Supplementary information for

Glucose-6-Phosphate Dehydrogenase Maintains Redox Homeostasis and Biosynthesis in *LKB1*-Deficient *KRAS*-Driven Lung Cancer

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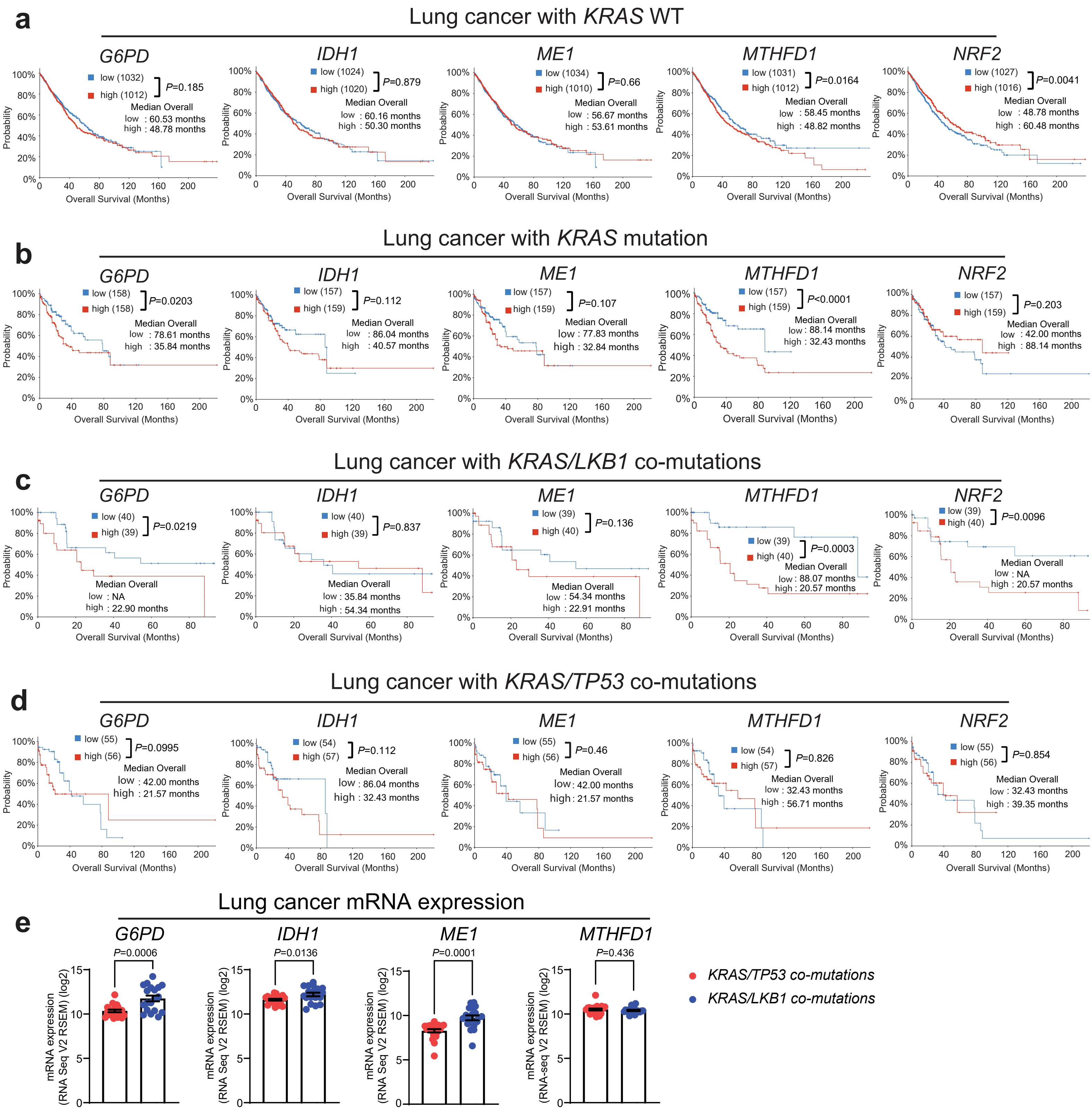
Supplementary Fig. 1

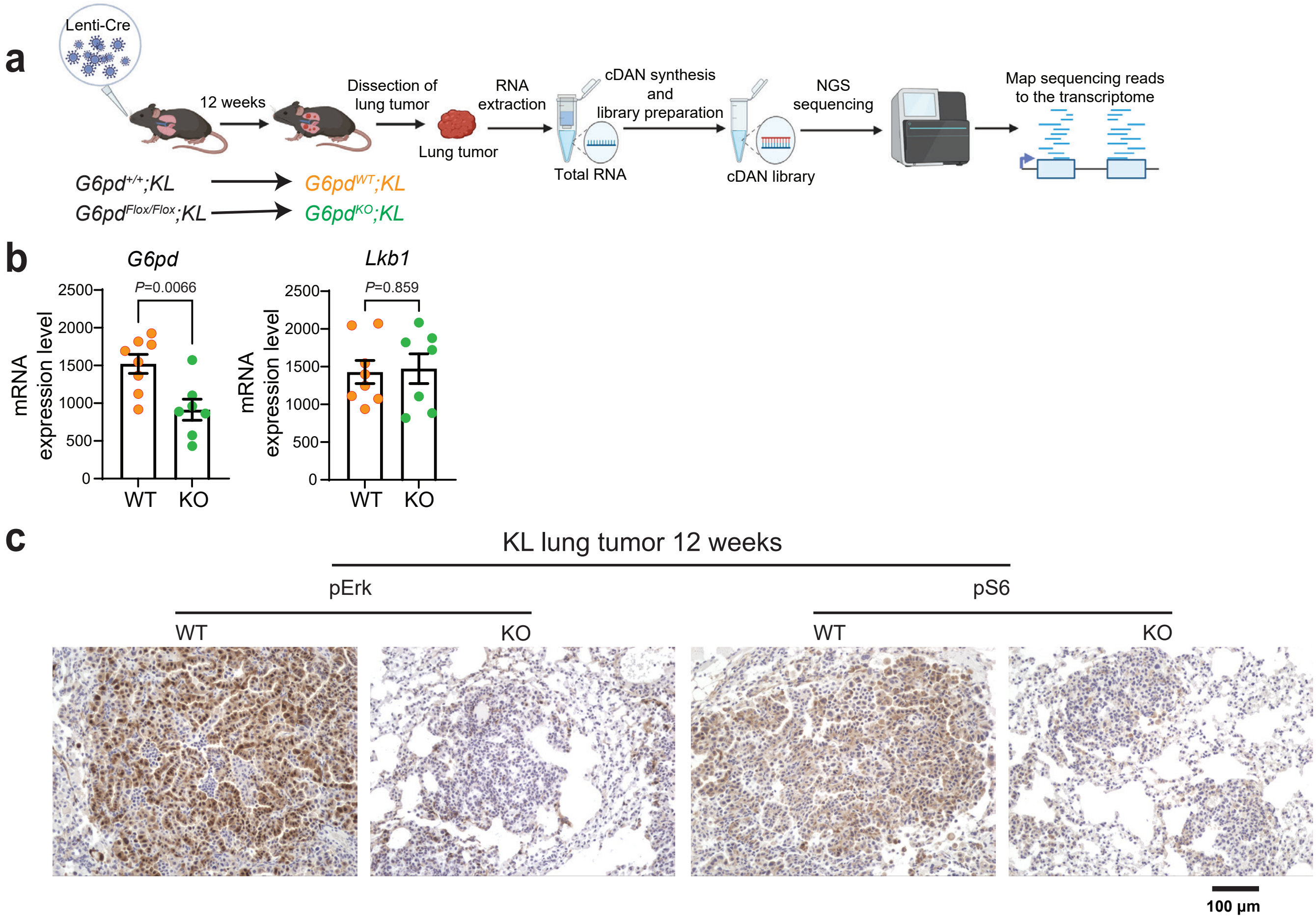


Supplementary Fig. 1: High *G6PD* expression correlates with poor survival of lung cancer patients with *KRAS/LKB1* co-mutations.

a. Overall survival comparison between low and high mRNA expression group of *G6PD*, *IDH1*, *ME1*, *MTHFD1*, and *NRF2* in lung cancer patients with *KRAS* wild type (WT), using data obtained from cBioPortal datasets on December 09, 2023. n = 2044 patients for G6PD, IDH1, ME1, MTHFD1, n = 2043 patients for NRF2.
b. Overall survival comparison between low and high mRNA expression group of *G6PD*, *IDH1*, *ME1*, *MTHFD1*, and *NRF2* in lung cancer patients with *KRAS* mutation, using data obtained from cBioPortal datasets on December 09, 2023. n = 316 patients for each gene.
c. Overall survival comparison between low and high mRNA expression group of *G6PD*, *IDH1*, *ME1*, *MTHFD1*, and *NRF2* in lung cancer patients with *KRAS/LKB1* co-mutations, using data obtained from cBioPortal datasets on December 09, 2023. n = 79 patients for each gene.
d. Overall survival comparison between low and high mRNA expression group of *G6PD*, *IDH1*, *ME1*, *MTHFD1*, and *NRF2* in lung cancer patients with KRAS/TP53 co-mutations, using data obtained from cBioPortal datasets on December 09, 2023. n = 111 patients for each gene.
e. The mRNA expression levels of *G6PD*, *ME1*, *IDH1*, and *MTHFD1* in lung cancer patients with *KRAS/TP53* co-mutations (n = 22 patients) and KRAS/LKB1 co-mutations (n = 19 patients), using data obtained from cBioPortal datasets on December 09, 2023.

Data are presented as mean ± SEM, significance was performed by cBioPortal (version 6.0.1). Source data are provided as a Source Data file.

Supplementary Fig. 2



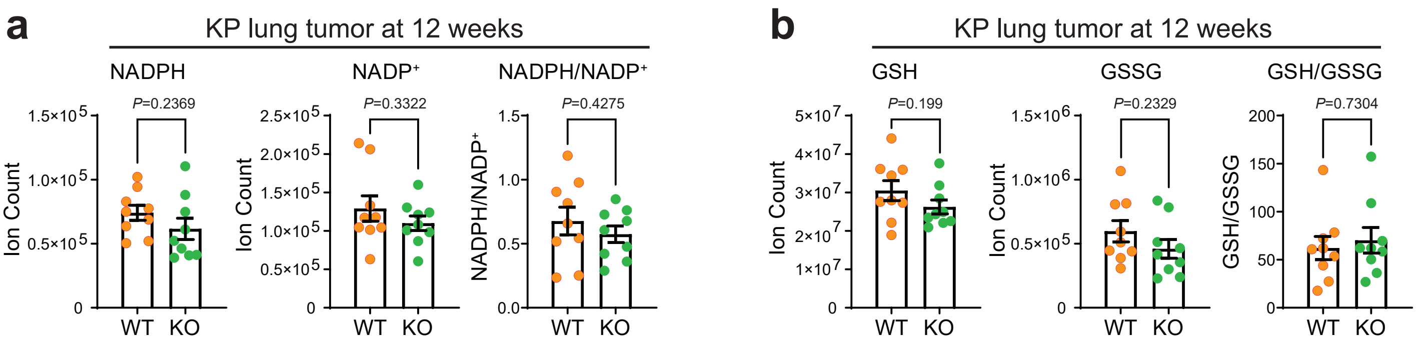
Supplementary Fig. 2: *G6pd* and *Lkb1* mRNA expression from bulk tumor RNA-seq and IHC staining of pErk and pS6 of KL lung tumors.

a. Scheme illustrating the bulk tumor RNA-seq of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* lung tumors at 12 weeks post-tumor induction (Created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license).

b. *G6pd* and *Lkb1* mRNA expression of *G6pd*^{KO}*;KL* (n = 7 mice) and *G6pd*^{WT}*;KL* (n = 8 mice) lung tumors.

c. Lower magnification of IHC staining of pERK (n = 24 images for *G6pd*^{WT}*;KL*, n = 12 images for *G6pd*^{KO}*;KL*) and pS6 (n = 18 images for *G6pd*^{WT}*;KL*, n = 15 images for *G6pd*^{KO}*;KL*) of KL lung tumors at 12 weeks post-tumor induction to show reduced growth signaling of KL lung tumors by G6PD depletion.

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (b). Source data are provided as a Source Data file.


a
KP lung tumor at 12 weeks
NADPH
NADP⁺
NADPH/NADP⁺
P=0.2369
P=0.3322
P=0.4275
Ion Count
NADPH/NADP⁺
WT KO
b
KP lung tumor at 12 weeks
GSH
GSSG
GSH/GSSG
P=0.199
P=0.2329
P=0.7304
Ion Count
GSH/GSSG
WT KO

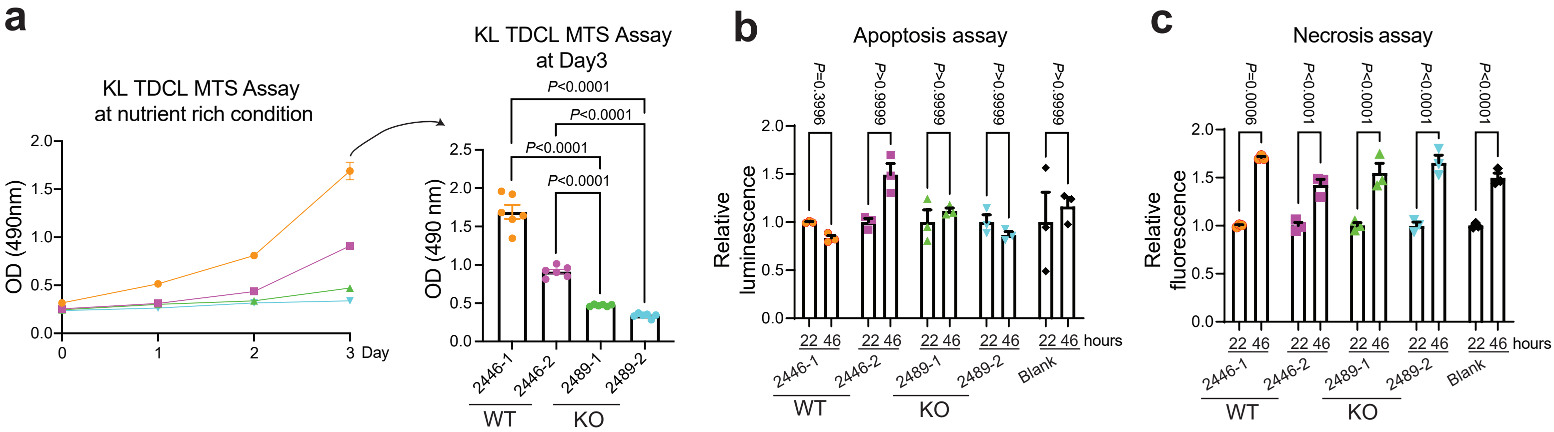
Supplementary Fig. 3: G6PD loss has no effect on KP lung tumor redox homeostasis.

a. Pool size of NADPH and NADP+, and NADPH/NADP+ ratio in *G6pd*^{WT}*;KP* (n = 9 mice) and *G6pd*^{KO}*;KP* (n = 9 mice) lung tumors at 12 weeks post-tumor induction.

b. Pool size of glutathione (GSH) and glutathione disulfide (GSSG), and GSH/GSSG ratio in *G6pd*^{WT}*;KP* (n = 9 mice) and *G6pd*^{KO}*;KP* (n = 9 mice) lung tumors at 12 weeks post-tumor induction.

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (a, GSH and GSSG in panel b), or Mann Whitney test (GSH/GSSG in panel b). Source data are provided as a Source Data file.

Supplementary Fig. 4



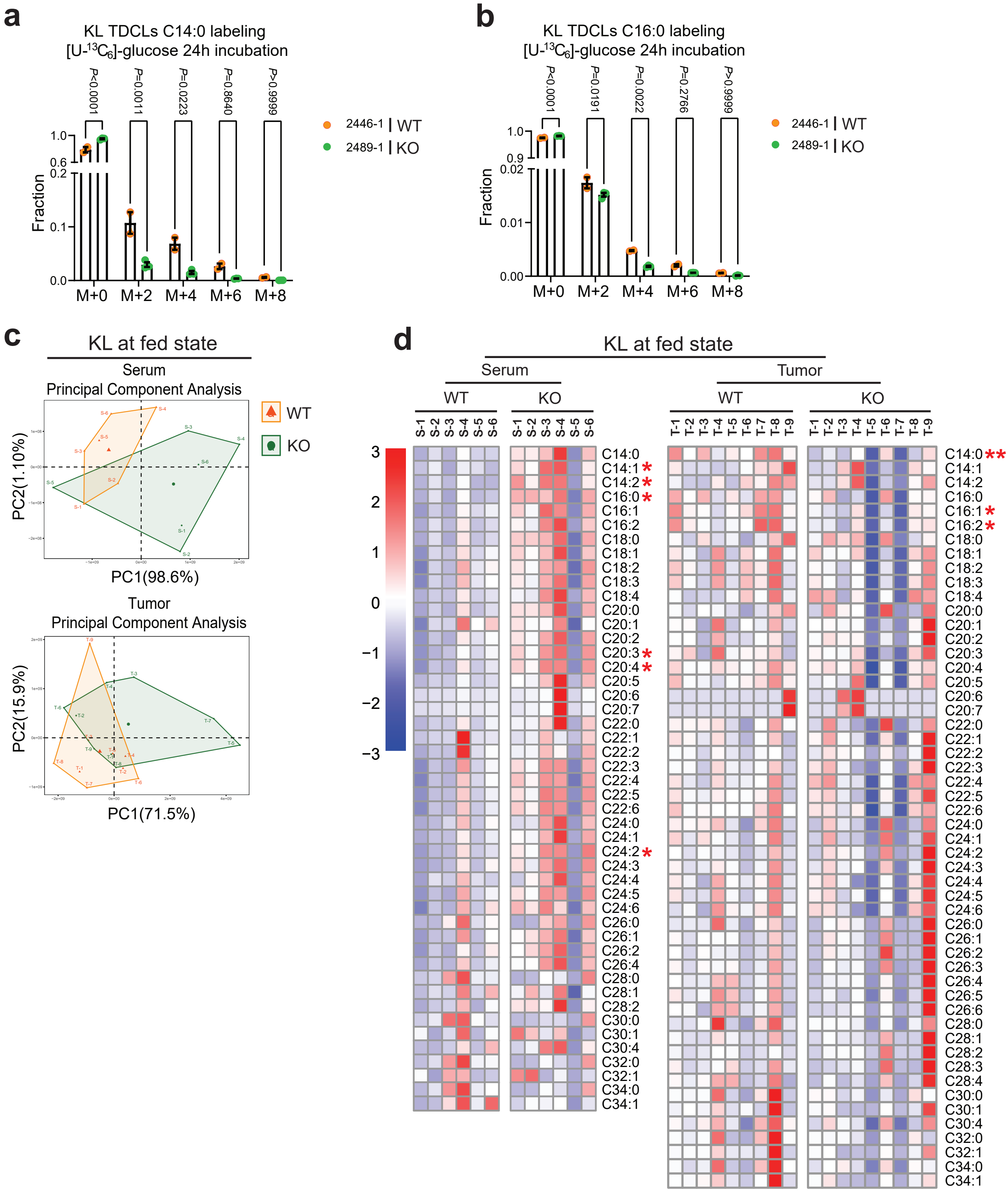
Supplementary Fig. 4: Reduced *G6pd*^{KO}*;KL* TDCLs proliferation is independent of apoptosis and necrosis.

a. MTS assay of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs in nutrient rich conditions. n = 6 replicates for each clone at indicated timepoint, except 2489-2 with n = 5 replicates at day 0 and n = 6 replicates at day 1, 2, and 3.

b & c. Apoptosis (b) and necrosis (c) assay of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs in nutrient rich conditions. n = 3 replicates for each clone at indicated timepoint.

Data are presented as mean ± SEM, significance was calculated by one-way ANOVA followed by Bonferroni's multiple comparisons test (a, b, c). Source data are provided as a Source Data file.

Supplementary Fig. 5



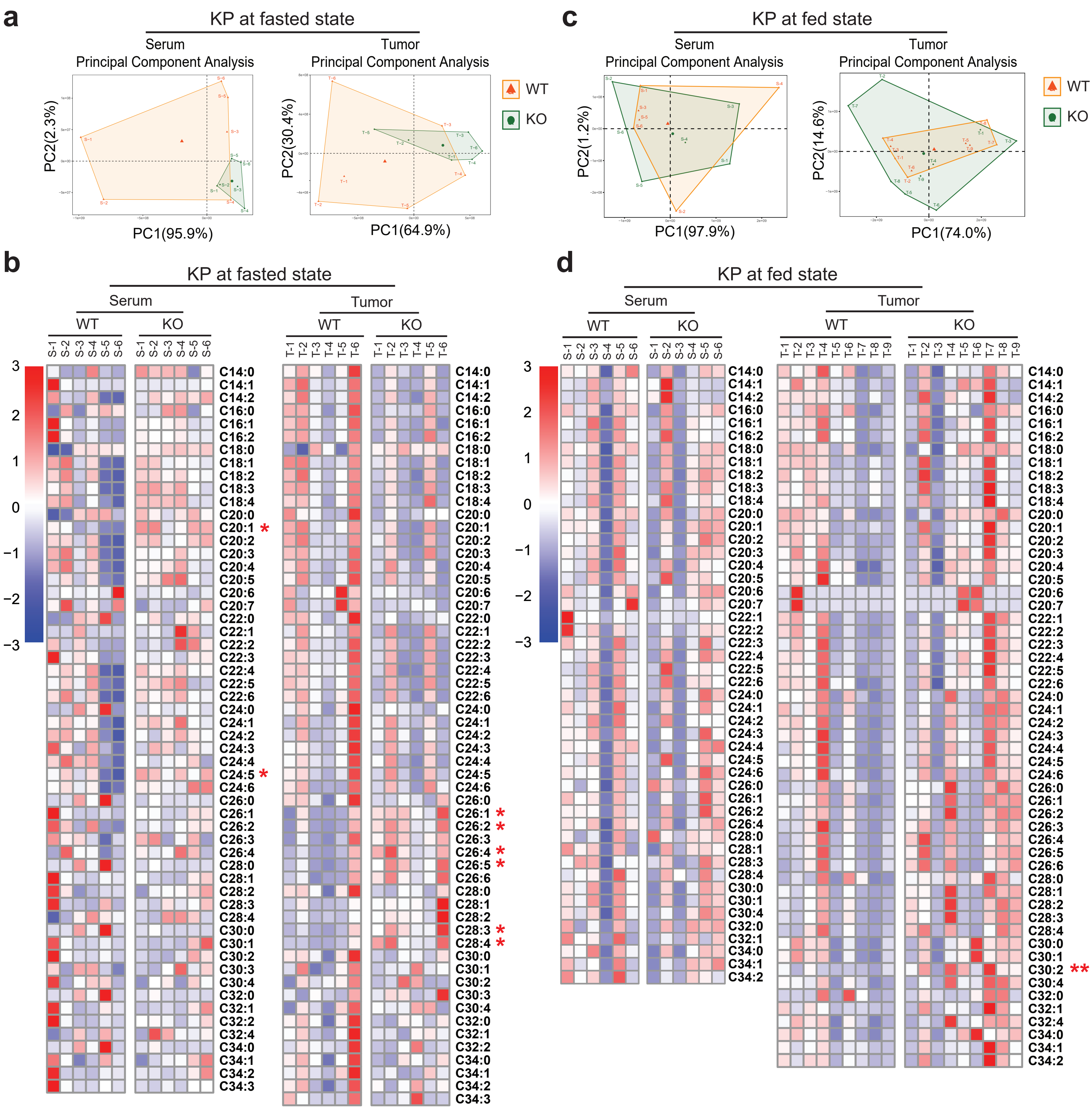
Supplementary Fig. 5: G6PD deficiency affects KL lung tumor lipid metabolism.

a & b. C14:0 (a) and C16:0 (b) ^{2}H labeling fraction of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs after 24 hours [U-$^{13}C_6$]-glucose labeling. n = 2 replicates for *G6pd*^{WT}*;KL* TDCL, n = 3 replicates for *G6pd*^{KO}*;KL* TDCL.

c & d. Principal Component Analysis (PCA) (c) and Heatmap (d) of saponified fatty acid pool size of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* lung tumors and serum from KL tumor bearing mice in fed state (food was kept in cages, and mice were euthanized with samples collected at 8:00AM) at 12 weeks post-tumor induction. n = 6 mice for tumor and serum samples.

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (c, d) or two-way ANOVA followed by Bonferroni's multiple comparisons test (a, b). *$P < 0.05$; **$P < 0.01$. Source data are provided as a Source Data file.

Supplementary Fig. 6



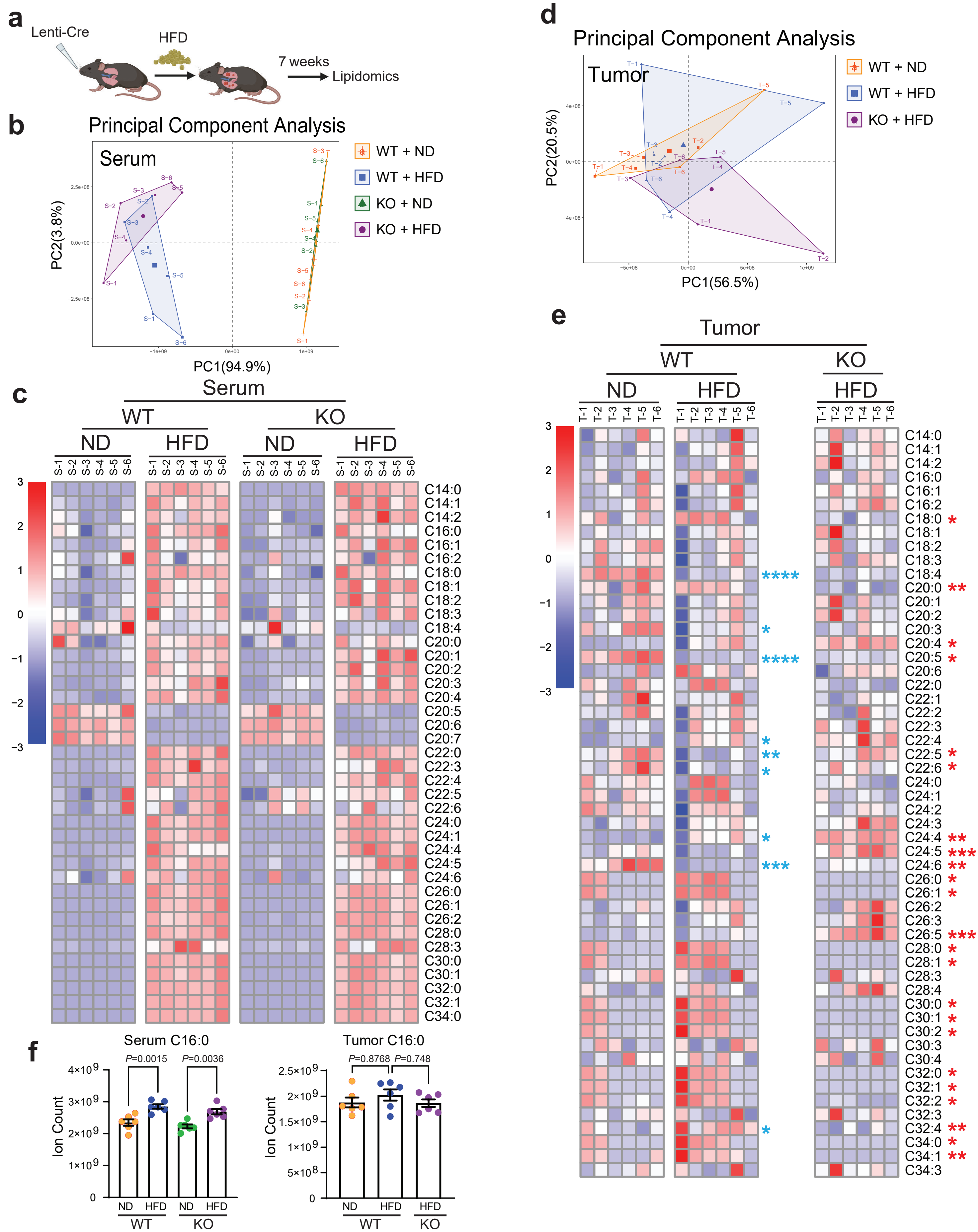
Supplementary Fig. 6: G6PD deficiency does not impact KP lung tumor lipid metabolism.

a & b. Principal Component Analysis (PCA) (a) and Heatmap (b) of saponified fatty acid pool size of *G6pd*^{WT}*;KP* and *G6pd*^{KO}*;KP* lung tumors and serum from KP lung tumor bearing mice in fasted state (food was removed from the mice at approximately 9:00AM, and mice were euthanized with samples collected at 3:00PM) at 12 weeks post-tumor induction. n = 6 mice for tumor and serum samples.

c & d. Principal Component Analysis (PCA) (c) and Heatmap (d) of saponified fatty acid pool size of *G6pd*^{WT}*;KP* and *G6pd*^{KO}*;KP* lung tumors and serum from KP lung tumor bearing mice in fed state (food was kept in cages, and mice were euthanized with samples collected at 8:00AM) at 12 weeks post-tumor induction. n = 6 mice for tumor and serum samples.

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (a, b, c, d). *P< 0.05; **P< 0.01. Source data are provided as a Source Data file.

Supplementary Fig.7



Supplementary Fig. 7: HFD partially rescues the alterations in fatty acyl group composition resulting from G6PD loss.

a. Scheme to examine the effect of HFD on fatty acyl group composition of KL lung tumors (Created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license).

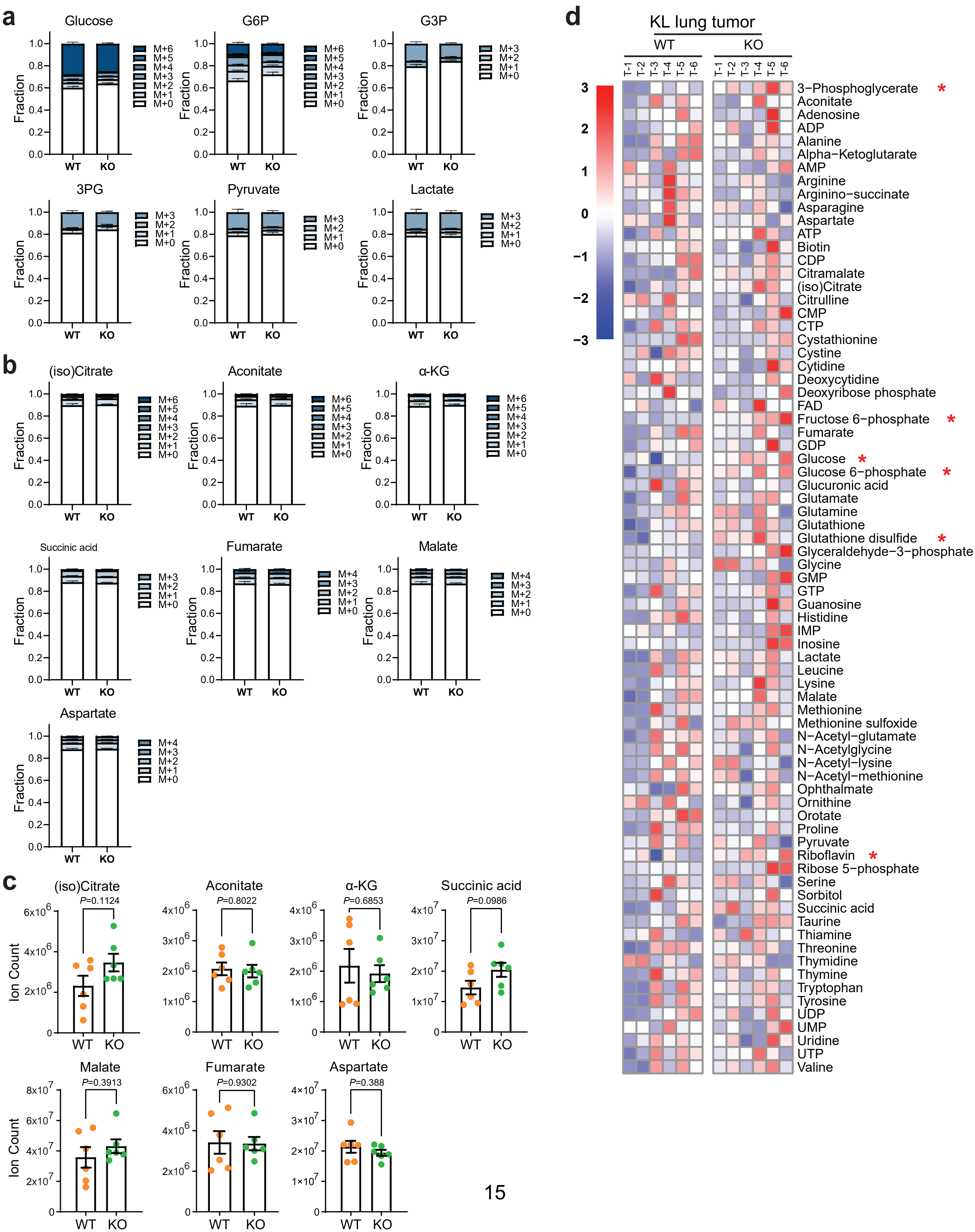
b & c. PCA (a) and heatmap (b) of saponified fatty acid pool size of serum from $G6pd^{WT}$*;KL* or $G6pd^{KO}$*;KL* lung tumor bearing mice fed with normal diet (ND, for 7 weeks) or high fat diet (HFD, for 7 weeks) in fasted state (food was removed from the mice at approximately 9:00AM, and mice were euthanized with samples collected at 3:00PM) at 7 weeks post-tumor induction. n = 6 mice for each group.

d & e. PCA (c) and heatmap (d) of saponified fatty acid pool size of $G6pd^{WT}$*;KL* or $G6pd^{KO}$*;KL* lung tumors from tumor bearing mice fed with normal diet (ND, for 7 weeks) or high fat diet (HFD, for 7 weeks) in fasted state (food was removed from the mice at approximately 9:00AM, and mice were euthanized with samples collected at 3:00PM) at 7 weeks post-tumor induction. n = 6 mice for each group. Blue asterisks denote significance between ND and HFD in $G6pd^{WT}$*;KL* tumors, while red asterisks denote significance between $G6pd^{WT}$*;KL* and $G6pd^{KO}$*;KL* tumors in HFD.

f. Pool size of C16:0 of $G6pd^{WT}$*;KL* and $G6pd^{KO}$*;KL* tumors and serum from (b) and (d).

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (d) one-way ANOVA followed by Bonferroni's multiple comparisons test (e). *$P< 0.05$; **$P< 0.01$; ***$P< 0.001$; ****$P< 0.0001$. Source data are provided as a Source Data file.

Supplementary Fig. 8



Supplementary Fig. 8: G6PD loss does not affect KL lung tumor TCA cycle metabolism.

a. ^{13}C labeling fraction of glycolytic intermediates from glucose of *G6pd*^{WT}*;KL* (n = 6 mice) and *G6pd*^{KO}*;KL* (n = 6 mice) lung tumors in fasted state (food was removed from the mice at approximately 9:00AM, and mice were euthanized with samples collected at 3:00PM) at 12 weeks post-tumor induction. Glucose 6-phosphate (G6P), glyceraldehyde 3-phosphate (G3P), 3-phosphoglycerate (3-PG),

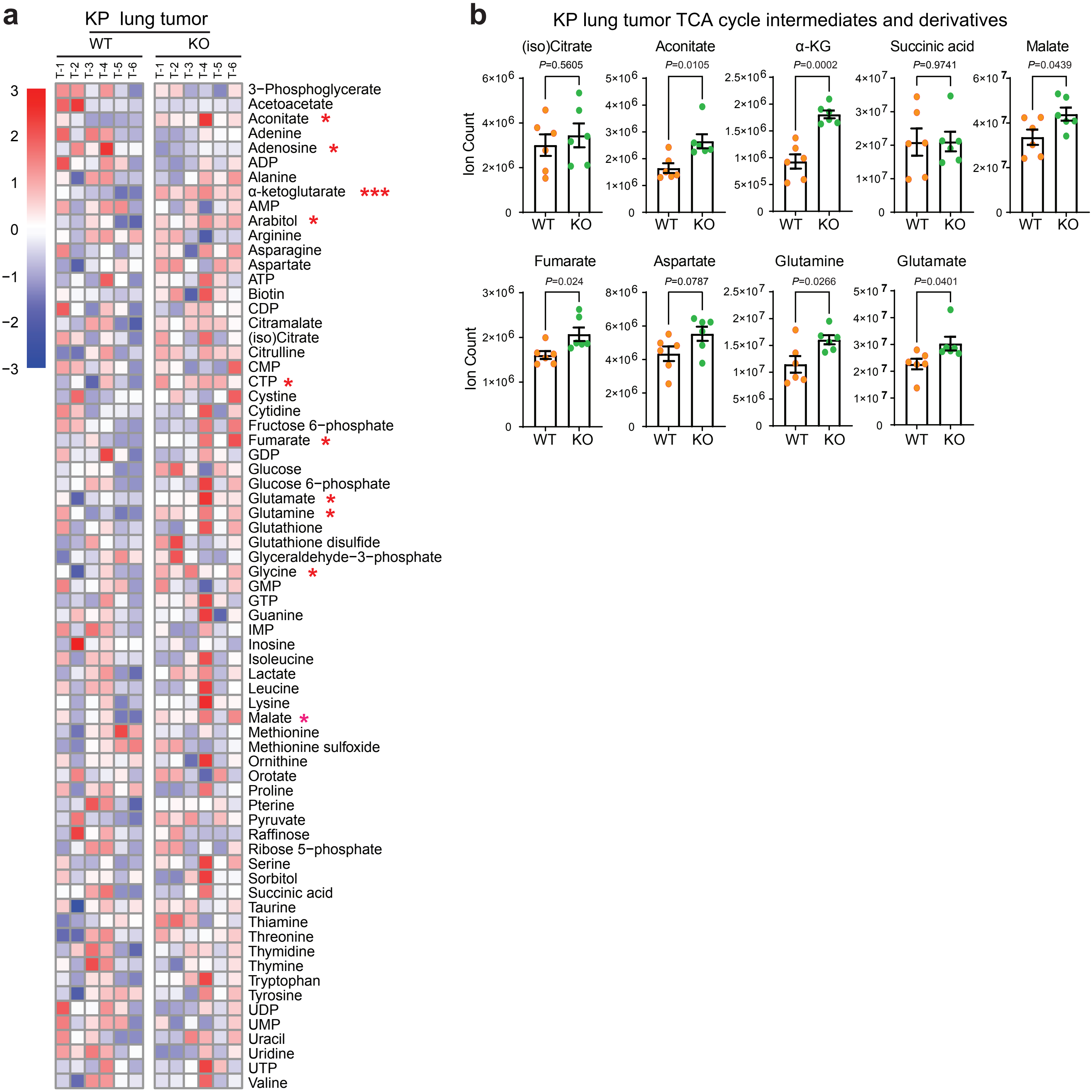
b. ^{13}C labeling fraction of TCA cycle intermediates from glucose of tumors same with (a). α-ketoglutarate (α-KG).

c. Pool size of TCA cycle intermediates of tumors same with (a).

d. A heatmap of water-soluble metabolites of tumors same with (a).

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (c). *$P < 0.05$. Source data are provided as a Source Data file.

Supplementary Fig. 9



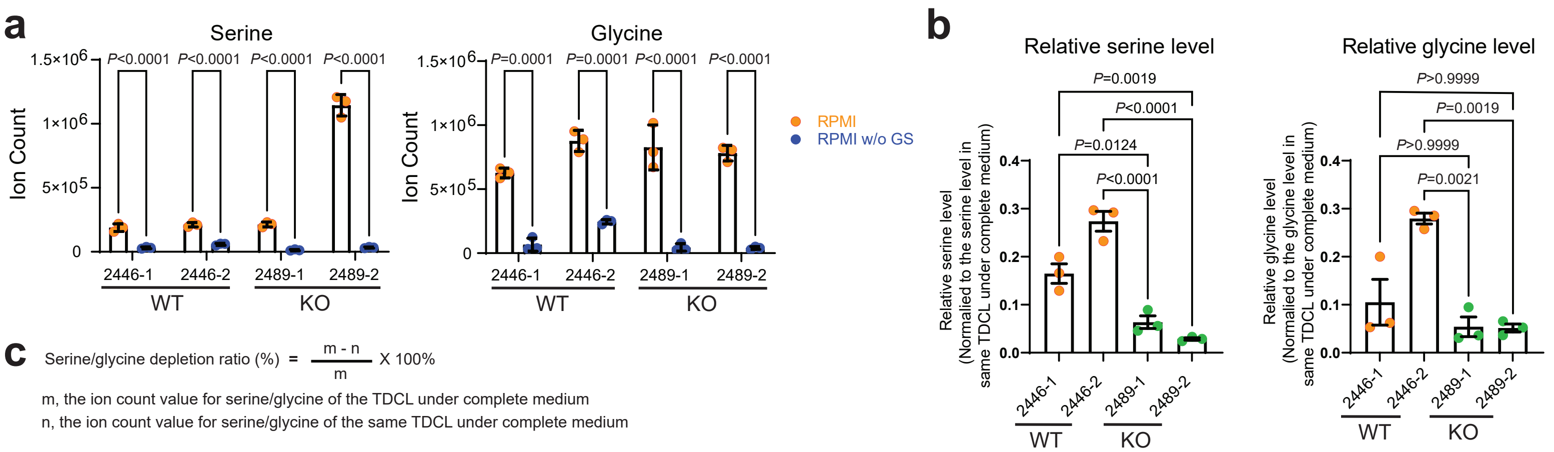
Supplementary Fig. 9: G6PD loss increases the pool size levels of TCA cycle intermediates in KP lung tumors.

a. Heatmap of water-soluble metabolites of *G6pd*^{WT}*;KP* (n = 6 mice) and *G6pd*^{KO}*;KP* (n = 6 mice) lung tumors in fasted state (food was removed from the mice at approximately 9:00AM, and mice were euthanized with samples collected at 3:00PM) at 12 weeks post-tumor induction.

b. Pool size of TCA cycle intermediates and derivatives of *G6pd*^{WT}*;KP* and *G6pd*^{KO}*;KP* tumors from (a). α-ketoglutarate (α-KG).

Data are presented as mean ± SEM, significance was calculated by two-tailed unpaired *t*-test (a, b). *$P < 0.05$; ***$P < 0.001$. Source data are provided as a Source Data file.

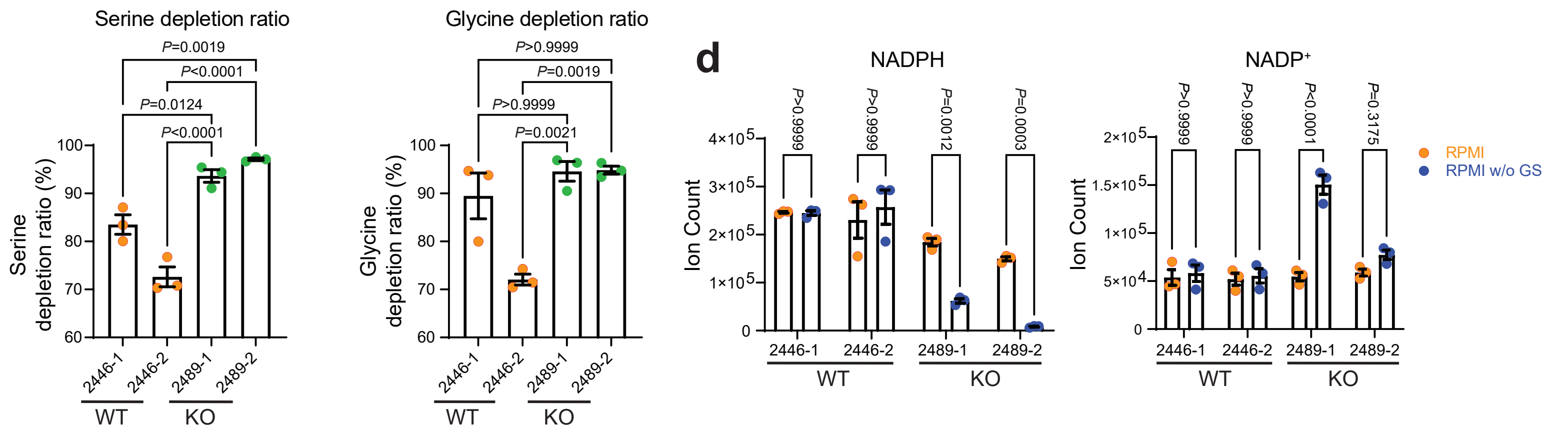
Supplementary Fig. 10



c Serine/glycine depletion ratio (%) = $\frac{m - n}{m}$ X 100%

m, the ion count value for serine/glycine of the TDCL under complete medium

n, the ion count value for serine/glycine of the same TDCL under complete medium



Supplementary Fig. 10: Serine/glycine depletion in KL TDCLs.

a. Pool size of intracellular serine and glycine of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs after 24 hours cultured in RPMI medium with or without serine and glycine. RPMI denotes cells cultured in complete RPMI medium, RPMI w/o GS denotes cells cultured in complete RPMI medium without serine and glycine. n = 3 replicates for each clone under different conditions.

b. Relative intracellular serine and glycine pool size levels of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs after 24 hours cultured in RPMI medium without serine and glycine. RPMI denotes cells cultured in complete RPMI medium, RPMI w/o GS denotes cells cultured in complete RPMI medium without serine and glycine. n = 3 replicates for each clone under different conditions.

c. Intracellular serine and glycine depletion ratio of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs after 24 hours cultured in RPMI medium without serine/glycine depleted medium. RPMI denotes cells cultured in complete RPMI medium, RPMI w/o GS denotes cells cultured in complete RPMI medium without serine and glycine. n = 3 replicates for each clone under different conditions.

d. Pool size levels of NADPH and NADP+ of *G6pd*^{WT}*;KL* and *G6pd*^{KO}*;KL* TDCLs after 24 hours cultured in RPMI medium with or without serine and glycine. RPMI denotes cells cultured in complete RPMI medium, RPMI w/o GS denotes cells cultured in complete RPMI medium without serine and glycine. n = 3 replicates for each clone under different conditions.

Data are presented as mean ± SEM, significance was calculated by two-way ANOVA followed by Bonferroni's multiple comparisons test (a, b, c, d, e). Source data are provided as a Source Data file.

Supplementary Table 1: List of the 28 studies involved in the overall survival analysis for lung cancer patients in the cBioPortal datasets

NO.	Study
1	Lung Adenocarcinoma (Broad, Cell 2012)
2	Lung Adenocarcinoma (CPTAC, Cell 2020)
3	Lung Adenocarcinoma (MSK Mind,Nature Cancer 2022)
4	Lung Adenocarcinoma (MSK, 2021)
5	Lung Adenocarcinoma (MSK, J Thorac Oncol 2020)
6	Lung Adenocarcinoma (MSK, NPJ Precision Oncology 2021)
7	Lung Adenocarcinoma (MSK, Science 2015)
8	Lung Adenocarcinoma (OncoSG, Nat Genet 2020)
9	Lung Adenocarcinoma (TCGA, Firehose Legacy)
10	Lung Adenocarcinoma (TCGA, Nature 2014)
11	Lung Adenocarcinoma (TCGA, PanCancer Atlas)
12	Lung Adenocarcinoma (TSP, Nature 2008)
13	Lung Adenocarcinoma Met Organotropism (MSK, Cancer cell 2023)
14	Lung Cancer (SMC, Cancer Research 2016)
15	Lung Cancer in Never Smokers (NCI, Nature Genetics 2021)
16	Lung Squamous Cell Carcinoma (CPTAC, Cell 2021)
17	Lung Squamous Cell Carcinoma (TCGA, Firehose Legacy)
18	Lung Squamous Cell Carcinoma (TCGA, Nature 2012)
19	Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas)
20	Metastatic Non-Small Cell Lung Cancer (MSK, Nature Medicine 2022)
21	Non-Small Cell Cancer (MSK, Cancer Discov 2017)
22	Non-Small Cell Lung Cancer (MSK, Cancer Cell 2018)
23	Non-Small Cell Lung Cancer (MSK, J Clin Oncol 2018)
24	Non-Small Cell Lung Cancer (MSK, Science 2015)
25	Non-Small Cell Lung Cancer (TRACERx, NEJM & Nature 2017)
26	Non-Small Cell Lung Cancer (University of Turin, Lung Cancer 2017)
27	Non-Small Cell Lung Cancer Brain Metastasis (MSK, Nat Commun 2023)
28	Pan-Lung Cancer (TCGA, Nat Genet 2016)

Supplementary Table 2: Sequences of mouse genotyping primers used in this study

*Kras*_F	CCATGGCTTGAGTAAGTCTGC
*Kras*_R	CGCAGACTGTAGAGCAGCG
*G6pd*_F	TCCTGAGTACTGGAATCACAACTG
*G6pd*_R	CACAGAACAGATGCTCATTGTGAG
*Lkb1*_F	ATCGGAATGTGATCCAGCTT
*Lkb1*_R	ACGTAGGCTGTGCAACCTCT
*P53*_F	GGTTAAACCCAGCTTGACCA
*P53*_R	GGAGGCAGAGACAGTTGGAG