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Supplementary Information for

Metabolic supervision by PPIP5K, an inositol pyrophosphate kinase/phosphatase, controls proliferation of the HCT116 tumor cell line.

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

Datasets S1 to S4

Materials and Methods

Cells and Cell Culture. The origins of the WT and PPIP5K KO lines of HCT116 and HEK293 cells are as previously described [1]. For cell growth experiments, 2×10^5 cells were seeded in 12 well plates and cultured in complete medium (DMEM/F12 for HCT116 and DMEM for HEK293 cells plus 10% FBS and 1× penicillin and streptomycin), low glucose medium (DMEM without added glucose plus 10% FBS (which contributes about 0.5 mM glucose [2]) and 1× penicillin and streptomycin), or DMEM/F12 without glutamine plus 10% FBS and 1× penicillin and streptomycin), for the indicated time period. Cells were then trypsinized and cell number were counted using Countess (Thermo Fisher Scientific). For assays of colony formation on soft agar, 4000 cells were mixed with 0.3% agarose in low glucose medium and seeded on top of 0.5% agarose layer in 12 well plates. Fresh medium was added on the top every 2-3 days during a 10 days culture period and colonies were counted under a Zeiss Colibri inverted microscope. For colony formation on a solid surface, 500 cells were seeded in each well of a 6 well plate and cultured in low glucose medium for 10 days, with medium changes every 2-3 days. Colonies were stained with 0.1% crystal violet and quantified by Image J.

In vivo tumor growth. WT or PPIP5K KO HCT116 cells (2.0×10^6) was suspended in DMEM/F12 media and subcutaneously injected into the both flanks of female nude mice in a volume of 100 µL. Tumor volumes were measured every 3 days. Approximate tumor volumes were calculated as volume = height × length × width. Mice were sacrificed before the tumor length reached 2 cm. Animal studies were approved by the Institutional Animal Care and Use Committee and conducted in accordance to National Institutes of Health guidelines.

Metabolite Extraction. Cellular metabolite extraction was conducted as previously described [3, 4]. Briefly, 6-8 × 10^5 cells per well were seeded into six-well plates and cultured in no glucose DMEM medium supplemented with 10% FBS and 1× penicillin and streptomycin for 24h. For isotope labeling experiment, 0.5mM [U-^{13}C] glucose or 2mM [U-^{13}C] serine (Cambridge Isotopes Laboratories, Inc.) was added for 24 h prior to analysis. Since the media also contained 2 mM non-labeled serine and approximately 0.5 mM non-labeled glucose, about 50% of these two molecules were ^{13}C-labeled. Cell confluence was similar between cell lines at the time of extraction. Medium was aspirated and 1 mL of −80 °C cooled extraction solvent (80% methanol/water) was immediately added to each well prior to transferring the plates to −80 °C for 15 min. The plates were then placed on dry ice, and cells were scraped into the extraction solvent and transferred to Eppendorf tubes. Metabolite extractions were centrifuged at 20,000 × g at 4 °C for 10 min. The supernatant of each sample was then transferred to a new Eppendorf tube and evaporated using a speed vacuum at room temperature. To measure AMP, ADP and ATP concentrations, cells were cultured low glucose medium for 2 h, and external standards of 10 ng $^{15}N_5$-AMP, $^{15}N_5$-ADP, or 50 ng $^{15}N_5$-ATP (Sigma-Aldrich) was added to metabolite extracts prior to evaporation. For metabolite analysis, the metabolite pellets were first dissolved in 30 µl sample solvent (water:methanol:acetonitrile, 2:1:1, v/v) and 3 µl was further analyzed by liquid chromatography-mass spectrometry (LC-MS).

High-Performance Liquid Chromatography. Compound separation was performed using an XBridge amide column (100 × 2.1mm; 3.5 µm; Waters) attached to a Dionex Ultimate 3000 UHPLC at room temperature. Mobile phase A: water with 5mM ammonium acetate, pH 6.9; Mobile phase B: 100% acetonitrile. The following linear gradient was used: 0 min, 85% B; 1.5 min, 85% B; 5.5 min, 35% B; 10 min, 35% B; 10.5 min, 10% B; 12.5, 10% B; 13.5 min, 85% B; 20 min, 85% B. Flow rate was 0.15 ml/min from 0 to 5.5 min, 0.17 ml/min from 6.9 to 10.5 min, 0.3

ml/min from 10.6 to 17.9 min, and 0.15 ml/min from 18 to 20 min. All solvents used were LC-MS grade, and were purchased from Fisher Scientific.

Mass Spectrometry. Mass spectrometry was performed using the Q Exactive plus (Thermo Scientific) instrument is equipped with a heated electrospray ionization probe (HESI) as previously described The parameters set as below: heater temperature, 120 °C; sheath gas, 30; auxiliary gas, 10; sweep gas, 3; spray voltage, 3.0 kV for the positive mode and 2.5 kV for the negative mode; capillary temperature, 320 °C; S-lens, 55; A scan range (m/z) of 70 to 900 was used in positive mode from 1.31 to 12.5 minutes. For negative mode, a scan range of 70 to 900 was used from 1.31 to 6.6 minutes and then 100 to 1,000 from 6.61 to 12.5 minutes; resolution: 70000; automated gain control (AGC), 3 × 106 ions. Customized mass calibration was performed before data acquisition.

Metabolite Peak Extraction and Data Analysis. Raw peak data collected from LC-MS were processed on Sieve 2.2 software (Thermo Fisher Scientific) with peak alignment and detection performed according to the manufacturer's protocol. For targeted metabolite analysis, the method "peak alignment and frame extraction" was applied. An input file of theoretical m/z and detected retention time was used for targeted metabolite analysis, and the m/z width was set to 5 ppm. An output file was obtained after data processing that included detected m/z and relative intensity in the different samples. To calculate the fold changes between different experimental groups, integrated peak intensities generated from the raw data were used. For isotope tracing experiments, the mass isotopomer distributions were calculated and normalized by comparing the ratio of labeled to unlabeled metabolites in each sample. Metabolite pathway impacts were determined by MetaboAnalyst pathway analysis [www.metaboanalyst.ca] using the following parameters: Over Representation Analysis- Hypergeometric Test. Volcano plots were generated using GraphPad Prism 8.

Measurement of intracellular ATP. 6×10^5 cells were seeded in a six-well plate in DMEM/F12 medium (i.e., glucose-replete) plus 10% FBS for 24h and then cultured in low glucose medium for 24h. Cells were washed three times in ice-cold buffer PBS, and then lysed by agitation in 1 ml of wash buffer containing 1% Triton X-100 for 5 min at 4 °C. The ATP was assayed using a commercial kit (Molecular Probes™, A22066).

Generation of PPIP5K1 Stable Expressing Cells. PPIP5K KO1 clonal lines that stably express either PPIP5K1 kinase-dead mutant PPIP5K1^{D332A} or phosphatase-dead mutant PPIP5K1^{R399A} were generated by using the pINDUCER20 system. Briefly, the PPIP5K1^{D332A} or PPIP5K1^{R399A} cDNA was inserted into a pINDUCER20 lentiviral vector. Virus was generated by co-transfection of the vectors with lentiviral packaging plasmids (Dharmacon) in HEK 293T cells. Collected virus particles were then transduced into PPIP5K KO cells and stable clones were selected with G418.

Assays of Intracellular Inositol Phosphates. HCT116 cells (1×10^6 cells per dish) were seeded in 10-cm dishes in media containing 10 µCi/mL [^{3}H] inositol (American Radiolabeled Chemicals). After 3 d, cells were quenched, and then cell extracts were assayed by HPLC [5].

Assay of Intracellular Proteins. To knockdown enzymes in the glucose metabolism pathways, siRNA of target genes (Origene) were transfected into HCT116 cells which were harvested after 48h. The knockdown efficiency was examined by lysing cells in RIPA buffer before Western analysis using antibodies against hexokinase-1 (HK1) (Cell Signaling Technology), hexokinase-2 (HK2) (Cell Signaling Technology), glucose-6-phosphate dehydrogenase (G6PD) (Cell Signaling Technology), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology),

or phosphoglycerate dehydrogenase (PHGDH) (Cell Signaling Technology) followed by HRP conjugated 2nd antibodies. To examine the expression level of one carbon cycle related enzymes, cells were cultured in low glucose medium for 24 hr and lysed with RIPA buffer (Thermo Fisher Scientific) before being analyzed by SDS-PAGE and Western blot analysis. Primary antibodies against methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) (Santa Cruz Biotechnology), methylenetetrahydrofolate dehydrogenase 1 like (MTHFD1L) (Sigma Aldrich), methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) (Abcam), Serine hydroxymethyltransferase 1 (SHMT1)(Cell Signaling Technology), Serine hydroxymethyltransferase 2 (SHMT2) (Cell Signaling Technology), PHGDH (Cell Signaling Technology) and phosphoribosylglycinamide formyltransferase (GART) (Thermo Fisher Scientific) were used followed by HRP conjugated 2nd antibodies. For AMPK activation, 70% confluent HCT116 cells were treated with low glucose medium plus 10% FBS with or without 10 mM glucose for 2 hr. Cells were lysed in RIPA buffer (Thermo Fisher Scientific) and AMPK phosphorylation was detected by SDS-PAGE and western blot using the p-AMPK (T172) antibodies (Cell Signaling Technology), the AMPK antibodies (Cell Signaling Technology, P-S6K (T389) antibodies and S6K antibodies (Cell Signaling Technology). Phosphoribosyl Pyrophosphate Synthetase 1 (PRPS1) phosphorylation was examined when cells were cultured in low glucose medium for 2h followed by separation of the cell extract in a native gel. Briefly, cells were lysed in non-reducing buffer (50mM tris pH7.4, 1% triton X-100, 150mM NaCl and 5% glycerol) and the extract was loaded to TGX Stain-Free gel (Biorad) with additional non-reducing loading buffer. The gel was separated and transferred in 1×Tris/glycine buffer (Biorad) and PRPS was detected using p-PRPS1 (S180) antibodies (antibodies-online) and pan-PRPS antibodies (Santa Cruz Biotechnology). For all the assays, cells were lysed in buffers with 1× Halt™ protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific) and further homogenized using a Minilys personal homogenizer. Typically, 20 µg of protein was loaded onto gel for immunoblotting. To quantify the level of MTHFD1, PHGDH and p-AMPK, the intensity of each MTHFD1, PHGDH band was calculated relative to Actin while p-AMPK band was calculated relative to total AMPK, and those resultant ratios were then normalized to the sum of all ratios obtained from a single gel.

Assay of SHMT activity. SHMT enzymatic activity in cell lysates was measured by determination of the formation of C-1 unit in N^5N^{10}-methylene tetrahydrofolate which equilibrates with carrier formaldehyde and be trapped with dimedon [6-8]. Briefly, 10 µg of cell lysate in 2 mM Tris(2-carboxyethyl)phosphine and 50 mM potassium phosphate pH7.4 was incubated with 10µM serine (with 0.5µCi L-[^{3}H(G)]-Serine (PerkinElmer)), 0.25 mM pyridoxal-5-phosphate (Santa Cruz Biotechnology), and 2 mM tetrahydrofolate (Cayman) in a total volume of 100 µL at 37°C for 20 min. The reaction was terminated with 75 µL of 1 M sodium acetate pH 4.5, 50 µL of 0.1 M formaldehyde, and 75 µL of 0.4 M dimedone. The vials were heated in a boiling water bath for 5 min to form the HCHO dimedon derivative. The tubes were then cooled for 5 min in an ice bath and briefly centrifuged before the dimedone compound was extracted by vigorous shaking with 1 mL of toluene. After two minutes of centrifugation, 0.8 mL of upper phase were removed for scintillation counting analysis.

Assay of PRPS activity. PRPS activity in cell lysates was assayed using a commercial kit from NovoCIB according to the manufactory's instruction. 10 µg lysate were incubated with reaction buffer and incubated at 37°C for 90 min before the reaction was read at OD340.

Other Assays: Glucose concentrations in culture media were assayed with a kit (Sigma; product GAHK20). Protein concentration was measured with a BCA kit (ThermoFisher).

Statistics. All experiments were derived from three or more biological replicates. Results are shown as means; error bars represent the standard errors of the mean. The unpaired Student's t-test was used to determine statistical significance of differences between means.

S1

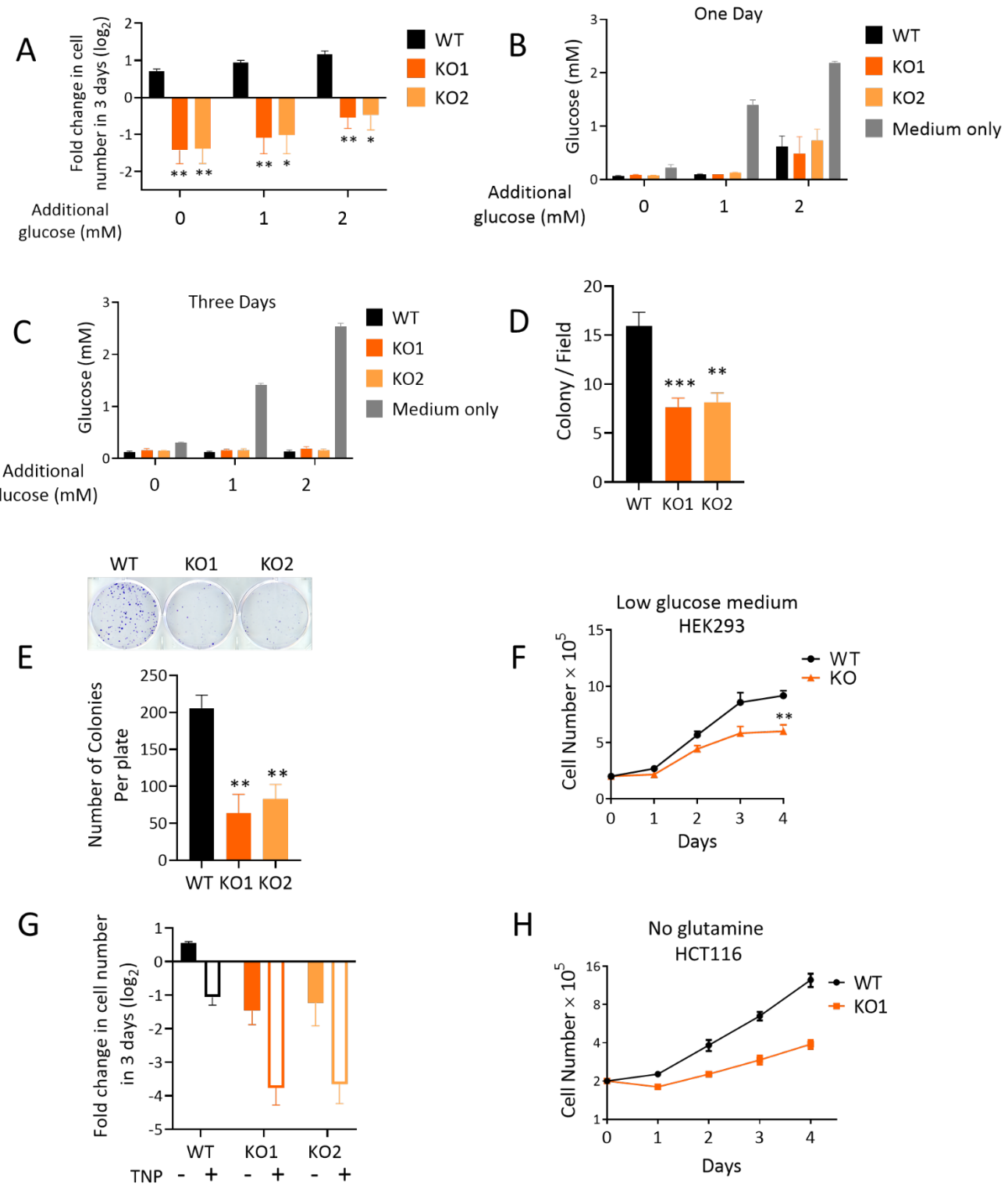
A
Fold change in cell number in 3 days (log2)
Additional glucose (mM)
WT
KO1
KO2
B
One Day
Glucose (mM)
Medium only
C
Three Days
D
Colony / Field
E
Number of Colonies Per plate
F
Low glucose medium
HEK293
Cell Number × 10^5
Days
G
Fold change in cell number in 3 days (log2)
TNP
H
No glutamine
HCT116

Fig. S1 Cell growth parameters for WT and PPIP5K KO HCT116 cells.
(A) Cell growth of WT HCT116 cells (black bars) and corresponding PPIP5K KO1 cells (dark orange bars) and PPIP5K KO2 cells (light orange bars) were seeded (2×10^5 cells/well) in the low glucose medium with or without an additional 1mM or 2 mM glucose. (B, C) Glucose concentration in culture medium when either WT or PPIP5K KO cells were cultured in the low glucose condition with or without an additional 1mM or 2mM glucose for either one day (B) or three days (C); gray bars indicates the initial total glucose concentrations of the various culture conditions, as determined in parallel incubations that did not contain any cells. (D) Colony formation on soft agar of WT and PPIP5K KO HCT116 cells in low glucose medium for 10 days. (E) Colony formation on a solid surface of WT and PPIP5K KO HCT116 cells in low glucose medium for 10 days with medium change every 2-3 days. (F) Growth curve of WT and PPIP5K KO HEK293 cells cultured in low glucose medium. (G) Growth curve of WT and PPIP5K KO HCT116 cells cultured in low glucose medium with (open bars) or without (filled bars) cell-treatment with 10μM TNP for 3 days. (H) Growth curve of WT and PPIP5K KO1 HCT116 cells cultured in high-glucose medium but without glutamine. Student's t-test was used to determine statistical significance of differences between means ($P < 0.05$ [*], $P < 0.01$ [**], $P < 0.001$ [***]).

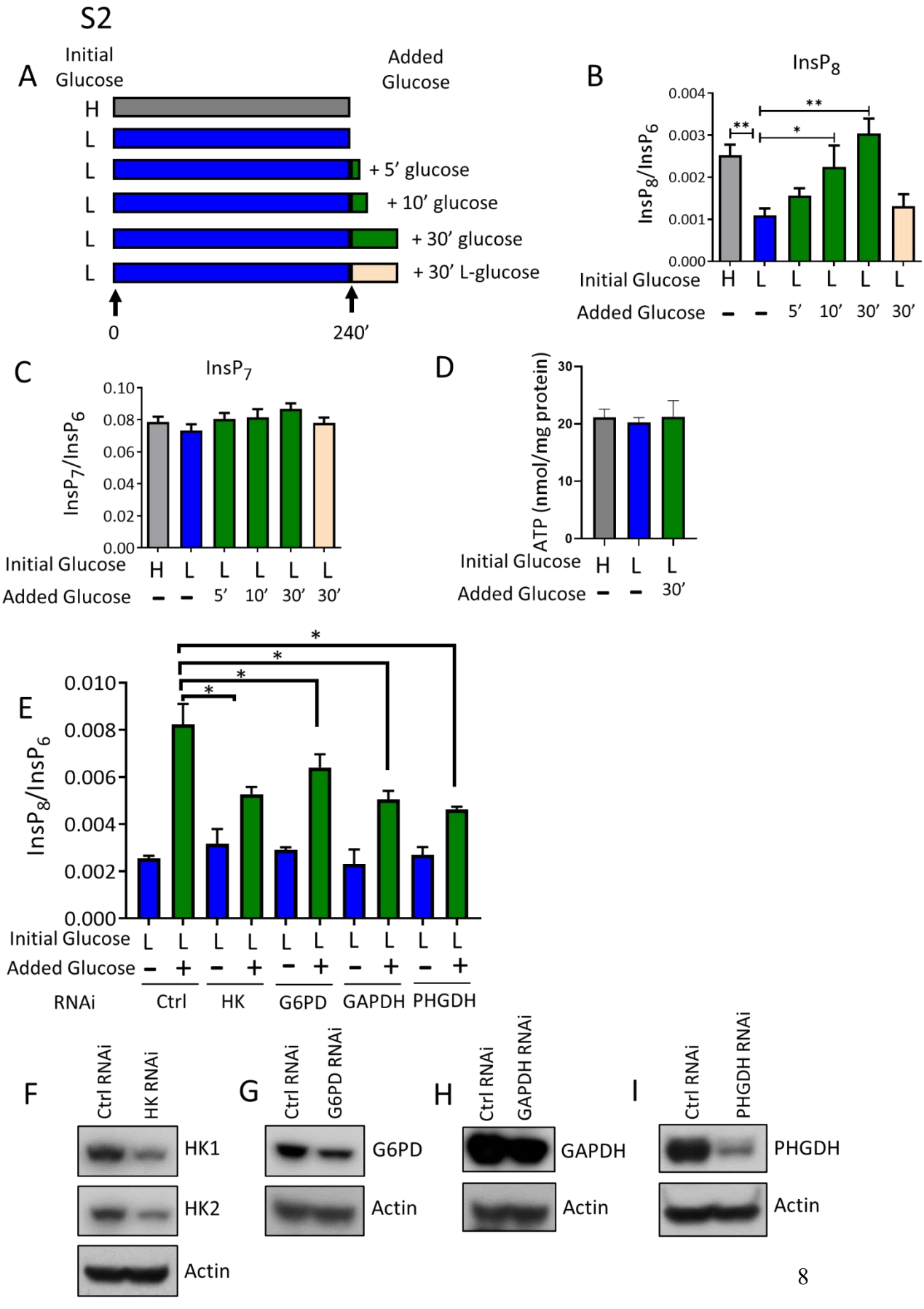
S2
A
Initial Glucose
Added Glucose
H
L
L
L
L
L
+ 5′ glucose
+ 10′ glucose
+ 30′ glucose
+ 30′ L-glucose
0
240′
B
InsP8
InsP8/InsP6
0.004
0.003
0.002
0.001
0.000
**
**
*
Initial Glucose H L L L L L
Added Glucose – – 5′ 10′ 30′ 30′
C
InsP7
InsP7/InsP6
0.10
0.08
0.06
0.04
0.02
0.00
Initial Glucose H L L L L L
Added Glucose – – 5′ 10′ 30′ 30′
D
ATP (nmol/mg protein)
30
20
10
0
Initial Glucose H L L
Added Glucose – – 30′
E
InsP8/InsP6
0.010
0.008
0.006
0.004
0.002
0.000
*
*
*
*
Initial Glucose L L L L L L L L L L
Added Glucose – + – + – + – + – +
RNAi Ctrl HK G6PD GAPDH PHGDH
F
Ctrl RNAi
HK RNAi
HK1
HK2
Actin
G
Ctrl RNAi
G6PD RNAi
G6PD
Actin
H
Ctrl RNAi
GAPDH RNAi
GAPDH
Actin
I
Ctrl RNAi
PHGDH RNAi
PHGDH
Actin

Fig. S2. Cross-talk between glucose metabolism and $InsP_8$ levels in WT HCT116 cells.
(A) Graphic depicting the glucose concentrations used in these experiments: Cells were cultured for 4 h in either high glucose medium ('H'; gray bar), or low glucose medium ('L'; blue bars). Then, as indicated in the panel, 10 mM of either D-glucose (green bars) or L-glucose (beige bar) was added for the indicated times. (B,C) Cellular $InsP_8$ and $InsP_7$ levels in cells cultured for 4h in high-glucose medium ('H'; grey bars), or low glucose medium ('L'; blue, green and beige bars), followed by addition of 10mM of either D-glucose (green bars) or L-glucose (beige bar) for the indicated times. (D) Cellular ATP levels during the treatments described for panel C. (E) Following RNAi knockdown of the indicated individual genes in glucose metabolism pathways, ^{3}H-inositol-labeled cells were cultured for 4 h in low glucose media, either without glucose re-addition ('L'; blue bars), or with re-addition of 10mM glucose for 30 min. Then $InsP_8$ levels were determined. (F, G, H, I) Validation of the extent of the RNAi knockdowns by Western analysis. Student's t-test was used to determine statistical significance of differences between means ($P < 0.05$ [*], $P < 0.01$ [**]).

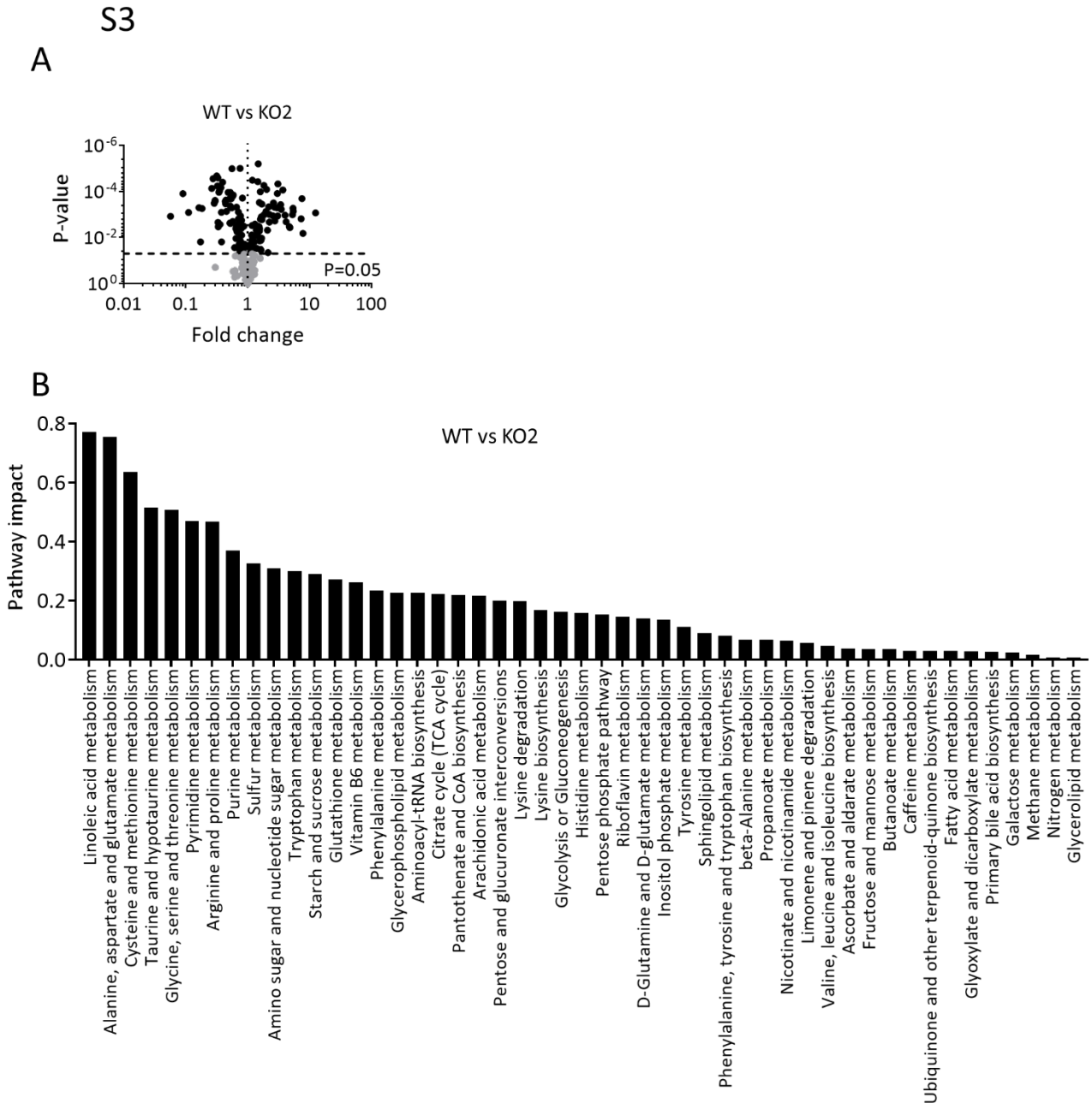

Fig. S3 Metabolomic analysis of PPIP5K KO cells
(A) Volcano plots of metabolites of WT cells and PPIP5K KO2 cells cultured in low glucose media.
(B) Network-based pathway analysis of statistically significant changes in metabolite levels in PPIP5K KO2 cells compared to WT cells.

S4

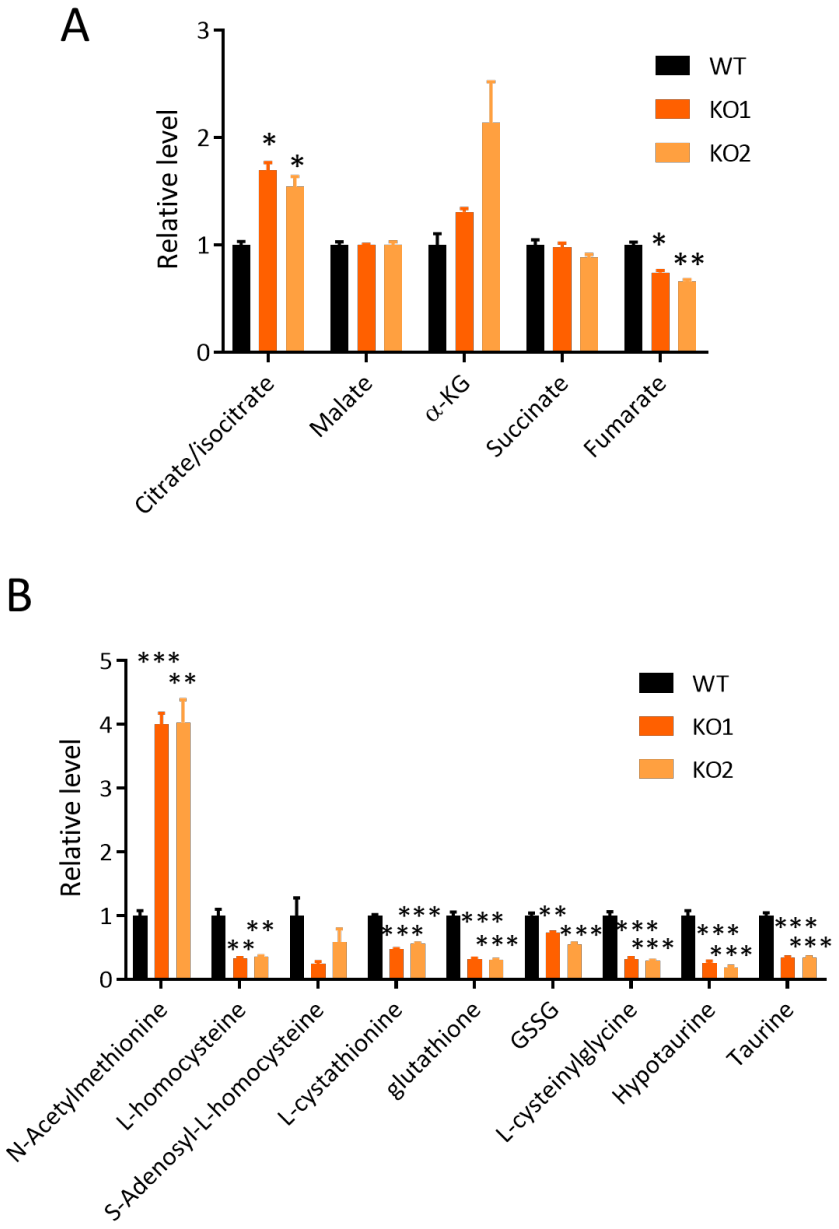

Fig. S4 Metabolomic analysis of TCA cycle and SGOC intermediates in WT and PPIP5KO HCT116 cells.

(A) Relative integrated peak intensities for TCA cycle metabolites in WT HCT116 cells (black bars), and corresponding PPIP5K KO1 (dark orange bars) and KO2 cells (light orange bars). cultured in low glucose media. (B) Relative integrated peak intensities for metabolites related to the SGOC-cycle in WT and PPIP5K KO HCT116 cells (color coded as in panel B) cultured in low-glucose media. Student's t-test was used to determine statistical significance of differences between means ($P < 0.01$ [**], $P < 0.001$ [***]).

S5

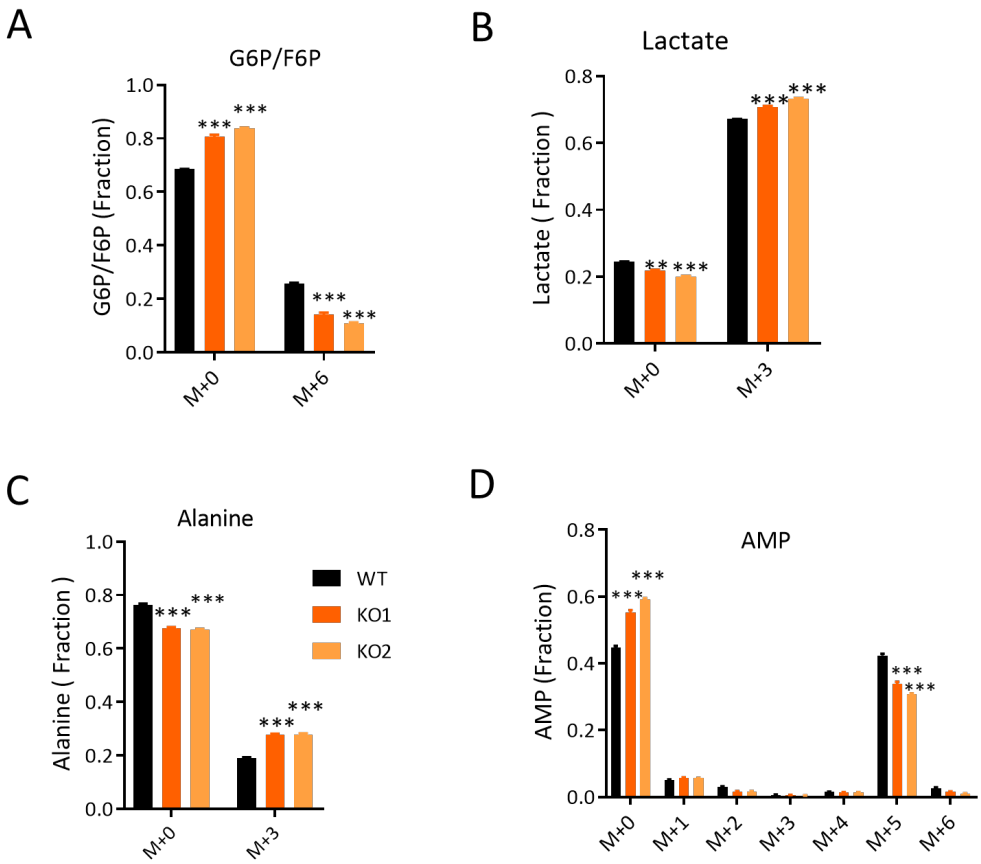

Fig. S5 [U-^{13}C]-glucose labeling of WT and PPIP5K KO HCT116 cells.
(A, B, C and D) Cells were incubated for 24 h in low-glucose media supplemented with [U-^{13}C]-glucose. The 'fraction' is that proportion of each indicated metabolite that is either non-labeled (i.e., M+0) or ^{13}C-labeled (i.e., M+n). Student's t-test was used to determine statistical significance of differences between means ($P < 0.01$ [**], $P < 0.001$ [***]).

S6

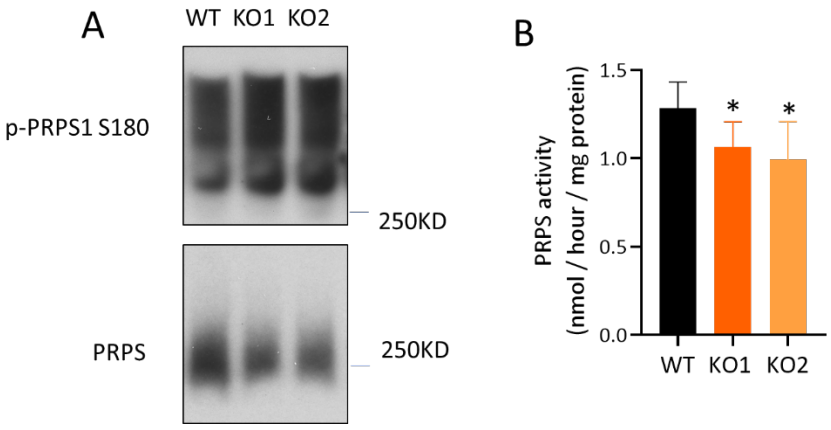

Fig. S6 Measurements of PRPS1 in WT and PPIP5K KO HCT116 cells.
(A) Western analysis of PRPS1 phosphorylation in non-denaturing gels after cells were cultured in low glucose medium for 2 h. (B)PRPS activity in cell lysate of WT and PPIP5K KO cells cultured in low glucose medium for 2 h. Student's t-test was used to determine statistical significance of differences between means ($P < 0.05$ [*]).

S7

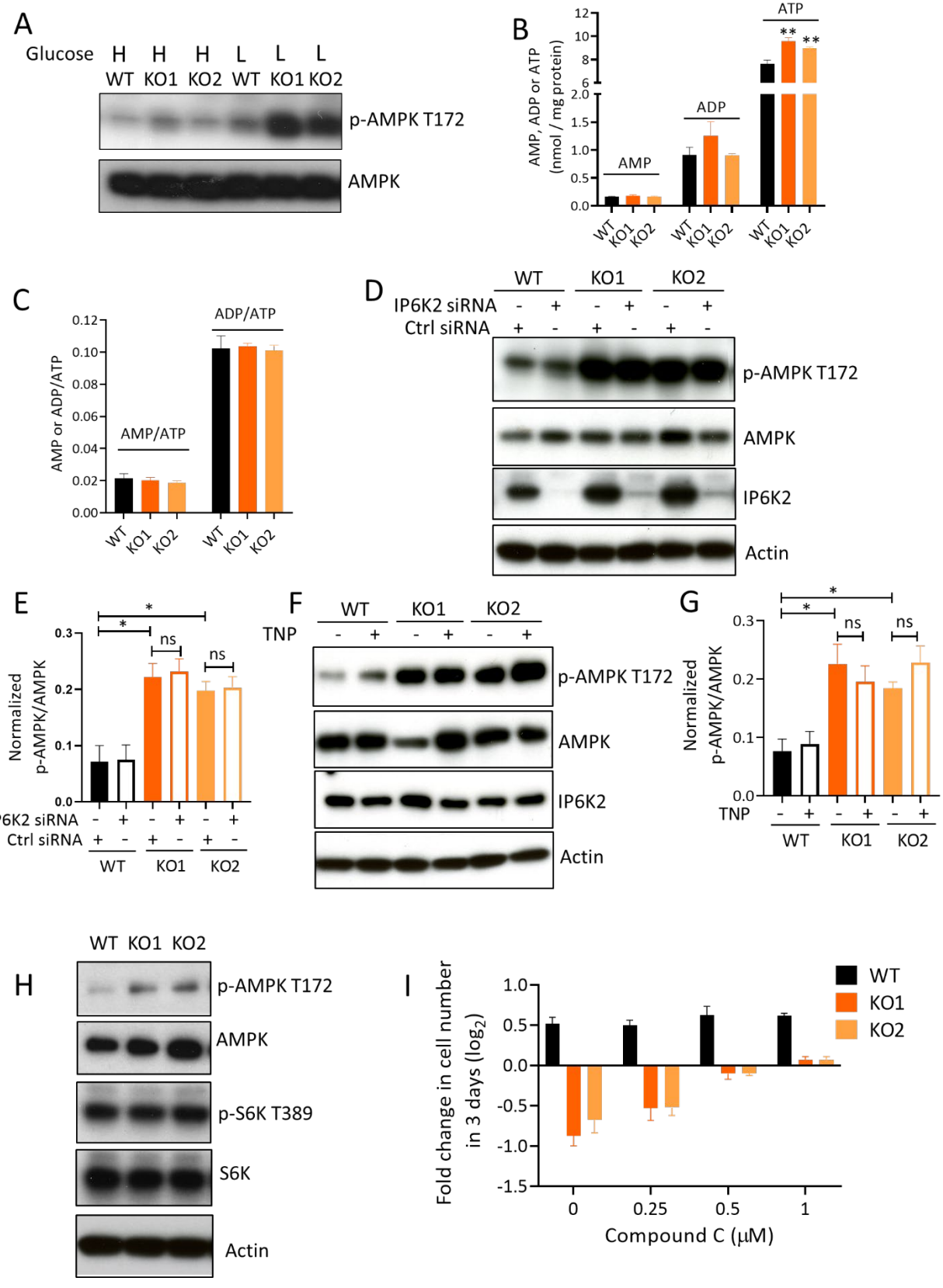
A
Glucose H H H L L L
WT KO1 KO2 WT KO1 KO2
p-AMPK T172
AMPK
B
AMP, ADP or ATP (nmol / mg protein)
AMP
ADP
ATP
**
**
WT KO1 KO2 WT KO1 KO2 WT KO1 KO2
C
AMP or ADP/ATP
AMP/ATP
ADP/ATP
WT KO1 KO2 WT KO1 KO2
D
WT KO1 KO2
IP6K2 siRNA - + - + - +
Ctrl siRNA + - + - + -
p-AMPK T172
AMPK
IP6K2
Actin
E
Normalized p-AMPK/AMPK
*
*
ns
ns
IP6K2 siRNA - + - + - +
Ctrl siRNA + - + - + -
WT KO1 KO2
F
WT KO1 KO2
TNP - + - + - +
p-AMPK T172
AMPK
IP6K2
Actin
G
Normalized p-AMPK/AMPK
*
*
ns
ns
TNP - + - + - +
WT KO1 KO2
H
WT KO1 KO2
p-AMPK T172
AMPK
p-S6K T389
S6K
Actin
I
Fold change in cell number in 3 days (log2)
WT
KO1
KO2
Compound C (μM)
0
0.25
0.5
1

Fig. S7. AMPK related parameters in WT and PPIP5K KO HCT116 cells.
(A) Western analysis of AMPK phosphorylation in WT and PPIP5K KO cells cultured in high glucose medium ('H') or low glucose medium ('L'). (B) Cellular AMP, ADP or ATP levels determined by liquid chromatography-mass spectrometry with internal standards, in WT HCT116 cells (black bars) and PPIP5K KO1 and KO2 HCT116 cells (dark orange and light orange bars, respectively), all cultured in low glucose medium for 2 h. (C) The ratio of AMP or ADP to ATP calculated from data in panel B. (D-G) Western analysis of AMPK phosphorylation upon IP6K2 either RNAi knockdown (D, E) or TNP treatment versus vehicle control (F, G). The relative ratio of p-AMPK/AMPK was calculated from three independent experiments (E, G). (H) Western analysis of phosphorylation status of AMPK and S6K. Cells were cultured in low glucose medium for 2 h (A-F). (I) Cell growth of WT and PPIP5K KO cells cultured in low-glucose medium in the presence of the AMPK inhibitor compound C. Student's t-test was used to determine statistical significance of differences between means ($P > 0.05$ [ns], $P < 0.05$ [*], $P < 0.01$ [**]).

S8

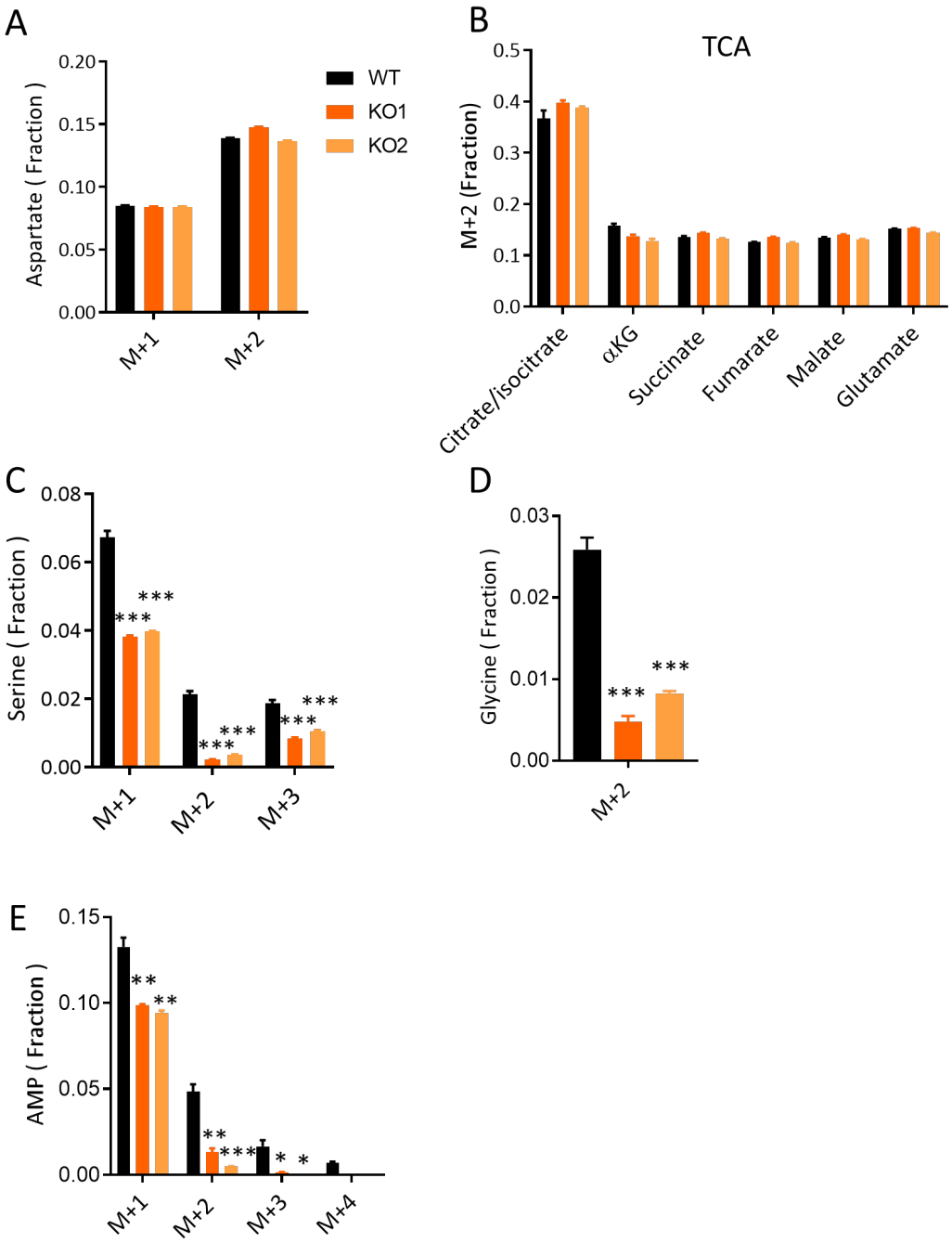

Fig. S8. Fractional labeling of selected metabolites in WT and PPIP5K KO HCT116 cells after incubation with [U-^{13}C]-glucose or [U-^{13}C]-serine.
(A-E) Cells were incubated for 24 h in low-glucose media supplemented with either [U-^{13}C]-glucose (panels A and B) or [U-^{13}C]-serine. The 'fraction' is that proportion of each indicated isotopologue that is ^{13}C-labeled (i.e., M+n). Student's t-test was used to determine statistical significance of differences between means ($P < 0.05$ [*], $P < 0.01$ [**], $P < 0.001$ [***]).

S9

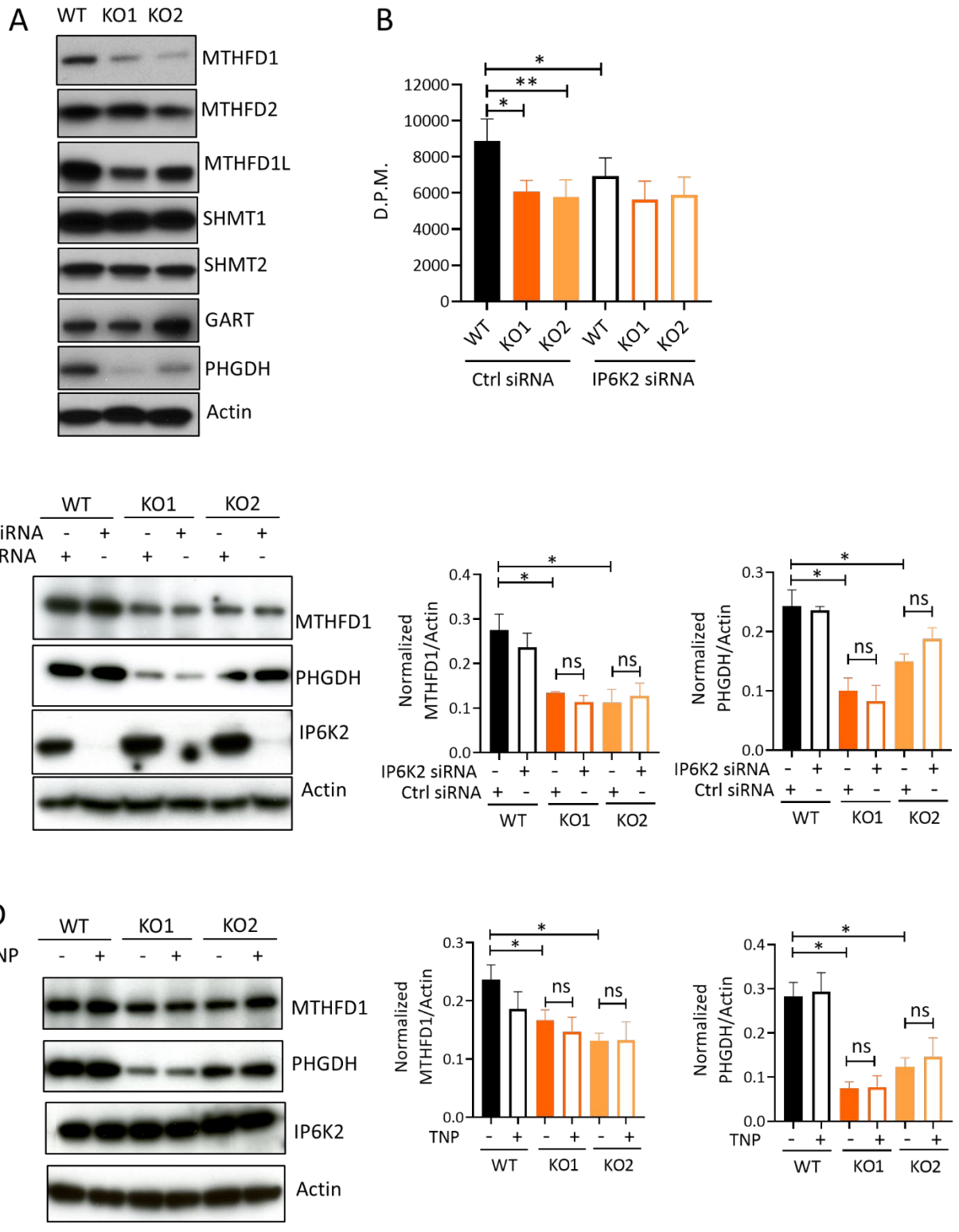
A
WT KO1 KO2
MTHFD1
MTHFD2
MTHFD1L
SHMT1
SHMT2
GART
PHGDH
Actin
B
D.P.M.
12000
10000
8000
6000
4000
2000
0
WT KO1 KO2 WT KO1 KO2
Ctrl siRNA
IP6K2 siRNA
C
WT KO1 KO2
IP6K2 siRNA
Ctrl siRNA
MTHFD1
PHGDH
IP6K2
Actin
Normalized MTHFD1/Actin
Normalized PHGDH/Actin
ns
D
TNP
WT KO1 KO2

Fig. S9. Expression levels and activities of enzymes providing one-carbon units towards purine synthesis in WT and PPIP5K KO HCT116 cells cultured in low glucose media.
(A) the expression of MTHFD1 (methylenetetrahydrofolate dehydrogenase 1), MTHFD2 (methylenetetrahydrofolate dehydrogenase 2), MTHFD1L (methylenetetrahydrofolate dehydrogenase 1 like), SHMT (Serine hydroxymethyltransferase), GART (phosphoribosylglycinamide formyltransferase) and PHGDH (Phosphoglycerate Dehydrogenase) in WT and PPIP5K KO cells. (B) SHMT activity in WT and PPIP5K KO cells transfected with IP6Ks siRNA. (C, D) the expression of MTHFD1 and PHGDH in WT and PPIP5K KO cells transfected with IP6K2 siRNA (C) or treated with TNP versus vehicle control (D) showing as representative western blots and the quantification from three independent experiments. Student's t-test was used to determine statistical significance of differences between means ($P > 0.05$ [ns], $P < 0.05$ [*], $P < 0.01$ [**]).

S10

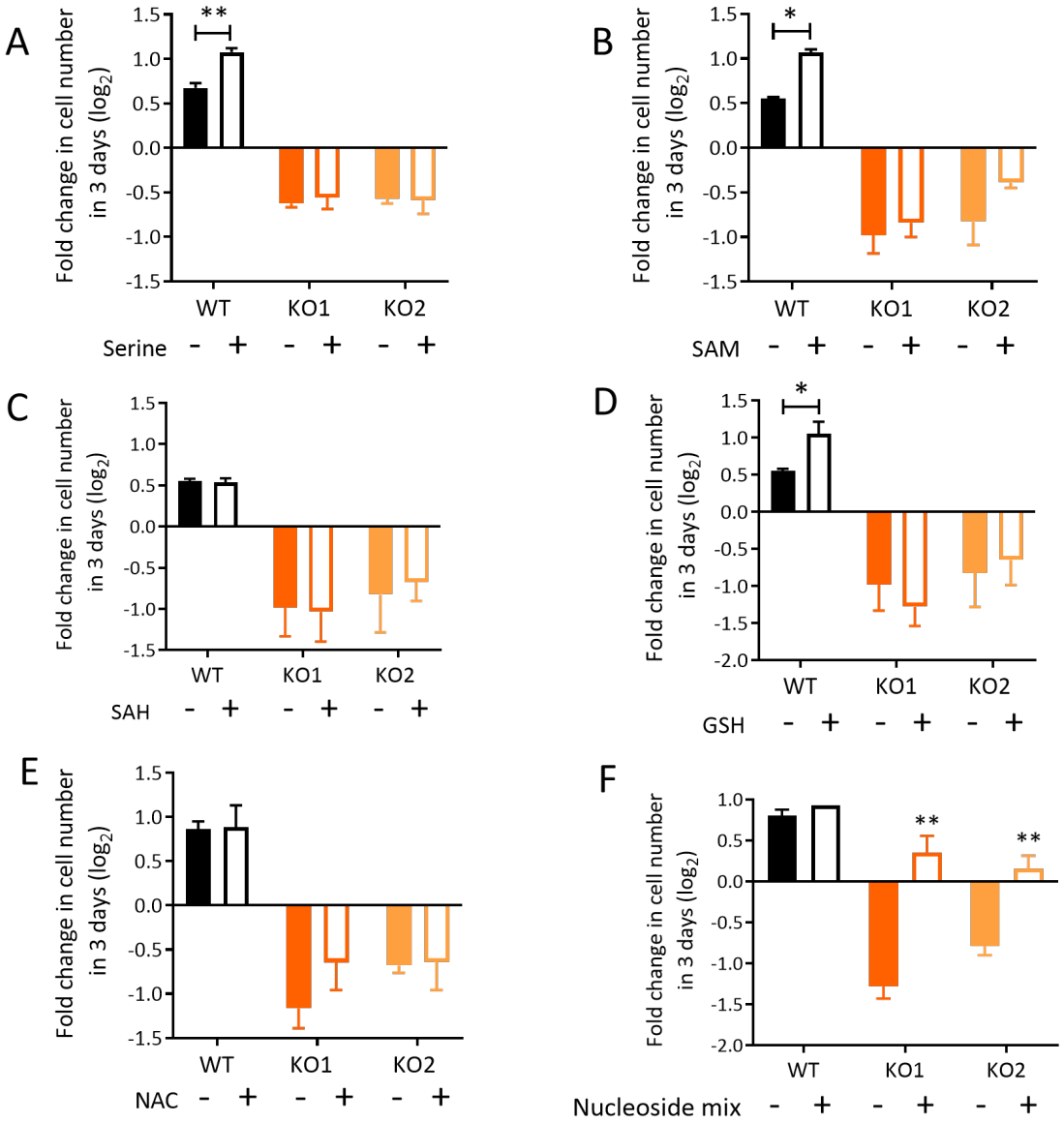

Fig. S10. The effects of various supplements to low-glucose media upon cell growth of WT and PPIP5K KO HCT116 cells.
(A-F) cell growth for 3 days in low glucose medium supplemented with either 4mM serine, 100uM SAM (S-Adenosylmethionine), 100uM SAH (S-adenosylhomocysteine), 5mM GSH (glutathione), 1mM NAC (N-acetyl-L-cysteine) or a nucleoside mixture. Student's t-test was used to determine statistical significance of differences between means ($P < 0.05$ [*], $P < 0.01$ [**]).

S11

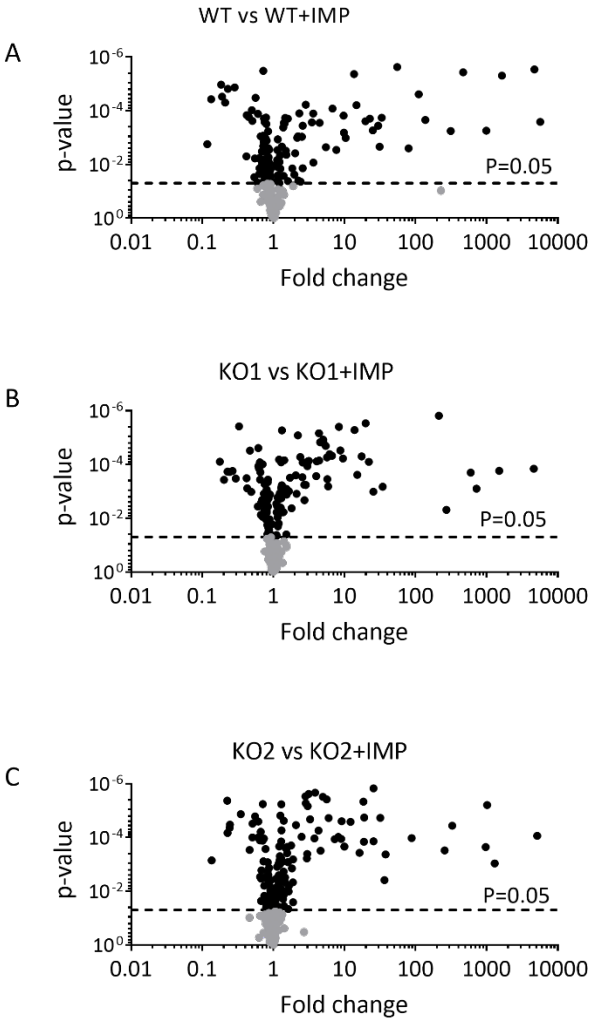

Fig. S11. Volcano plots showing the impact of 1 mM IMP supplementation upon detected metabolites from WT and PPIP5K KO HCT116 cells cultured in low glucose medium

S12

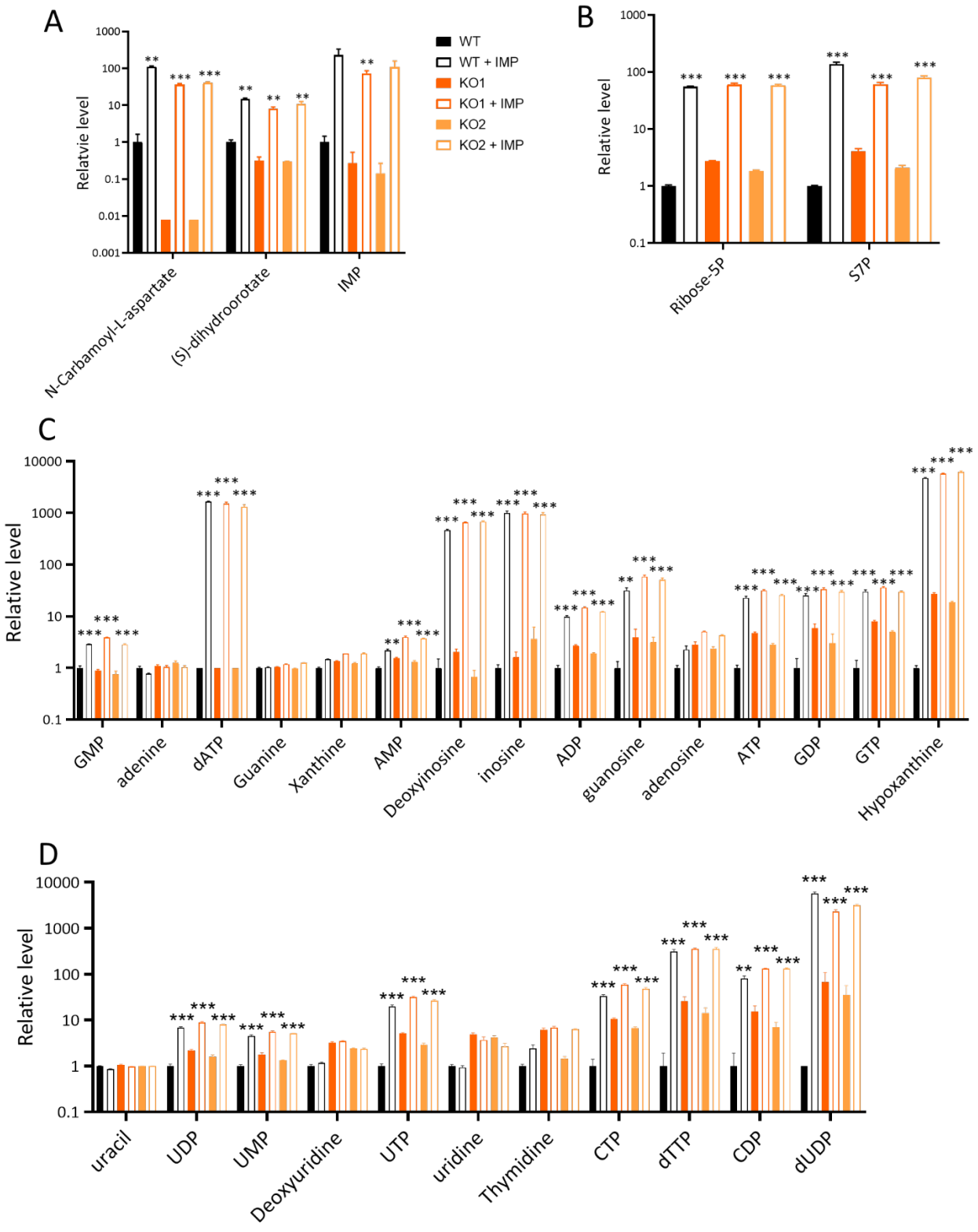
A
Relatvie level
WT
WT + IMP
KO1
KO1 + IMP
KO2
KO2 + IMP
N-Carbamoyl-L-aspartate
(S)-dihydroorotate
IMP
B
Relative level
Ribose-5P
S7P
C
Relative level
GMP
adenine
dATP
Guanine
Xanthine
AMP
Deoxyinosine
inosine
ADP
guanosine
adenosine
ATP
GDP
GTP
Hypoxanthine
D
Relative level
uracil
UDP
UMP
Deoxyuridine
UTP
uridine
Thymidine
CTP
dTTP
CDP
dUDP

Fig. S12. Levels of metabolites in WT and PPIP5K KO HCT116 cells cultured in low glucose medium supplemented with 1mM IMP
(A) Relative levels of metabolites involved in de novo nucleotide synthesis. (B) Relative levels of metabolites of purine metabolism. (C) Relative levels of metabolites of pyrimidine metabolism. (D). Relative levels of metabolites in the pentose phosphate pathway. Student's t-test was used to determine statistical significance of differences between means of cells cultured with or without IMP supplementation ($P < 0.01$ [**], $P < 0.001$ [***]).

S13

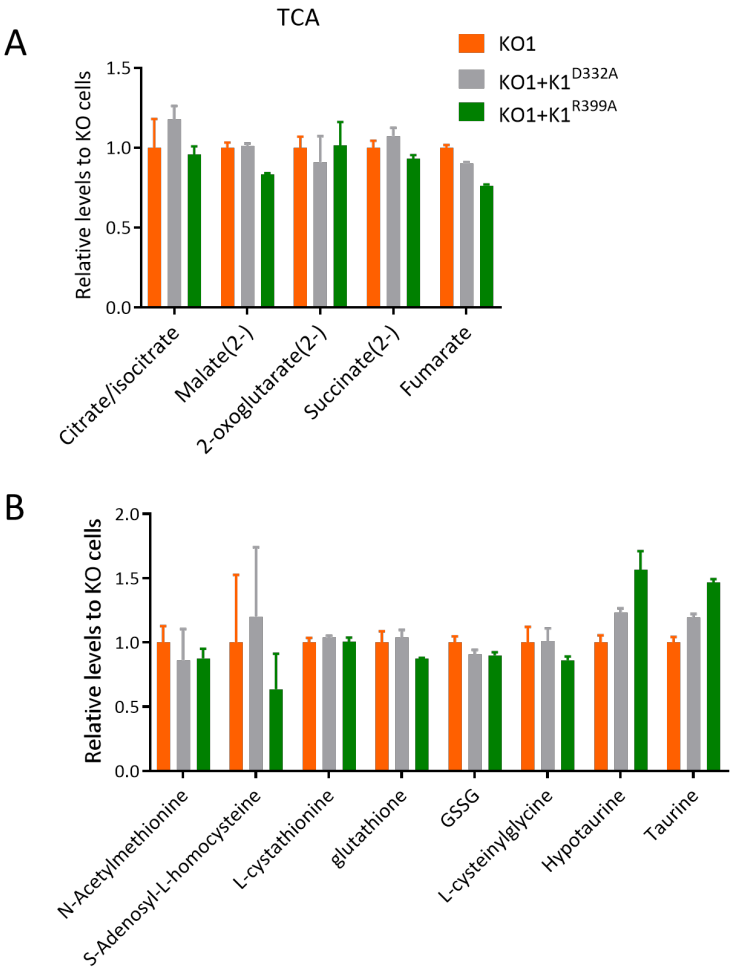

Fig. S13 Lack of effects of stable expression of either PPIP5K1^{D332A} or PPIP5K1^{R399A} mutants upon relative integrated peak intensities for TCA metabolites (A) and SGOC related metabolites (B) in PPIP5K KO1 HCT116 cells.

Dataset S1 (separate file). Steady-state metabolites of WT and PPIP5K KO HCT116 cells cultured in low glucose medium.

Dataset S2 (separate file). ^{13}C-glucose and ^{13}C-serine isotope tracing in WT and PPIP5K KO HCT116 cells cultured in low glucose medium.

Dataset S3 (separate file). Steady-state metabolites of WT and PPIP5K KO HCT116 cells cultured in low glucose medium supplemented with 1mM IMP.

Dataset S4 (separate file). Steady-state metabolites of PPIP5K KO HCT116 cells stably expressing PPIP5K1^{D332A} or PPIP5K1^{R399A} mutants cultured in low glucose medium.

1. Gu, C., et al., *KO of 5-InsP7 kinase activity transforms the HCT116 colon cancer cell line into a hypermetabolic, growth-inhibited phenotype.* Proc Natl Acad Sci U S A, 2017. **114**(45): p. 11968-11973.
2. Lindl, T.Z.-u.G., 5th ed., *Spektrum Akademischer Verlag:*. 2002, Heidelberg, Germany
3. Liu, X., Z. Ser, and J.W. Locasale, *Development and quantitative evaluation of a high-resolution metabolomics technology.* Anal Chem, 2014. **86**(4): p. 2175-84.
4. Liu, X., et al., *High-Resolution Metabolomics with Acyl-CoA Profiling Reveals Widespread Remodeling in Response to Diet.* Mol Cell Proteomics, 2015. **14**(6): p. 1489-500.
5. Gu, C., et al., *Inositol Pyrophosphate Profiling of Two HCT116 Cell Lines Uncovers Variation in InsP8 Levels.* PLoS One, 2016. **11**(10): p. e0165286.
6. Braman, J.C., M.J. Black, and J.H. Mangum, *Serine transhydroxymethylase: a simplified radioactive assay; purification and stabilization of enzyme activity employing Affi-Gel Blue.* Prep Biochem, 1981. **11**(1): p. 23-32.
7. Cao, J., et al., *HDAC11 regulates type I interferon signaling through defatty-acylation of SHMT2.* Proc Natl Acad Sci U S A, 2019. **116**(12): p. 5487-5492.
8. Robert T. Taylor, H.W., *Radioactive Assay for Serine Transhydroxymethylase.* Analytical Biochemistry, 1965. **13**(1): p. 80-84.