with the indicated antibodies.

(B) Indicated cells were cultured with or without S/G(-) media for 24 h in the presence or absence of compound C (10 μ M). Immunoblotting analyses were performed with the indicated antibodies.

(C) Indicated cells were cultured with or without S/G(-) media for 12 h in the presence or

absence of NAC (2 mM). Intracellular ROS levels were measured.

(D) HRE luciferase activities were measured in the indicated cells cultured with or without S/G(-) media for the indicated periods of time in the presence or absence of Compound C (10 μ M). NS, not significant.

(E and F) Indicated cells were cultured with or without S/G(-) media for the indicated periods of time in the presence or absence of compound C (10 μ M). Quantitative real-time PCR (E) and immunoblotting (F) analyses were performed with the indicated primers and antibodies, respectively.

The data represent the mean $\pm$ s.d. of three independent experiments **(C-E)**. * P < 0.05; ** P < 0.01; *** P < 0.001, based on the Student's t-test.

Figure S6 (related to Figure 6). AMPK-HIF-1 α signaling promotes *de novo* serine biosynthesis, proliferation, and survival of GBM cells upon serine/glycine deprivation

(A) Indicated cells were cultured with S/G(-) media for 5 d in the presence or absence of compound C (10 μ M) or PX-478 (5 μ M). Intracellular serine and glycine levels were measured.

(B-D) Indicated cells with or without the depletion of SSP genes were cultured with S/G(-) media for 5 d. Intracellular serine and glycine levels (B), NADPH and NADP⁺ levels (C), and ROS levels (D) were measured.

(E) Indicated cells with or without the reconstituted expression of SSP genes were cultured with S/G(-) media for 5 d in the presence or absence of compound C (10 μ M) or PX-478 (5 μ M). Intracellular NADPH and NADP⁺ levels (top panel), and ROS levels (bottom panel) were measured.

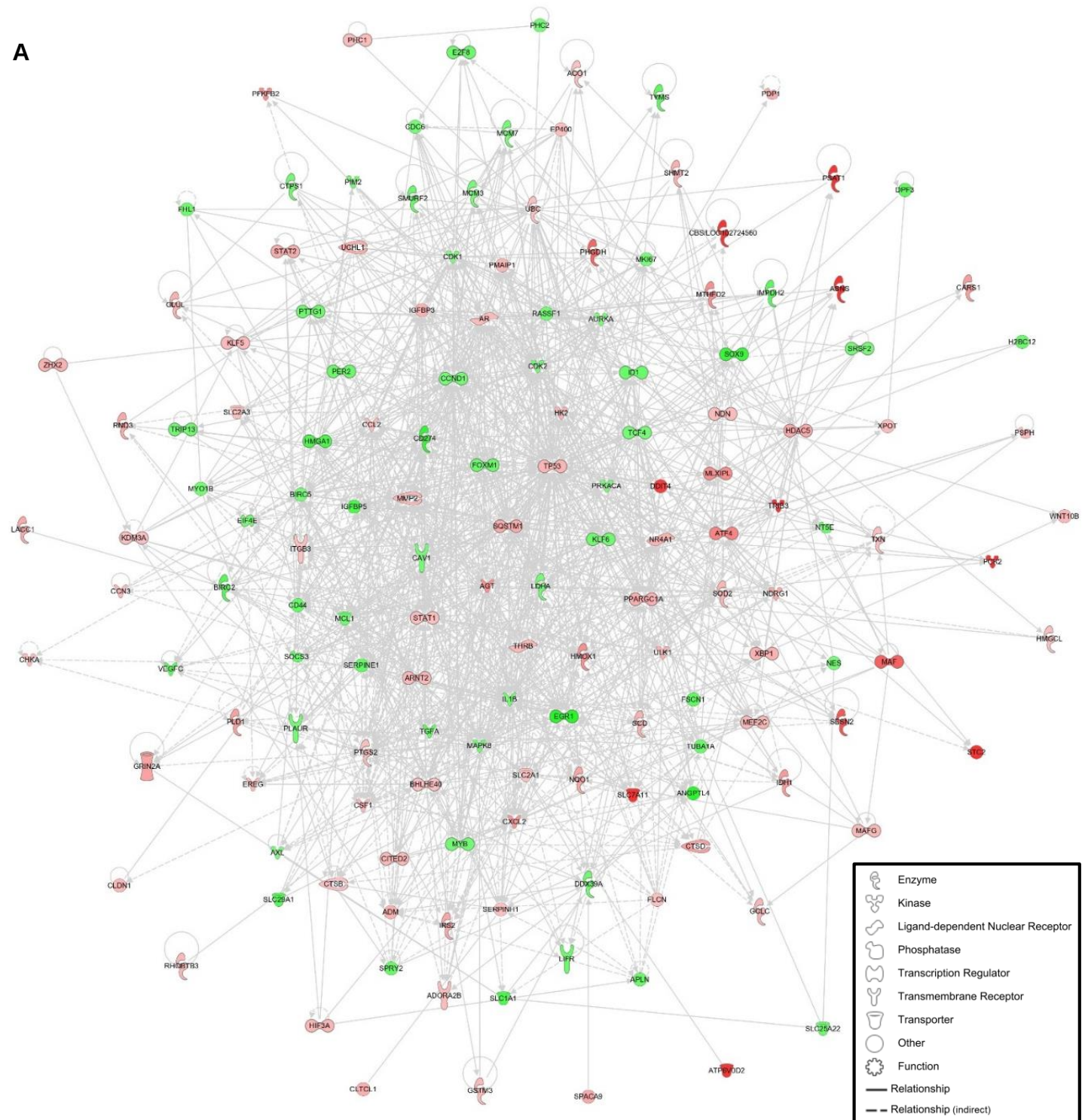
(F) Indicated cells were cultured with or without S/G(-) media for 6 d in the presence or absence of 2-DG (1 mM), and harvested for cell counting.

The data represent the mean $\pm$ s.d. of three independent experiments **(A-E)**. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, based on the Student's t-test or one-way ANOVA with Tukey's *post hoc* test.

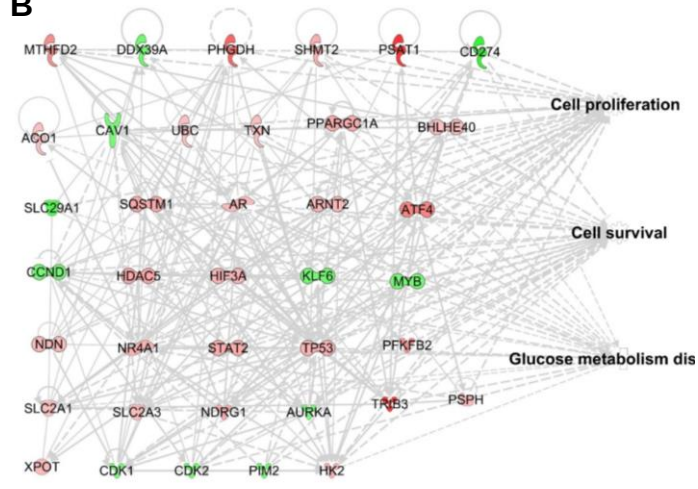
Figure S7. Full uncut blots are presented for the representative western blots featured in the present study.

Figure S1

A



B



C

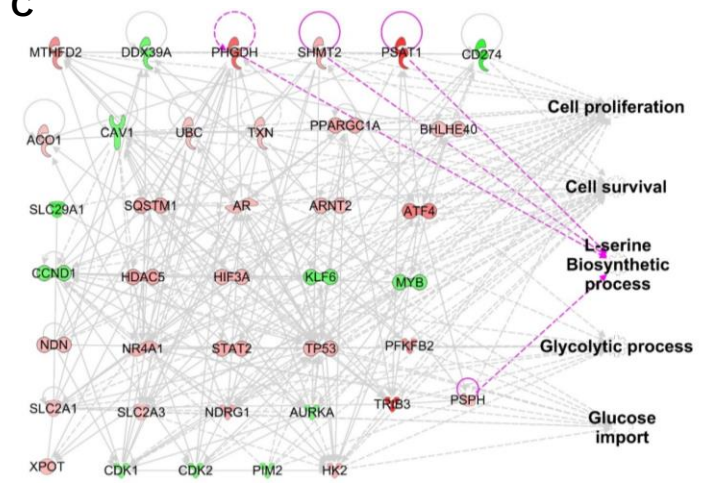


Figure S2

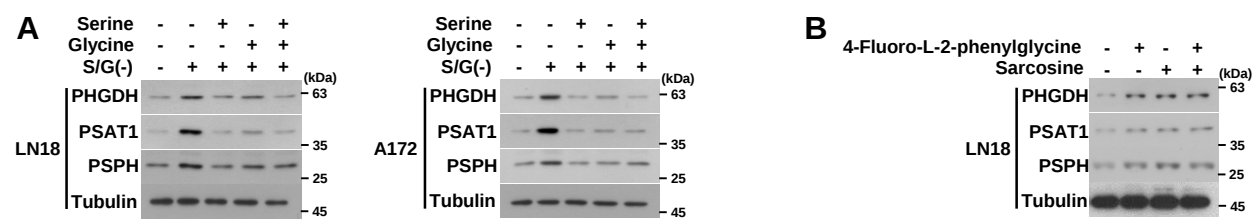


Figure S3

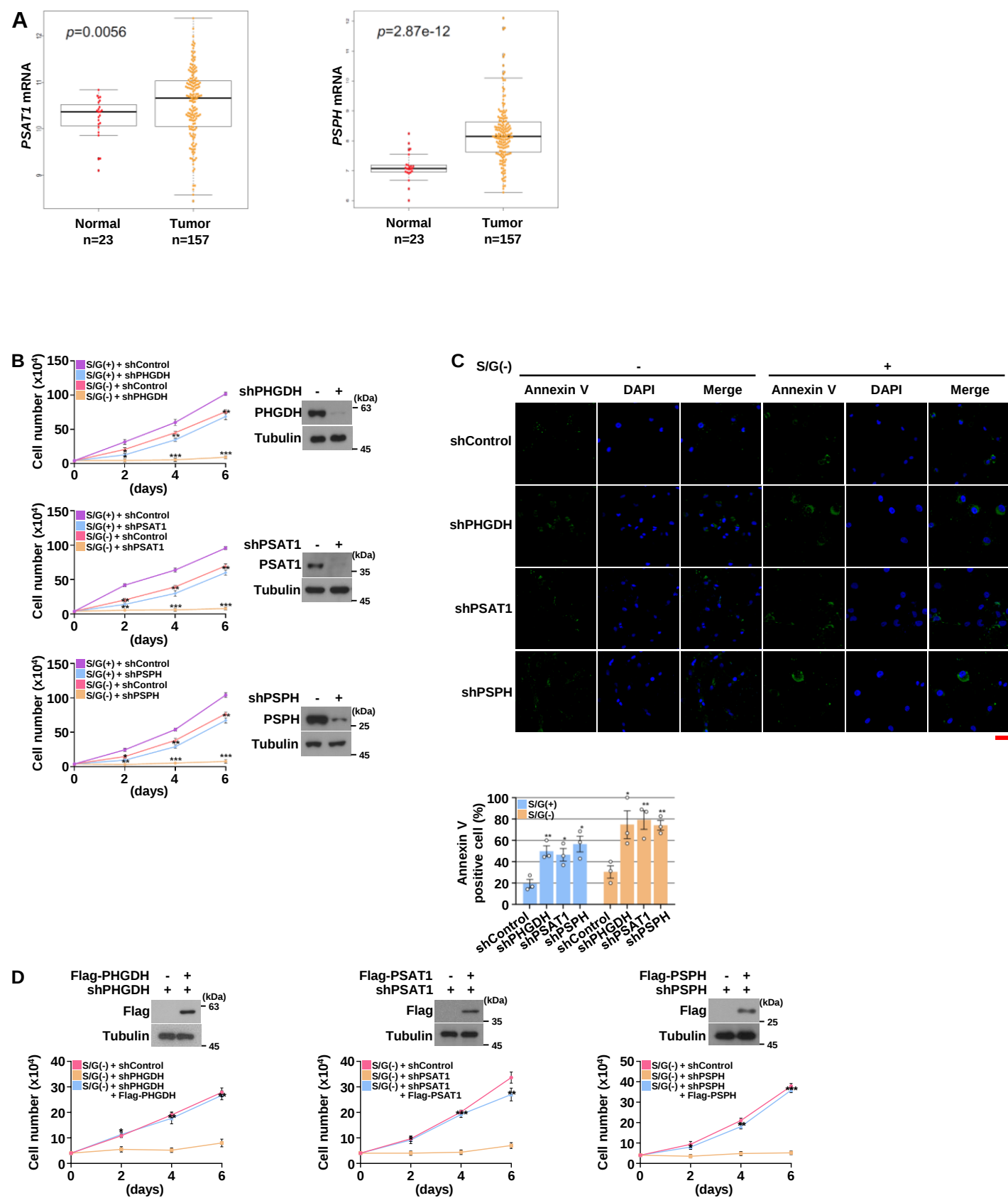


Figure S4

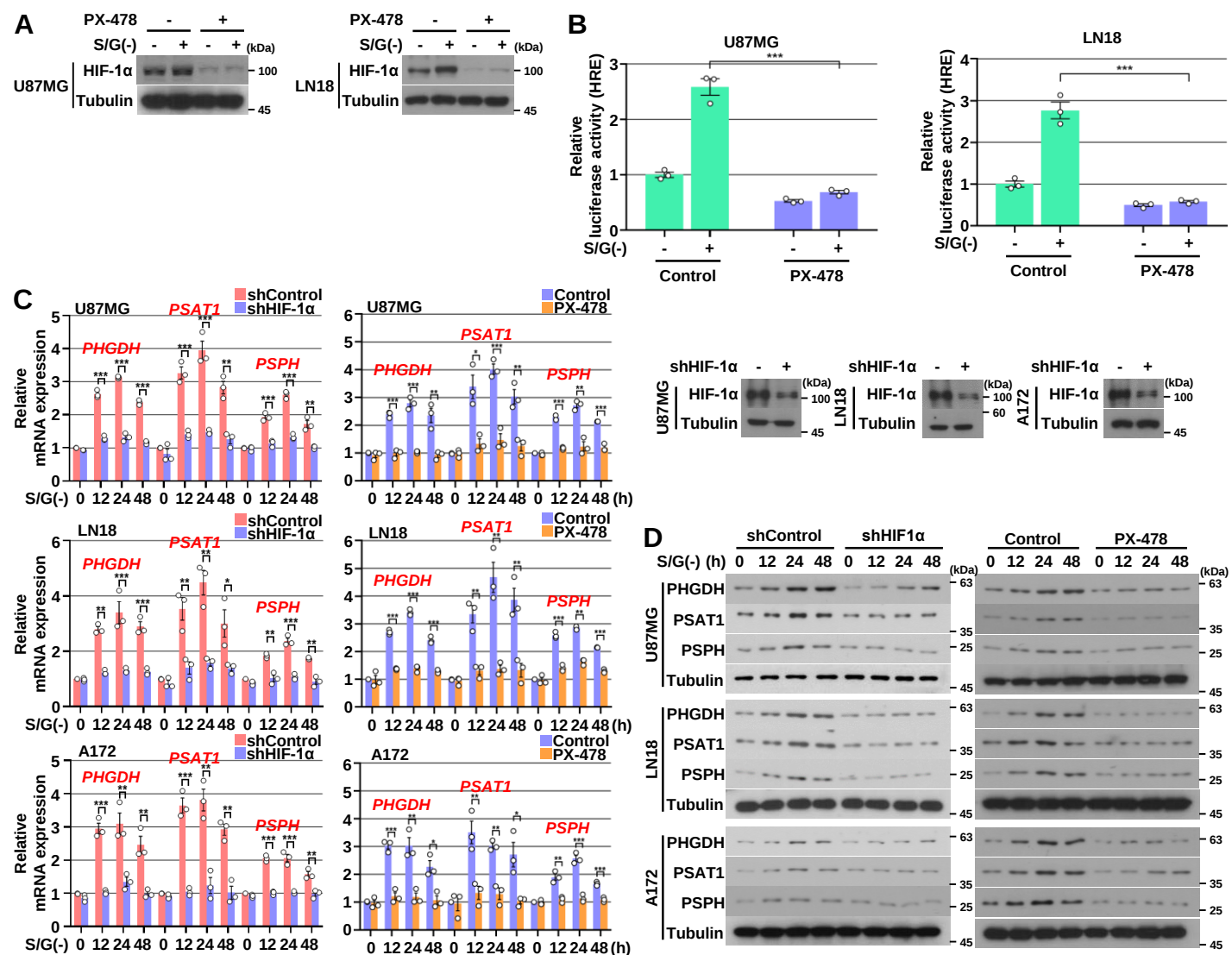


Figure S5

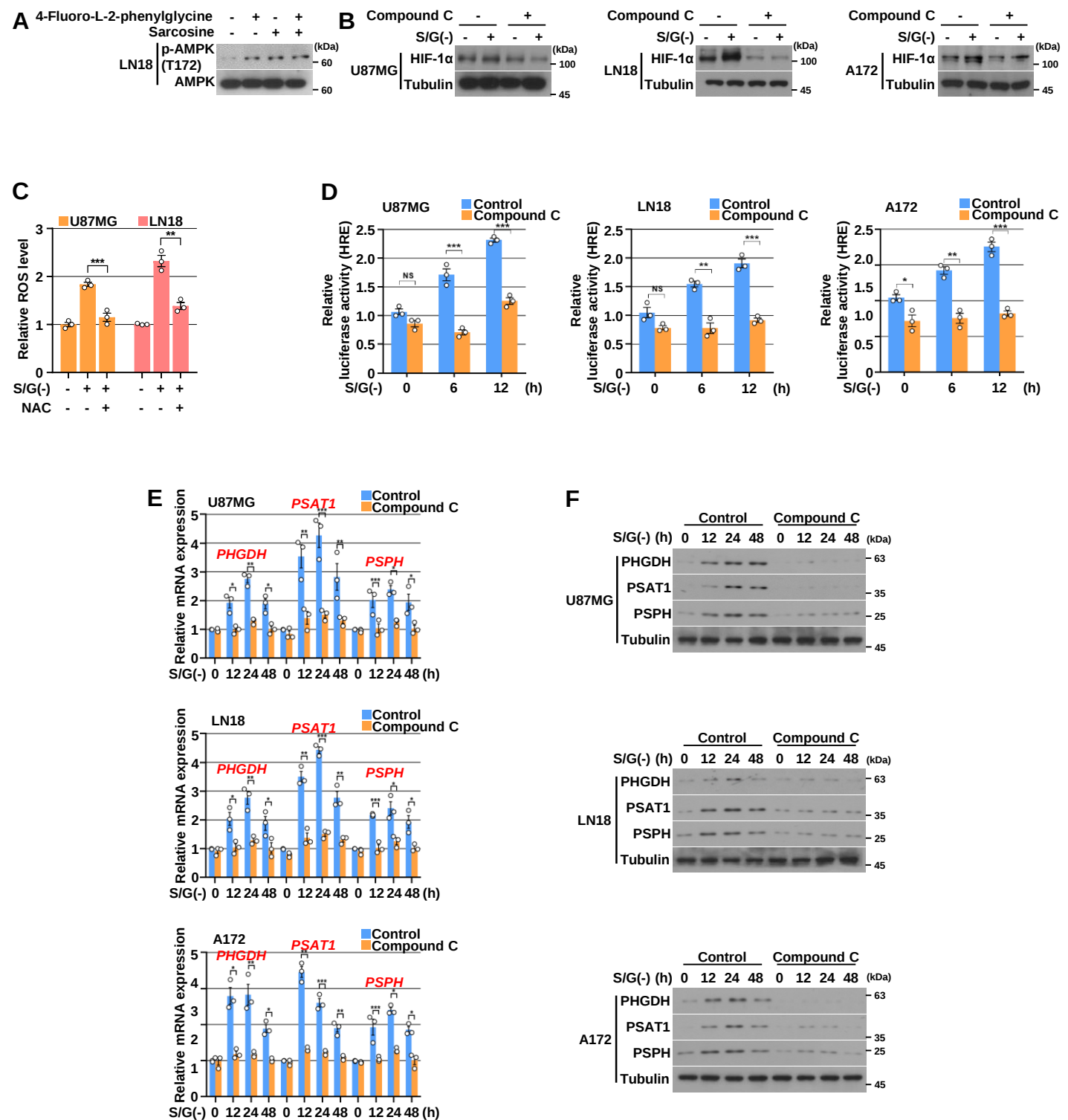


Figure S6

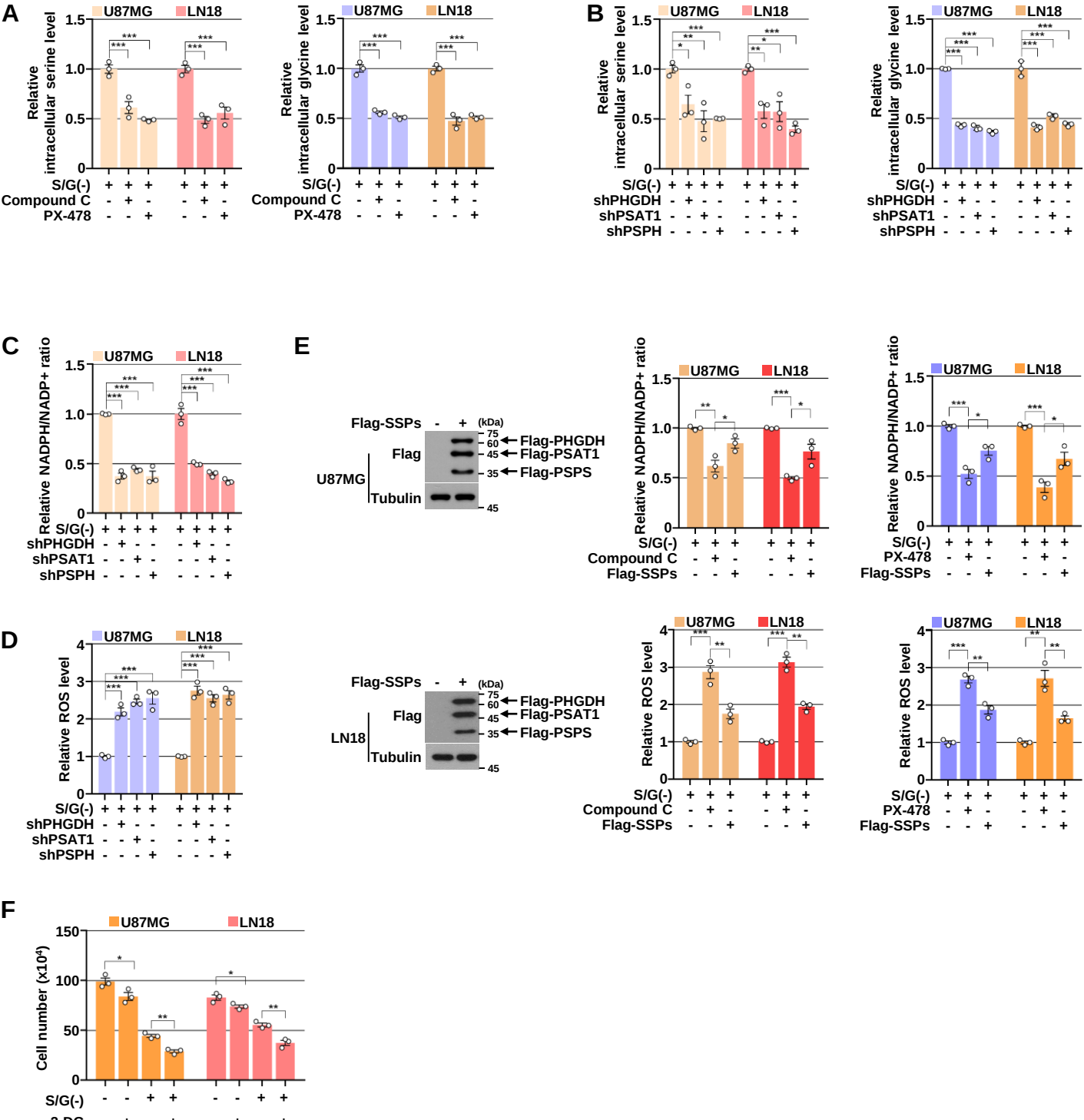


Figure S7

Figure 2.

F

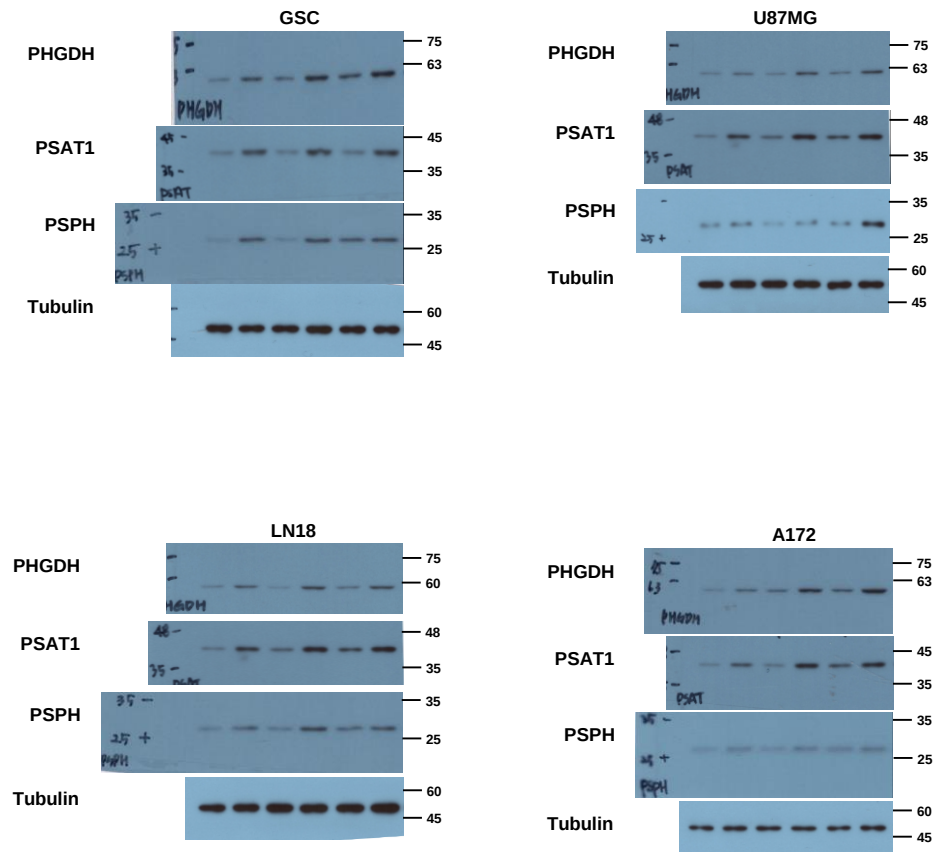
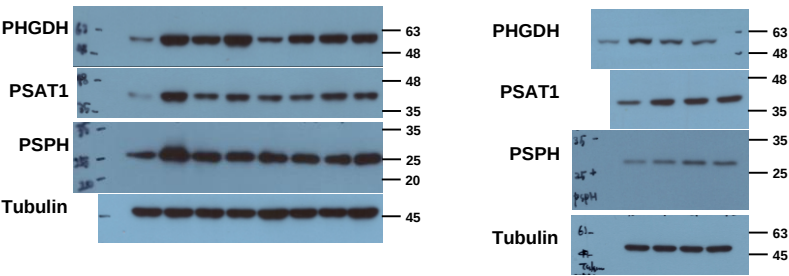


Figure 3.

B



C

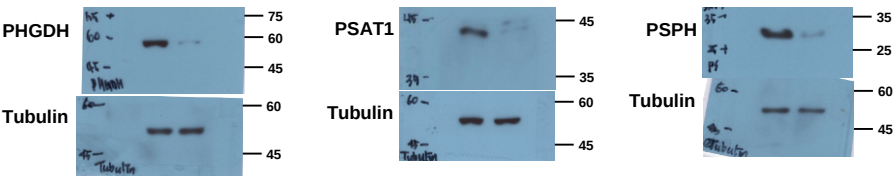
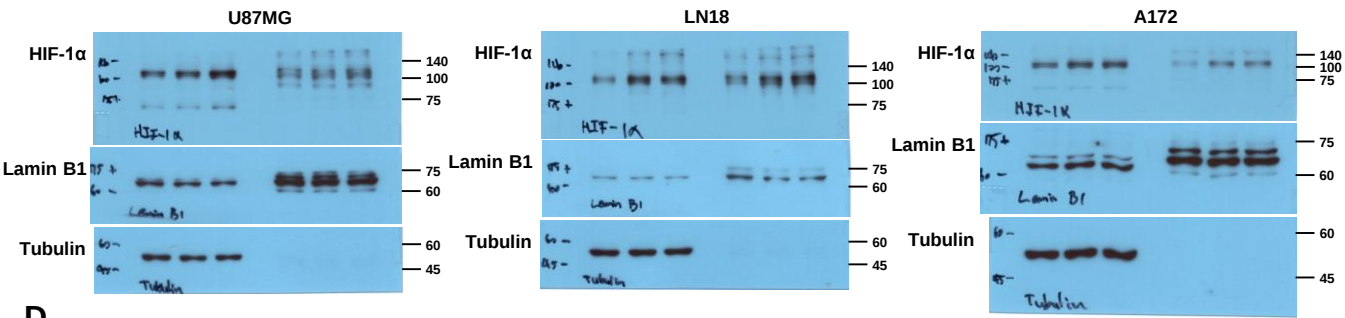
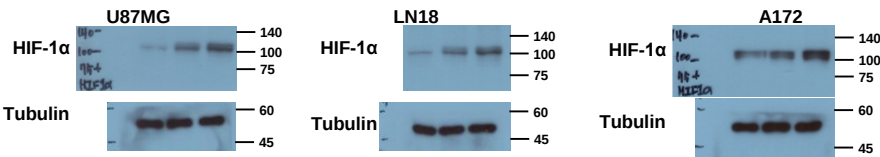


Figure 4.

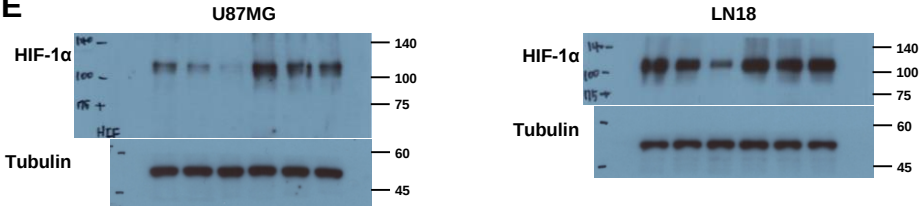
A



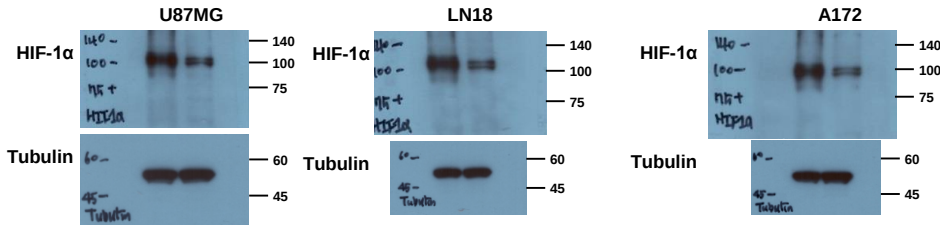
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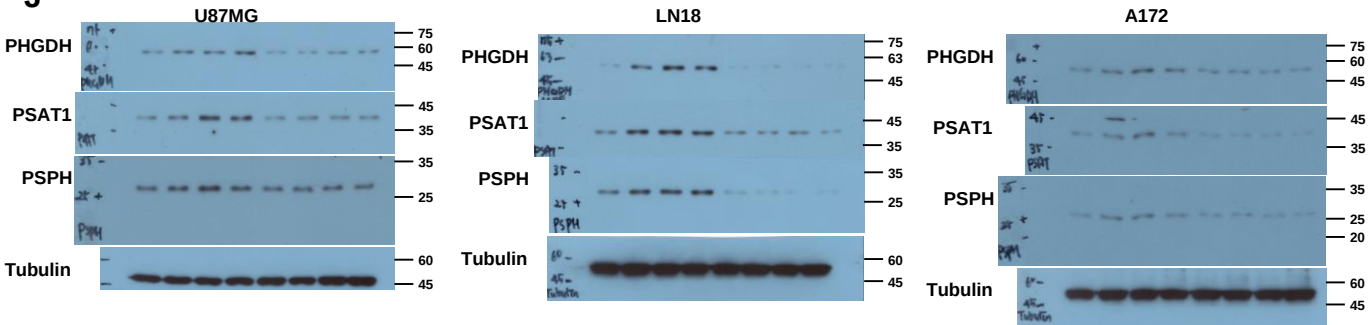
E



I



J



K

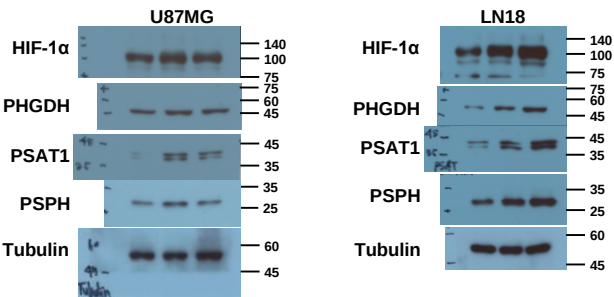


Figure 5.

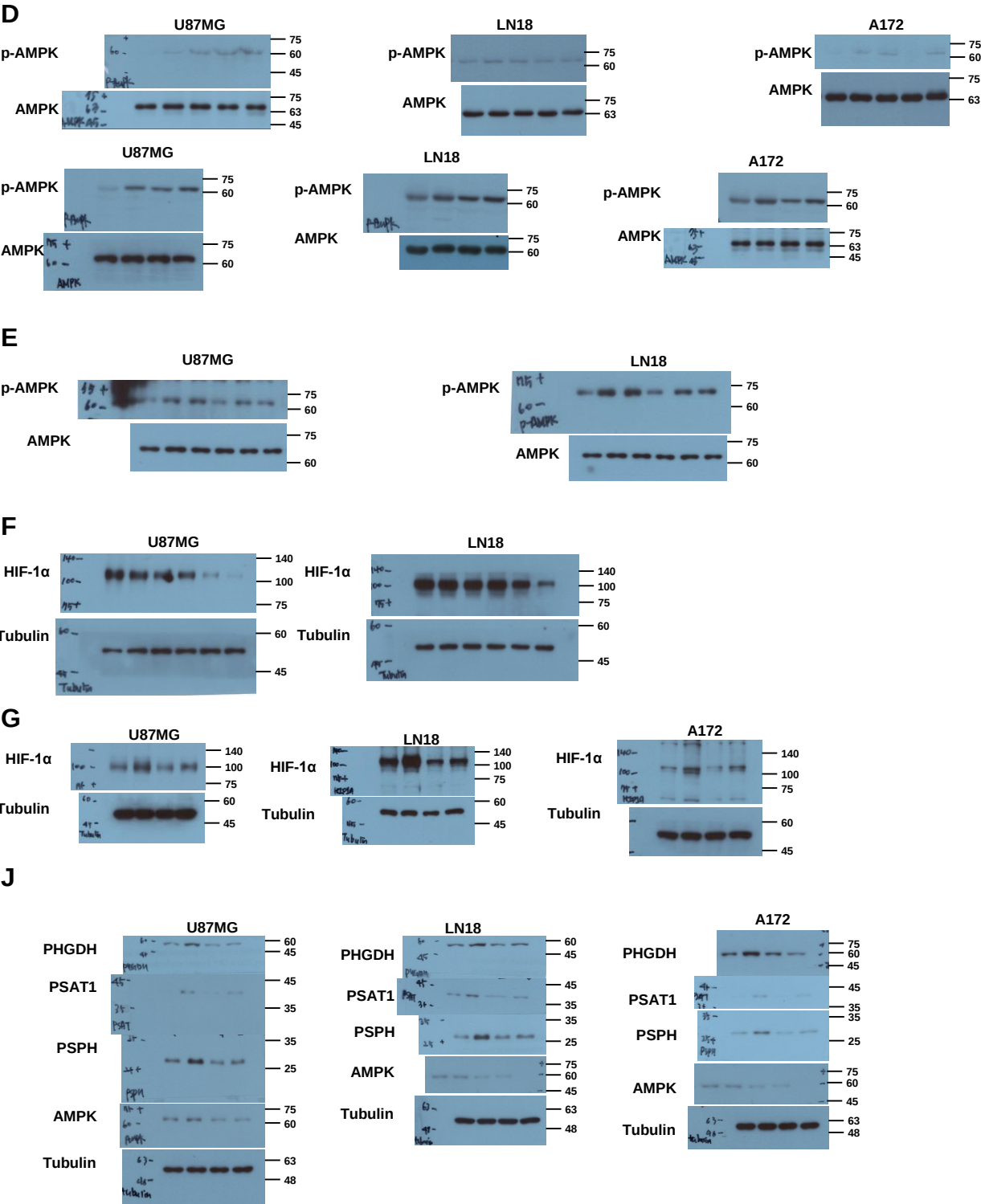


Figure S2

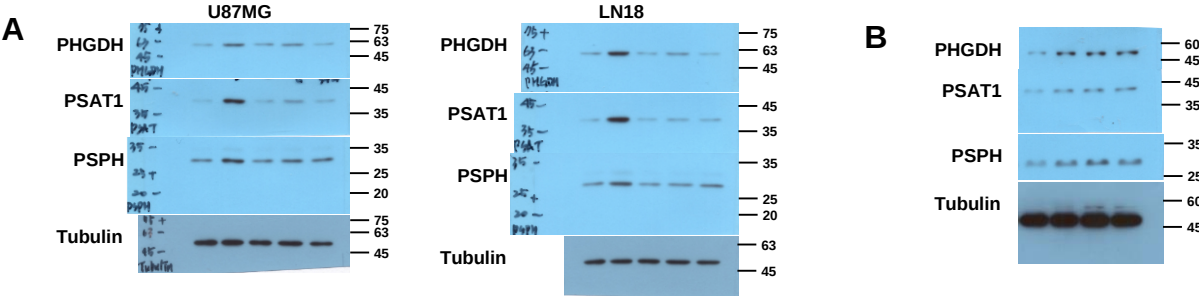


Figure S3

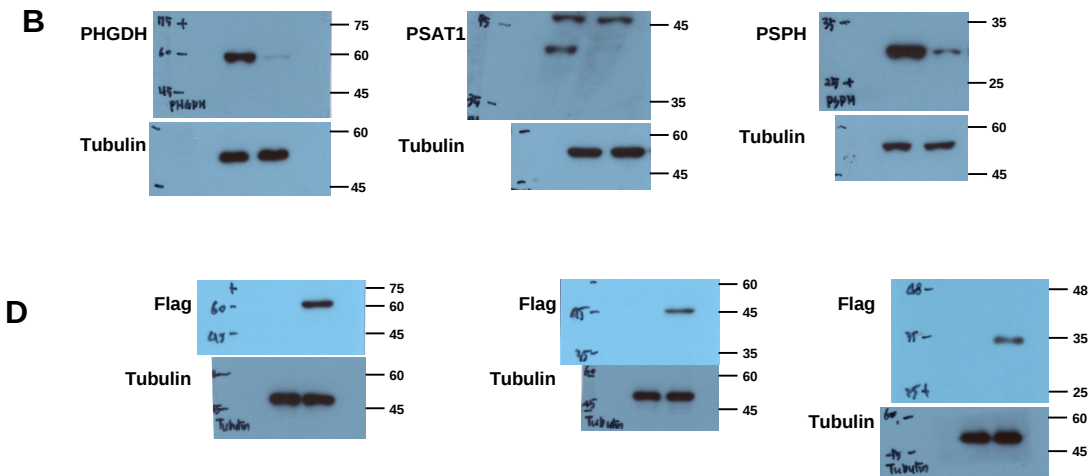


Figure S4.

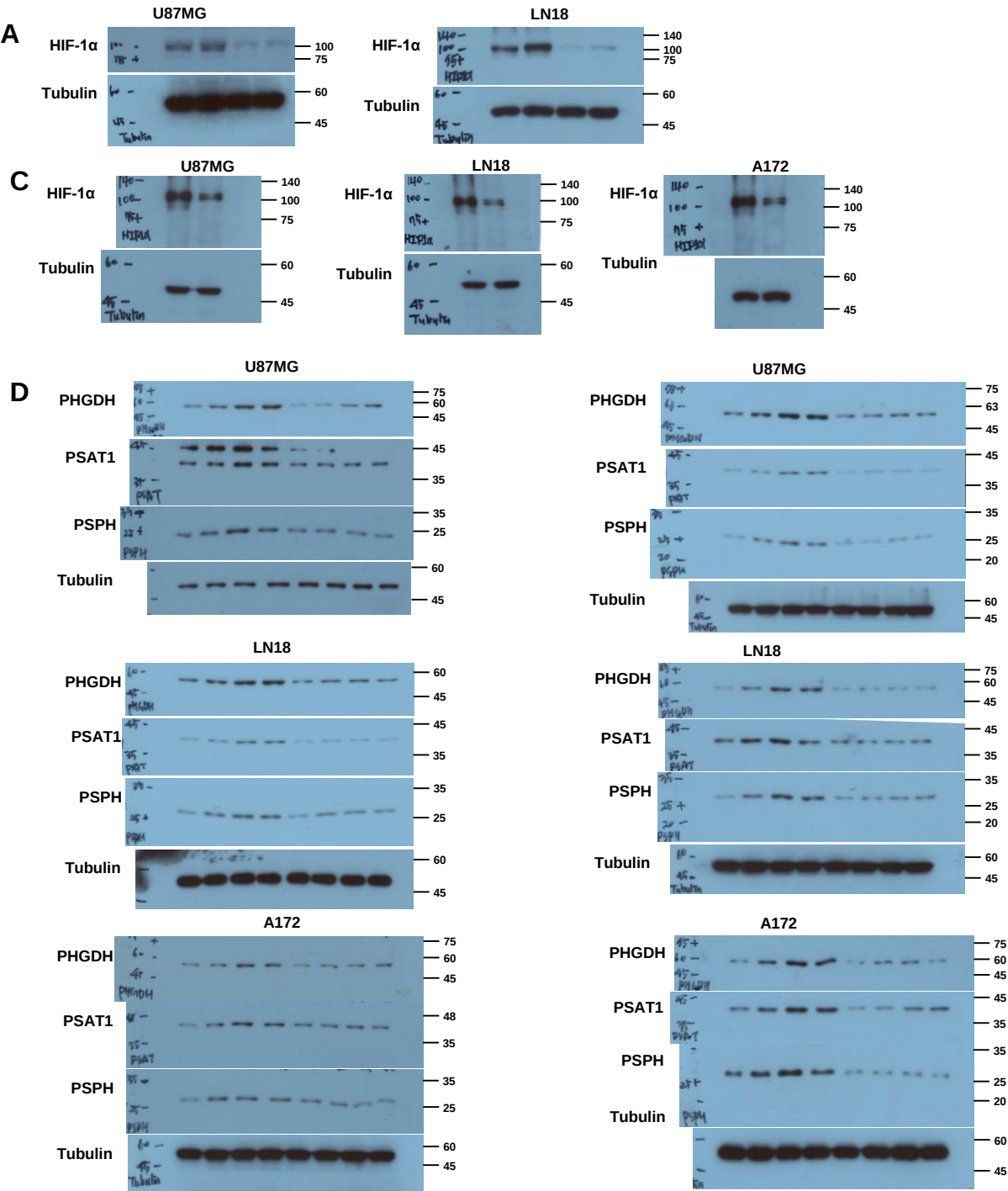


Figure S5

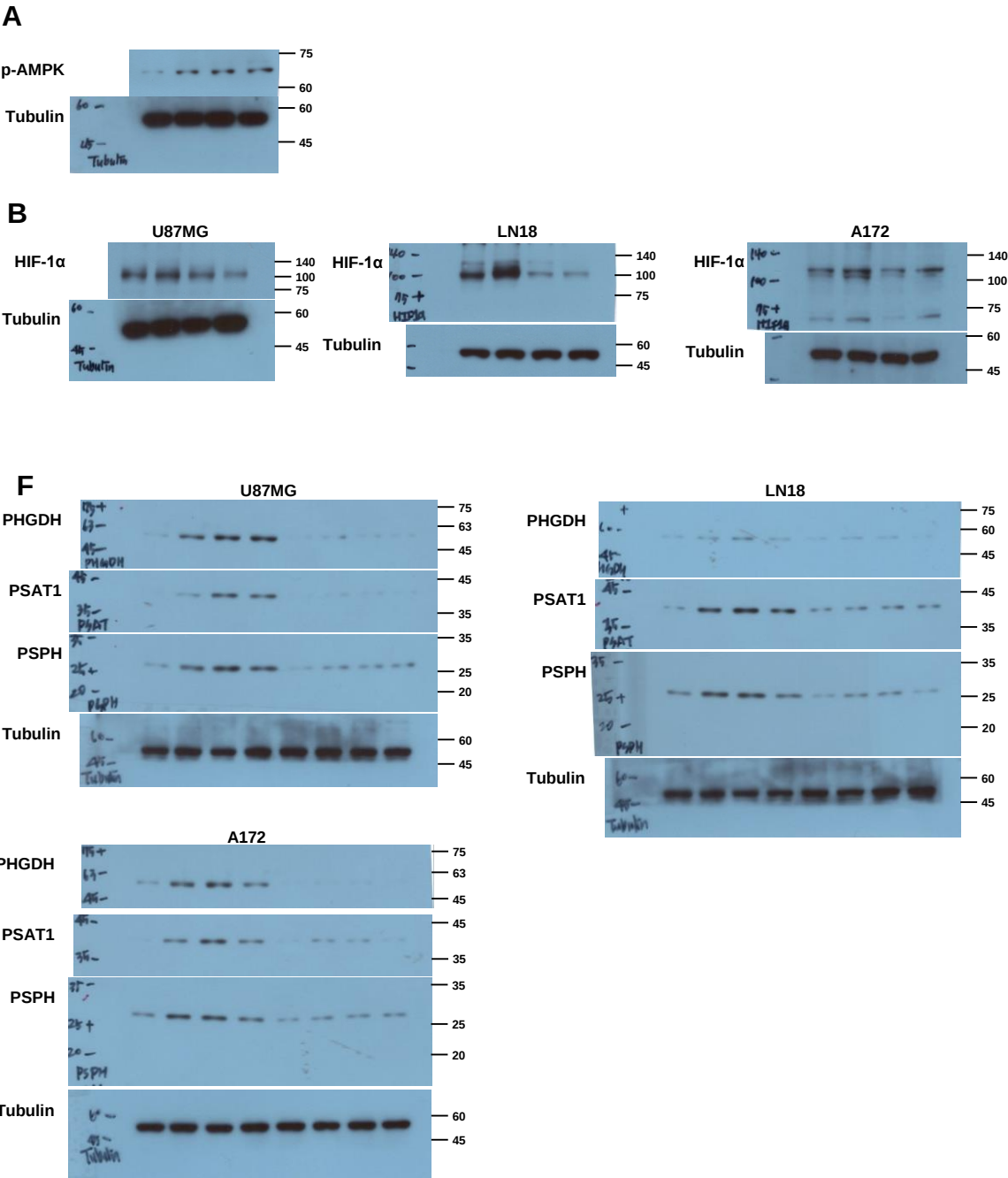


Figure S6

