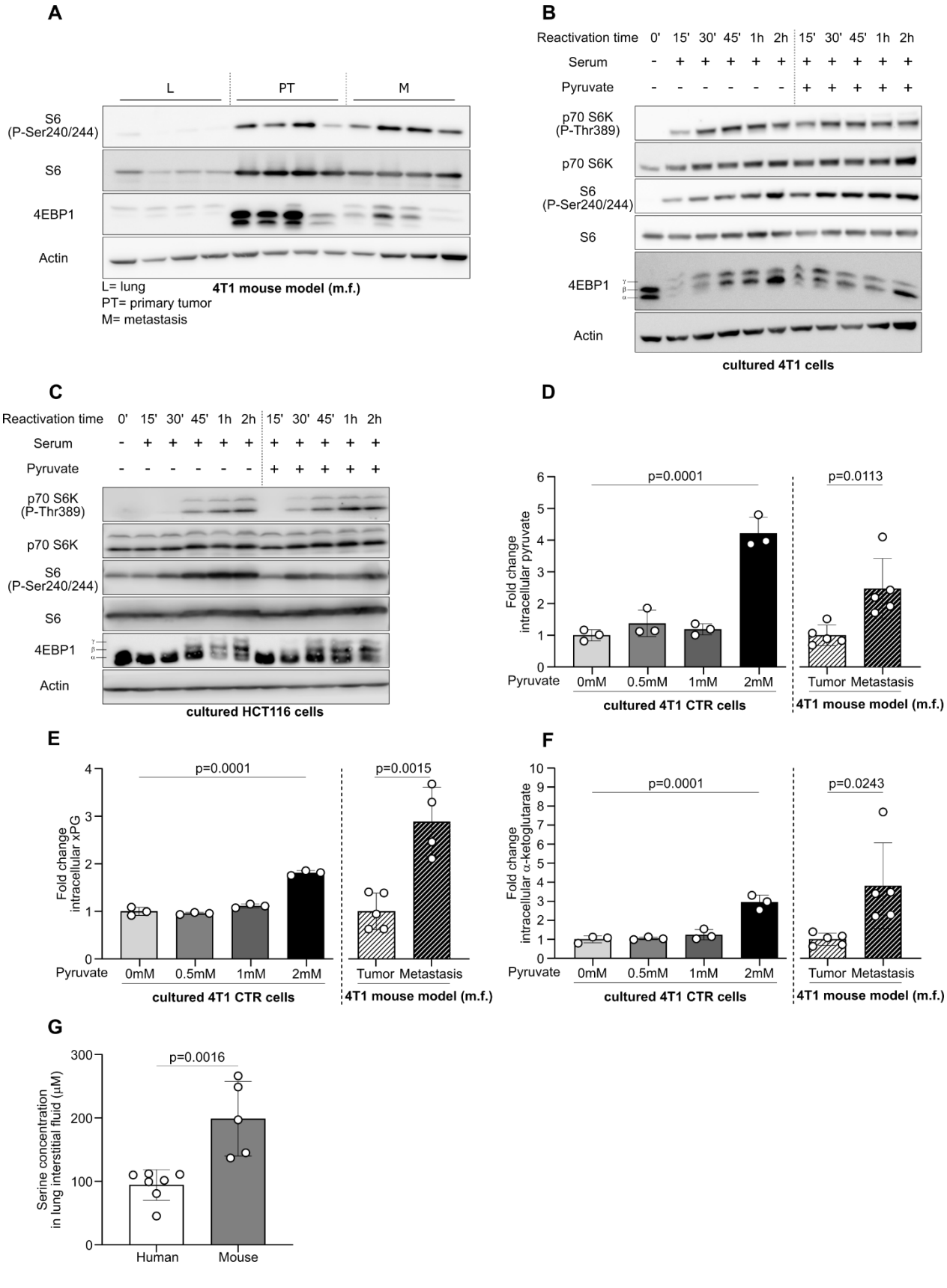
Supplemental Information


A
L
PT
M
S6 (P-Ser240/244)
S6
4EBP1
Actin
L= lung
PT= primary tumor
M= metastasis
4T1 mouse model (m.f.)
B
Reactivation time 0' 15' 30' 45' 1h 2h 15' 30' 45' 1h 2h
Serum - + + + + + + + + + +
Pyruvate - - - - - - + + + + +
p70 S6K (P-Thr389)
p70 S6K
S6 (P-Ser240/244)
S6
4EBP1
Actin
cultured 4T1 cells
C
Reactivation time 0' 15' 30' 45' 1h 2h 15' 30' 45' 1h 2h
Serum - + + + + + + + + + +
Pyruvate - - - - - - + + + + +
p70 S6K (P-Thr389)
p70 S6K
S6 (P-Ser240/244)
S6
4EBP1
Actin
cultured HCT116 cells
D
p=0.0001
p=0.0113
Fold change intracellular pyruvate
Pyruvate
0mM
0.5mM
1mM
2mM
cultured 4T1 CTR cells
Tumor
Metastasis
4T1 mouse model (m.f.)
E
p=0.0001
p=0.0015
Fold change intracellular xPG
Pyruvate
0mM
0.5mM
1mM
2mM
cultured 4T1 CTR cells
Tumor
Metastasis
4T1 mouse model (m.f.)
F
p=0.0001
p=0.0243
Fold change intracellular α-ketoglutarate
Pyruvate
0mM
0.5mM
1mM
2mM
cultured 4T1 CTR cells
Tumor
Metastasis
4T1 mouse model (m.f.)
G
p=0.0016
Serine concentration in lung interstitial fluid (μM)
Human
Mouse

Supplementary Fig 1. Pyruvate supplementation *in vitro* recapitulates some of the metabolic features of lung metastases compared to primary tumors, related to Figure 1.

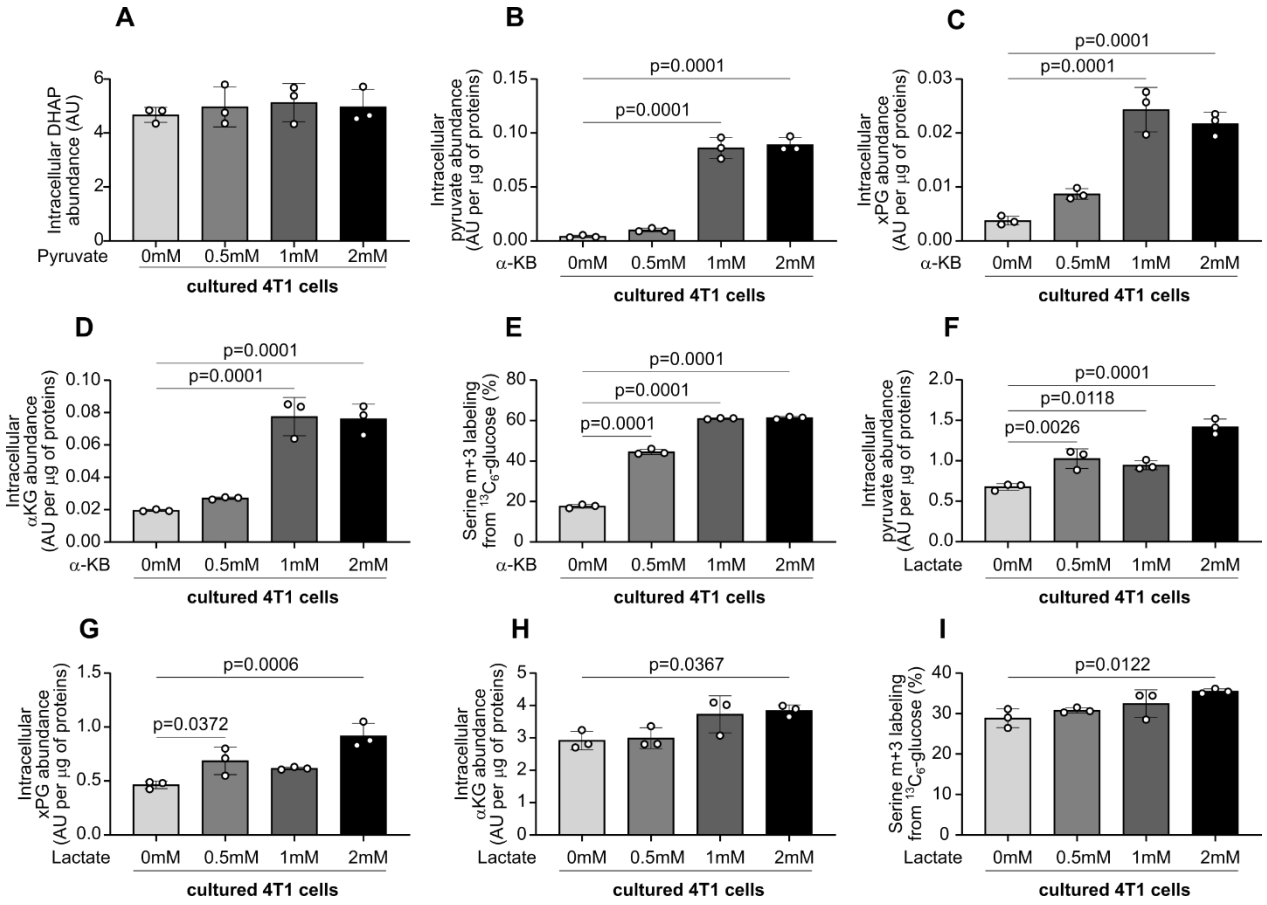
A, Phosphorylation of the mTORC1 targets S6 and 4EBP1 in the lung (L), primary tumor (PT) and metastasis (M) of BALB/c mice injected with 4T1 cells in the mammary fat pad (m.f.). Four additional mice related to figure 1a. *n=4*

B-C, Phosphorylation of the mTORC1 targets p70 S6K, S6 and 4EBP1 upon 16h of serum starvation, 1h starvation in HBSS and subsequent reactivation (from 15 minutes to 2h) in serum containing culture medium with or without 2mM sodium pyruvate in 4T1 or HCT116 cells. One representative image is shown, *n=3.*

D-F, Intracellular pyruvate, xPG and α-ketoglutarate abundance in 4T1 CTR cells incubated for 24h hour in medium containing increasing concentration of sodium pyruvate or in primary tumor and metastasis of BALB/c mice injected with 4T1 cells in the mammary fat pad (m.f.) . Data are presented as fold-change compared to the 0mM (in the case of cultured 4T1 CTR cells) or the tumor (in the case of 4T1 mouse model). One-way ANOVA with Dunnet's multiple comparison test (comparison between the 0mM conditions and all the others), *n=3* for the cultured 4T1 CTR cells. Two-tailed unpaired Student's t-test, *n=5* for the 4T1 mouse model.

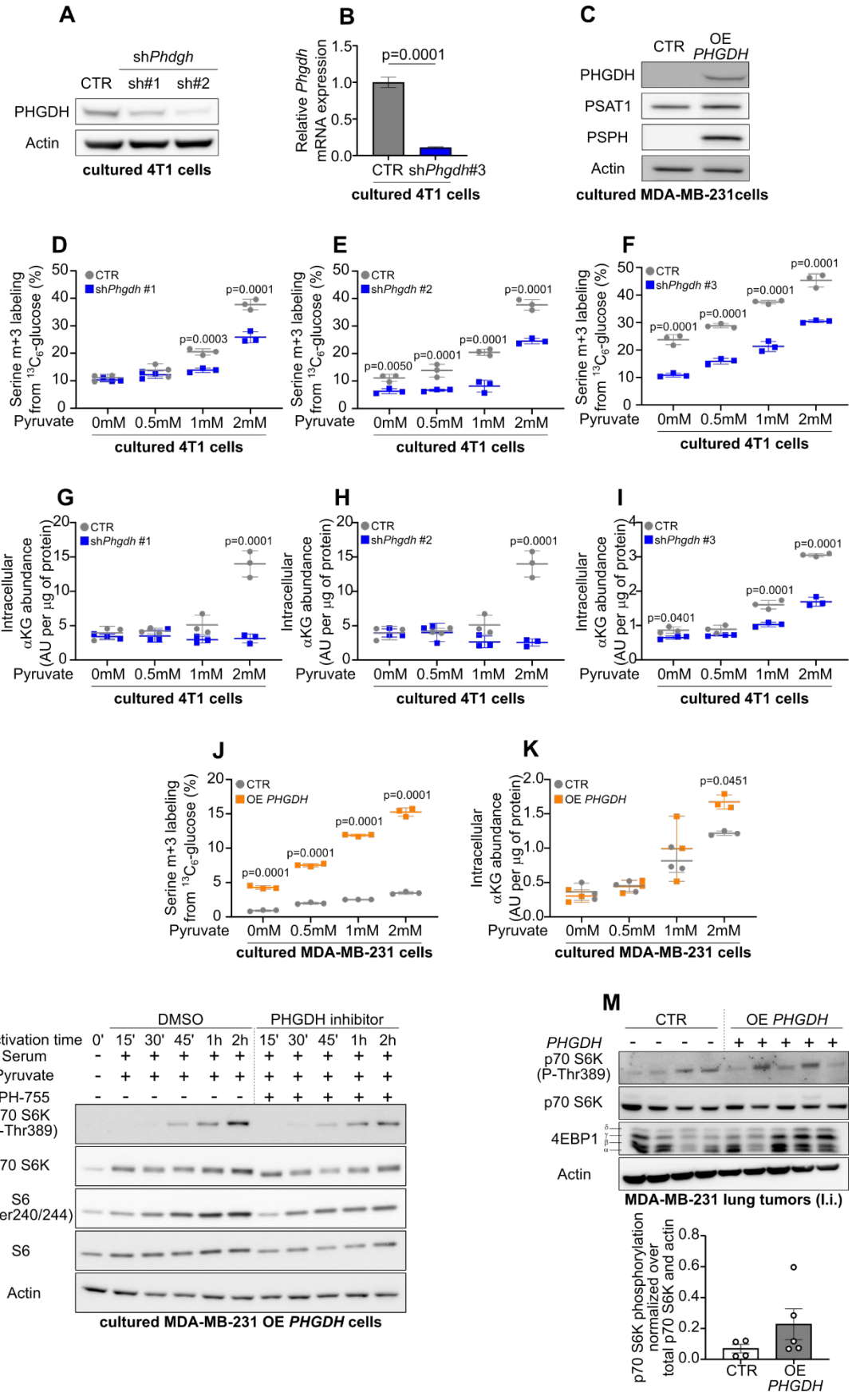
G, Concentration of serine in the interstitial fluid of lung samples collected from human patients and BALB/c mice. Two-tailed unpaired Student's t-test. Human n=7, Mouse n=5.

All error bars represent s.d. from mean of biologically independent samples or mice.



Supplementary Fig 2. Increased xPG levels and NAD+/NADH ratio support serine biosynthesis activation in presence of extracellular pyruvate, related to Figure 2

A-L, Intracellular DHAP, xPG, pyruvate, α-ketoglutarate abundance, in 4T1 CTR cells incubated for 24h hour in medium containing increasing concentration of sodium pyruvate, α-ketobutarate or sodium lactate. To assess d*e novo* serine biosynthesis, 4T1 cells were grown in culture medium containing $^{13}C_6$ glucose and increasing concentrations of sodium pyruvate, α-ketobutarate (AKB) or sodium lactate. One-way ANOVA with Dunnet's multiple comparison test (comparison between the 0mM conditions and all the others). *n=3* All error bars represent s.d. from mean of biologically independent samples.


A
shPhdgh
CTR
sh#1
sh#2
PHGDH
Actin
cultured 4T1 cells
B
Relative Phgdh mRNA expression
p=0.0001
CTR
shPhgdh#3
cultured 4T1 cells
C
CTR
OE PHGDH
PHGDH
PSAT1
PSPH
Actin
cultured MDA-MB-231cells
D
Serine m+3 labeling from 13C6-glucose (%)
CTR
shPhgdh #1
p=0.0001
p=0.0003
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
E
Serine m+3 labeling from 13C6-glucose (%)
CTR
shPhgdh #2
p=0.0001
p=0.0001
p=0.0001
p=0.0050
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
F
Serine m+3 labeling from 13C6-glucose (%)
CTR
shPhgdh #3
p=0.0001
p=0.0001
p=0.0001
p=0.0001
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
G
Intracellular αKG abundance (AU per µg of protein)
CTR
shPhgdh #1
p=0.0001
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
H
Intracellular αKG abundance (AU per µg of protein)
CTR
shPhgdh #2
p=0.0001
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
I
Intracellular αKG abundance (AU per µg of protein)
CTR
shPhgdh #3
p=0.0001
p=0.0401
p=0.0001
Pyruvate
0mM 0.5mM 1mM 2mM
cultured 4T1 cells
J
Serine m+3 labeling from 13C6-glucose (%)
CTR
OE PHGDH
p=0.0001
p=0.0001
p=0.0001
p=0.0001
Pyruvate
0mM 0.5mM 1mM 2mM
cultured MDA-MB-231 cells
K
Intracellular αKG abundance (AU per µg of protein)
CTR
OE PHGDH
p=0.0451
Pyruvate
0mM 0.5mM 1mM 2mM
cultured MDA-MB-231 cells
L
DMSO
PHGDH inhibitor
Reactivation time 0' 15' 30' 45' 1h 2h 15' 30' 45' 1h 2h
Serum
Pyruvate
PH-755
p70 S6K (P-Thr389)
p70 S6K
S6 (P-Ser240/244)
S6
Actin
cultured MDA-MB-231 OE PHGDH cells
M
CTR
OE PHGDH
PHGDH
p70 S6K (P-Thr389)
p70 S6K
4EBP1
Actin
MDA-MB-231 lung tumors (l.i.)
p70 S6K phosphorylation normalized over total p70 S6K and actin
CTR
OE PHGDH

Supplementary Fig 3. *PHGHD* expression modulates pyruvate-induced serine biosynthesis activation, related to Figure 3

A, PHGDH protein levels upon knockdown in 4T1 cells expressing the sh*Phgdh* sequences #1 and #2.

B, *Phgdh* gene expression upon knockdown in 4T1 cells expressing the *shPhgdh* sequence #3. Two-tailed unpaired Student's t-test. *n=3.*

C, Protein expression of serine biosynthesis enzymes upon overexpression (OE) of PHGDH in MDA-MB-231 cells.

D-F, Activation of *de novo* serine biosynthesis assessed through measurement of serine m+3 labeling enrichment after incubation of 4T1 control (CTR) or *Phgdh*-silenced (*shPhgdh* sequences #1,#2 and #3) cells for 24h in culture medium containing $^{13}C_6$ glucose and increasing concentrations of sodium pyruvate. 4T1 cells control (CTR) or knockdown for *Phgdh* (*shPhgdh*). Two-way ANOVA with Sidak's multiple comparison test *n=3*.

G-I, Intracellular α-ketoglutarate abundance in 4T1 control (CTR) or *Phgdh*-silenced (*shPhgdh* sequences #1,#2 and #3) cells after 24h incubation in culture medium containing increasing concentrations of sodium pyruvate. Two-way ANOVA with Sidak's multiple comparison test. *n=3*

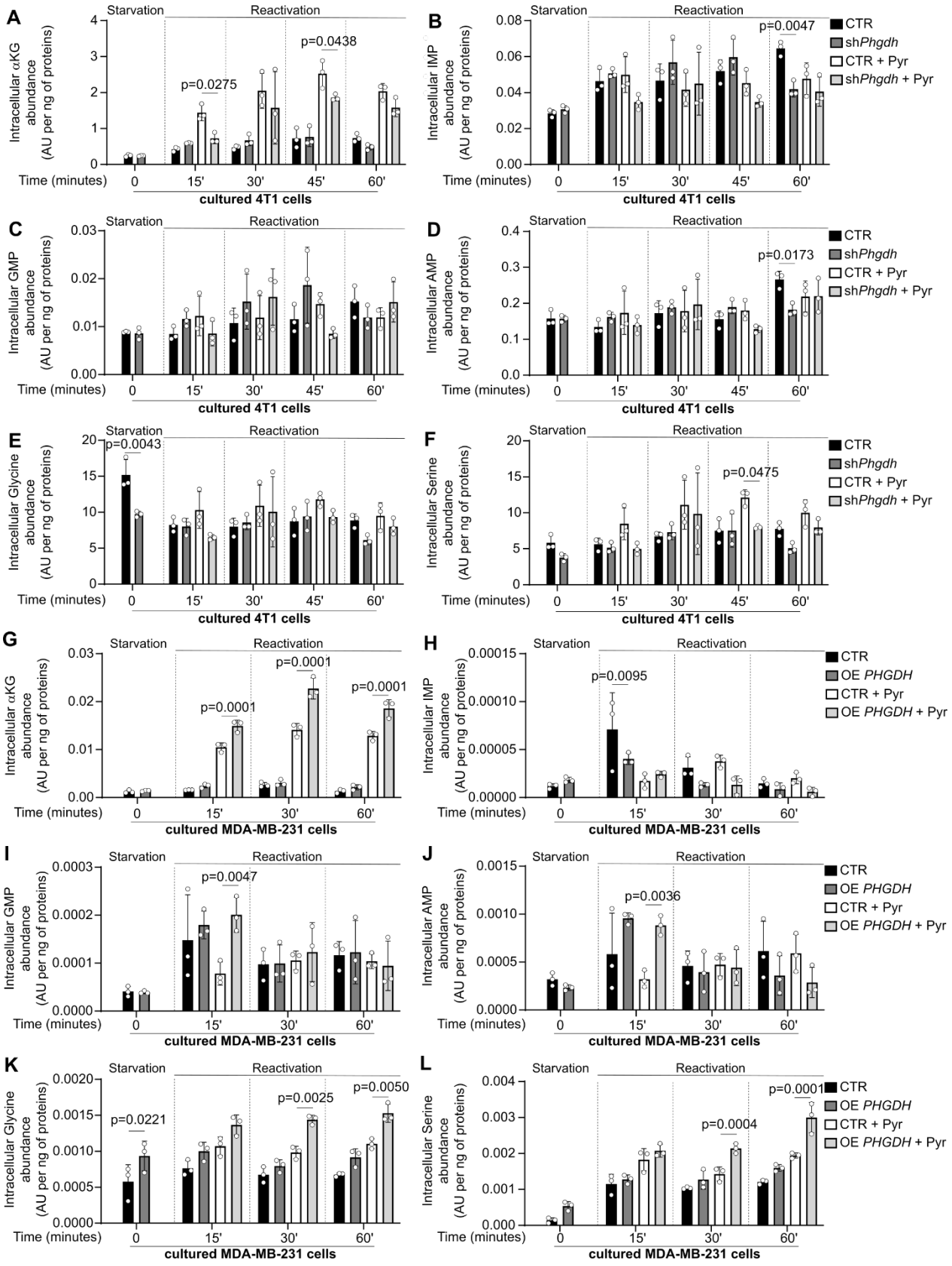
J, Activation of *de novo* serine biosynthesis assessed through measurement of serine m+3 labeling enrichment after incubation of MDA-MB-231 control (CTR) and *PHGDH*-overexpressing (OE) cells for 24h in culture medium containing $^{13}C_6$ glucose and increasing concentrations of sodium pyruvate. MDA-MB-231 cells control (CTR) or overexpressing *PHGDH* (OE *PHGDH*). Two-way ANOVA with Sidak's multiple comparison test. *n=3*

K, Intracellular α-ketoglutarate abundance in MDA-MB-231 control (CTR) or *PHGDH*-overexpressing (OE *PHGDH*) cells after 24h incubation in culture medium containing increasing concentrations of sodium pyruvate. Two-way ANOVA with Sidak's multiple comparison test. *n=3*

L, Phosphorylation of the mTORC1 targets p70 S6K, and S6 upon 16h of serum starvation, 1h starvation in HBSS and subsequent reactivation (from 15 minutes to 2h) in serum containing culture medium with 2mM sodium pyruvate and with or without 1µM of PHGDH inhibitor PH-755 in MDA-MB-468 cells. One representative image is shown, *n=3.*

M, Phosphorylation of the mTORC1 targets p70 S6K and 4EBP1 in MDA-MB-231 control (CTR) or *PHGDH*-overexpressing (OE *PHGDH*) lung metastases. l.i. refers to lung injection into NMRI nu/nu mice. Quantification of phospho-p70 S6K is shown in the graph. Data are normalized on total p70 S6K and Actin. Two-tailed unpaired Student's t-test. *n=4* for CTR group, *n=5* for OE *PHGDH* group.

Error bars represent s.d. from mean of biologically independent samples except in panel B where the error bars represent s.d. of technical replicates. Error bars in panel M represent s.e.m. of biologically independent samples.


A
Starvation
Reactivation
Intracellular αKG abundance (AU per ng of proteins)
p=0.0275
p=0.0438
Time (minutes)
0
15'
30'
45'
60'
cultured 4T1 cells
B
Intracellular IMP abundance (AU per ng of proteins)
p=0.0047
CTR
shPhgdh
CTR + Pyr
shPhgdh + Pyr
C
Intracellular GMP abundance (AU per ng of proteins)
D
Intracellular AMP abundance (AU per ng of proteins)
p=0.0173
E
Intracellular Glycine abundance (AU per ng of proteins)
p=0.0043
F
Intracellular Serine abundance (AU per ng of proteins)
p=0.0475
G
Intracellular αKG abundance (AU per ng of proteins)
p=0.0001
p=0.0001
p=0.0001
cultured MDA-MB-231 cells
H
Intracellular IMP abundance (AU per ng of proteins)
p=0.0095
OE PHGDH
OE PHGDH + Pyr
I
Intracellular GMP abundance (AU per ng of proteins)
p=0.0047
J
Intracellular AMP abundance (AU per ng of proteins)
p=0.0036
K
Intracellular Glycine abundance (AU per ng of proteins)
p=0.0221
p=0.0025
p=0.0050
L
Intracellular Serine abundance (AU per ng of proteins)
p=0.0004
p=0.0001

Supplementary Fig 4. Modulation of *PHGDH* expression does not consistently alter nucleotide abundances in the presence of pyruvate, related to Figure 4.

A-L, Metabolites abundance changes in 4T1 control (CTR) or *Phgdh*-silenced (*shPhgdh*) cells as well as MDA-MB-231 control (CTR) or *PHGHD* overexpressing (OE *PHGHD*) cells upon 16h of serum starvation, 1h starvation in HBSS and subsequent reactivation (15, 30, 45 and 60 minutes) in culture medium containing or not 2mM Sodium Pyruvate. *n=3*

Two-way ANOVA with Tukey's multiple comparison test. For clarity, only p values lower than 0.05 comparing CTR vs *shPhgdh*, CTR vs OE *PHGHD*, CTR + Pyr vs *shPhgdh* + Pyr or CTR +Pyr vs OE *PHGHD* + Pyr are shown on the graphs. Error bars represent s.d. from mean of biologically independent samples.

Supplementary Table 1, Metadata related to Figure 1C.

Patient ID in Figure 1c	SampleID	Sample type	AJCC Stage	Gender	Age at initial pathologic diagnosis	Year of initial pathologic diagnosis	Days to date of death	PAM50Call RNAseq	ER Status	HER2 Status	PR Status
#1	TCGA-BH-A1ES-01	Primary Tumor	Stage IIB	F	35	1999	3462	LumA	Positive	Negative	Positive
	TCGA-BH-A1ES-06	Metastatic		F	35	1999	3462	LumA			
#2	TCGA-BH-A1FE-01	Primary Tumor	Stage IIB	F	31	1998	2273	LumA	Positive	Negative	Positive
	TCGA-BH-A1FE-06	Metastatic		F	31	1998	2273	Normal			
#3	TCGA-E2-A15A-01	Primary Tumor	Stage IIIC	F	45	2009		LumB	Positive	Negative	Positive
	TCGA-E2-A15A-06	Metastatic		F	45	2009		LumB			
#4	TCGA-BH-A18V-01	Primary Tumor	Stage IIB	F	48	2001	1555	Basal	Negative	Negative	Negative
	TCGA-BH-A18V-06	Metastatic		F	48	2001	1555	Basal			
#5	TCGA-E2-A15K-01	Primary Tumor	Stage IIA	F	58	2010		LumB	Positive	Negative	Positive
	TCGA-E2-A15K-06	Metastatic		F	58	2010		LumB			

Supplementary Table 2, Metadata related to Figure 1H and Supplementary Figure1G.

Gender	Age (y)	Reason of surgery
M	68	volume reduction surgery in emphysema
M	69	volume reduction surgery in emphysema
M	58	peritumoral normal lung tissue from tumorectomy
F	64	volume reduction surgery in emphysema
F	56	peritumoral normal lung tissue from tumorectomy
F	34	peritumoral normal lung tissue from tumorectomy
M	66	peritumoral normal lung tissue from tumorectomy