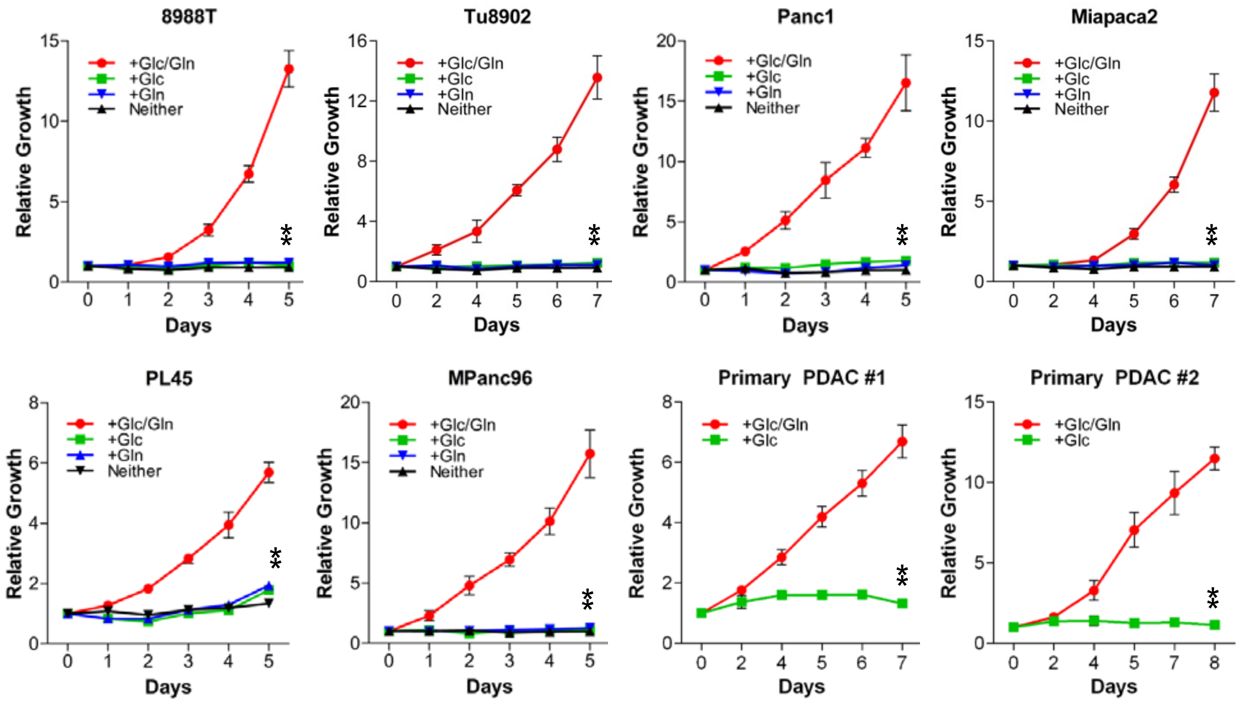
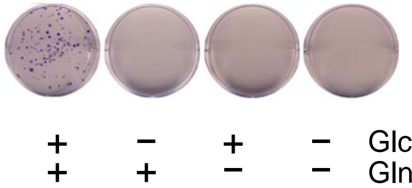
Supplementary Figure 1

a

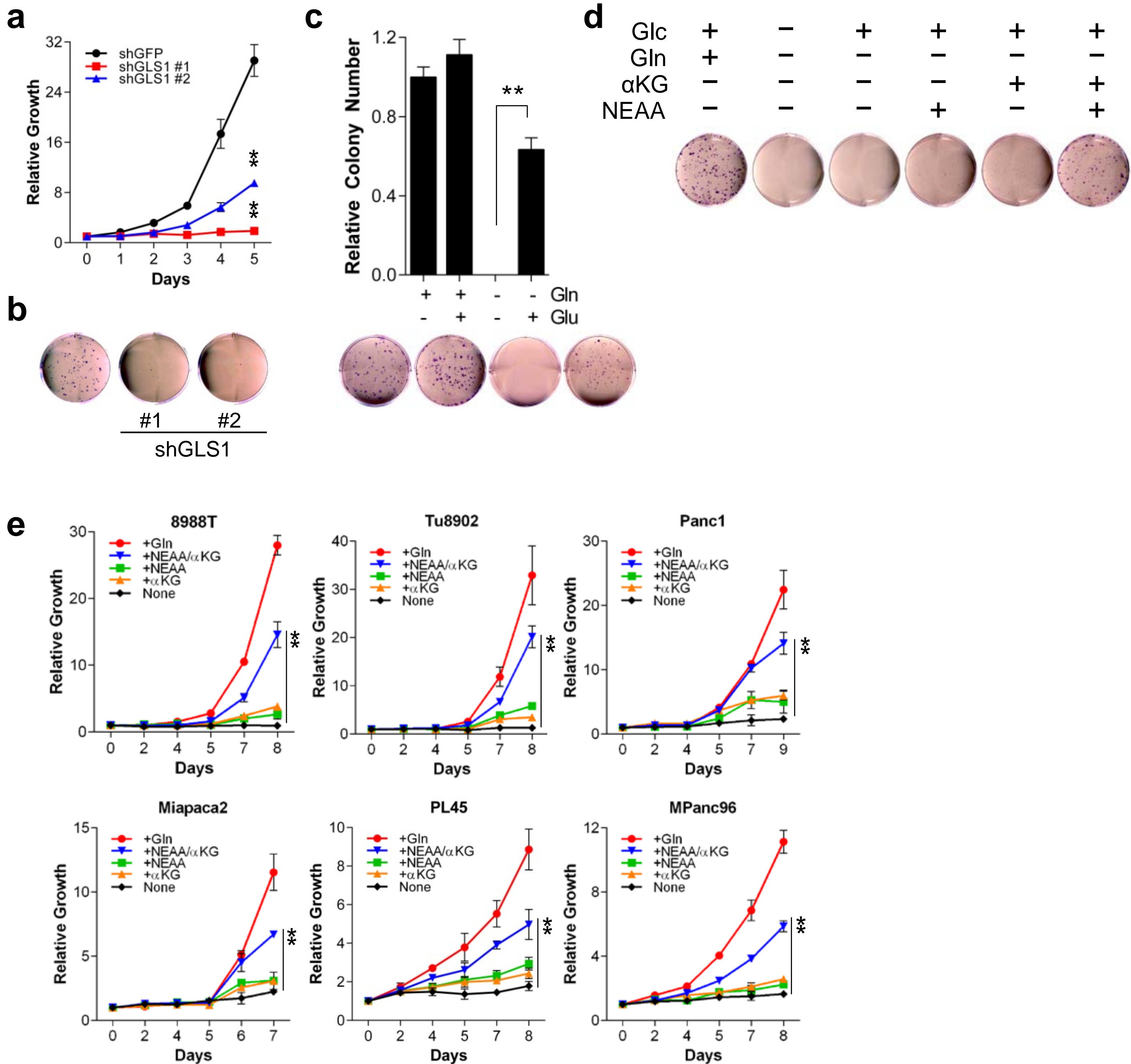


b



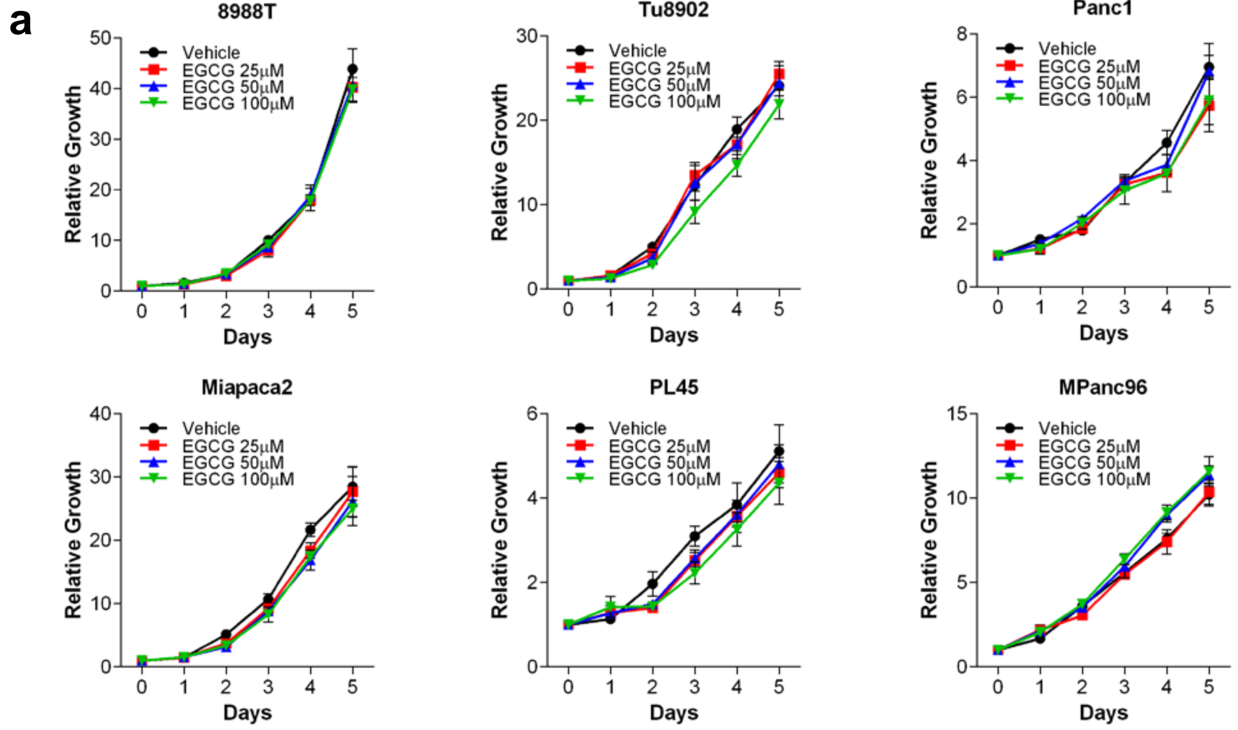
Supplementary Figure 1. a, Relative proliferation of PDAC cell lines (8988T, Tu8902, Panc1, Miapaca2, PL45 and MPanc96) and low passage primary human PDAC cell lines (#1 and #2) under conditions indicated. Cells were plated in complete culture media (10mM glucose and 2mM Gln) which was replaced the following day with glucose- or Gln-free medium supplemented with 10% dialyzed FBS and assayed for proliferation. At the indicated time points, cells were fixed in 10% formalin and stained with 0.1% crystal violet. Dye was extracted with 10% acetic acid and the relative proliferation was determined by OD at 595 nm. **b,** Representative wells of the clonogenic growth experiment depicted in Fig.1a. Error bars represent s.d. of triplicate wells from a representative experiment. ** $P < 0.01$.

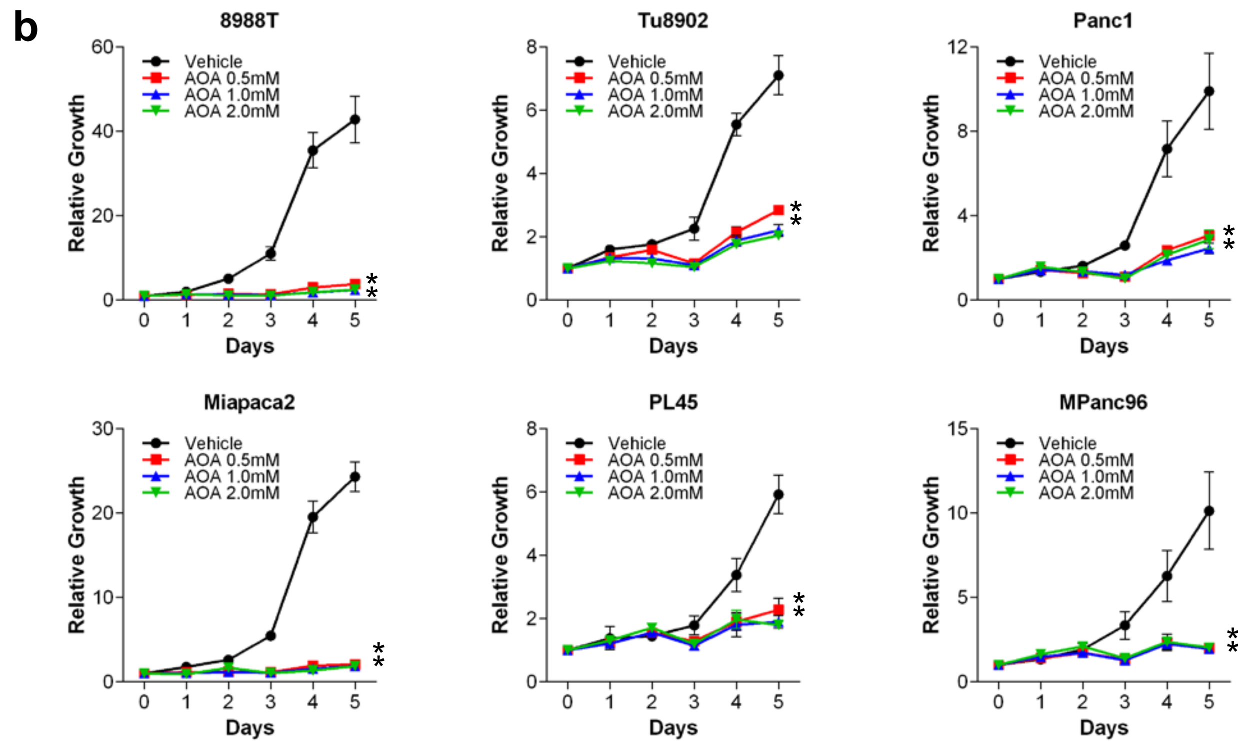
Supplementary Figure 2



Supplementary Figure 2. a, Relative proliferation of 8988T cells expressing a control shRNA (shGFP) or shRNAs to GLS (#1 and #2). **b,** Representative wells of the clonogenic growth experiment depicted in Fig.1b. **c,** Relative clonogenic growth of 8988T under conditions indicated. Glu (4mM) was added to media after Gln-withdrawal and clonogenic growth was assessed. **d,** Representative wells of the clonogenic growth experiment depicted in Fig.1d. **e,** Relative proliferation of PDAC cell lines (8988T, Tu8902, Panc1, Miapaca2, PL45 and MPanc96). NEAA mixture (0.1 mM glycine, alanine, aspartate, asparagine, proline and serine), dimethyl αKG (4mM), or the combination was added to media after Gln-withdrawal and cellular proliferation was assayed. Error bars represent s.d. of triplicate wells from a representative experiment. $^{**}P < 0.01$.

Supplementary Figure 3

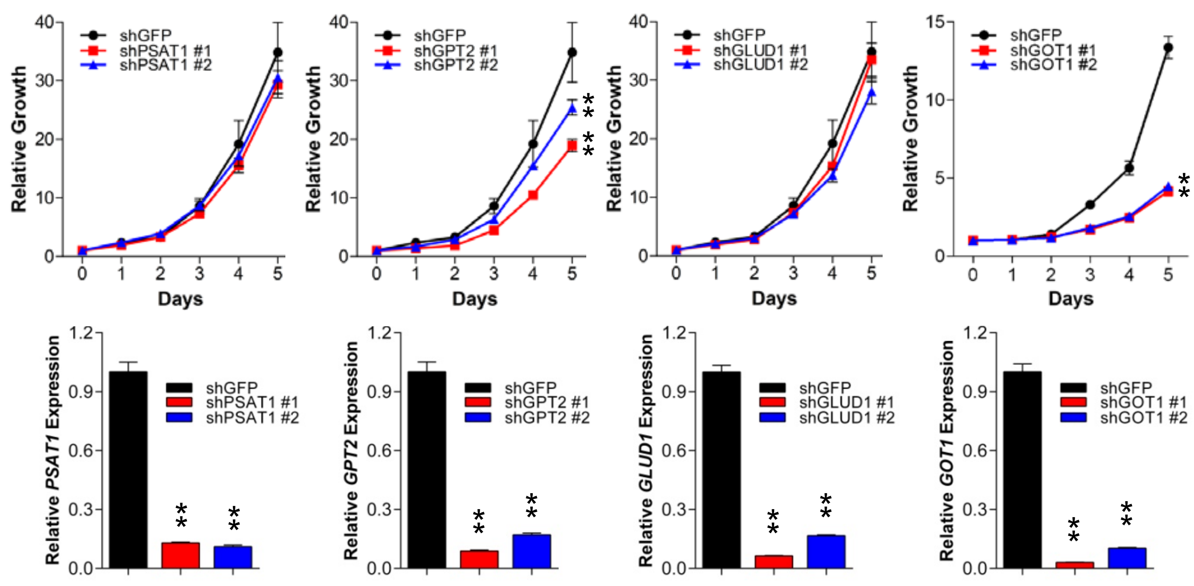




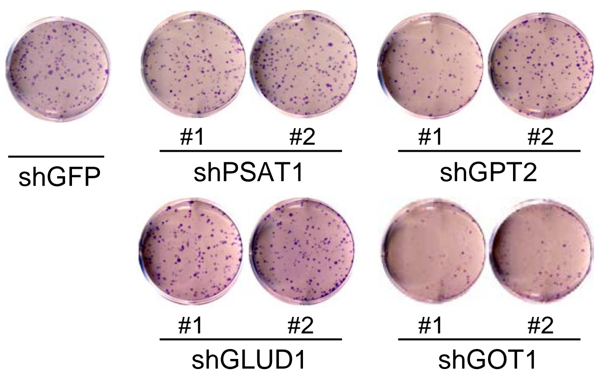
Supplementary Figure 3. a and b, PDAC cell lines (8988T, Tu8902, Panc1, Miapaca2, PL45 and MPanc96) were treated with either AOA (aminooxyacetate) or EGCG (epigallocatechin gallate) and assayed for cellular proliferation. Error bars represent s.d. of triplicate wells from a representative experiment. $^{**}P < 0.01$.

Supplementary Figure 4

a

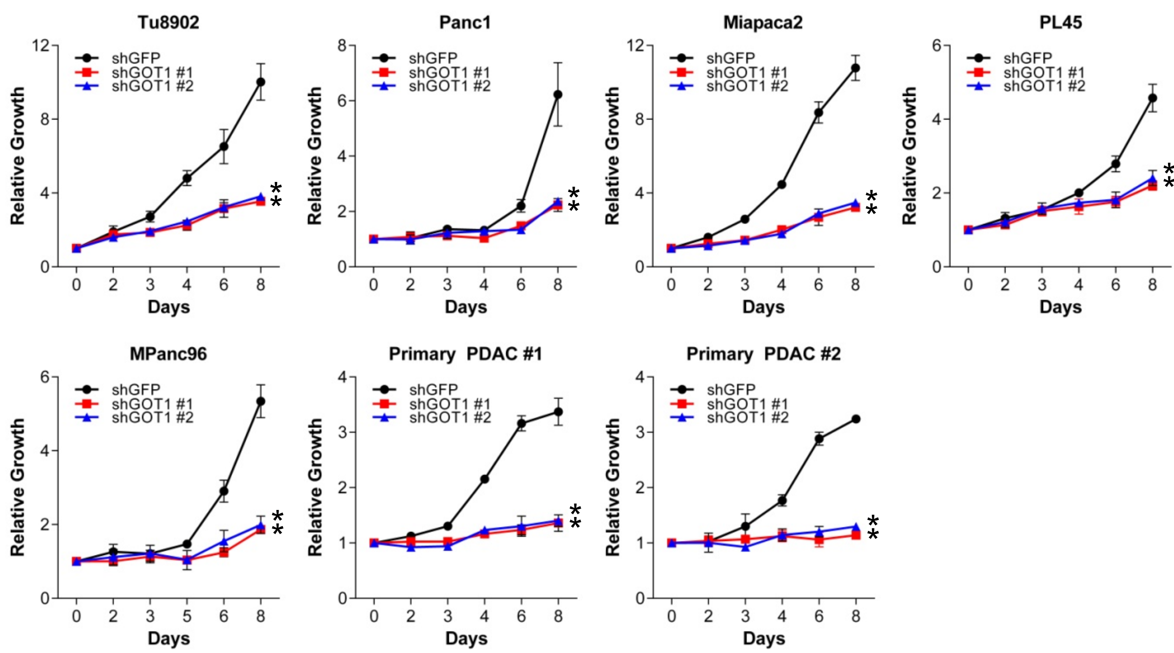


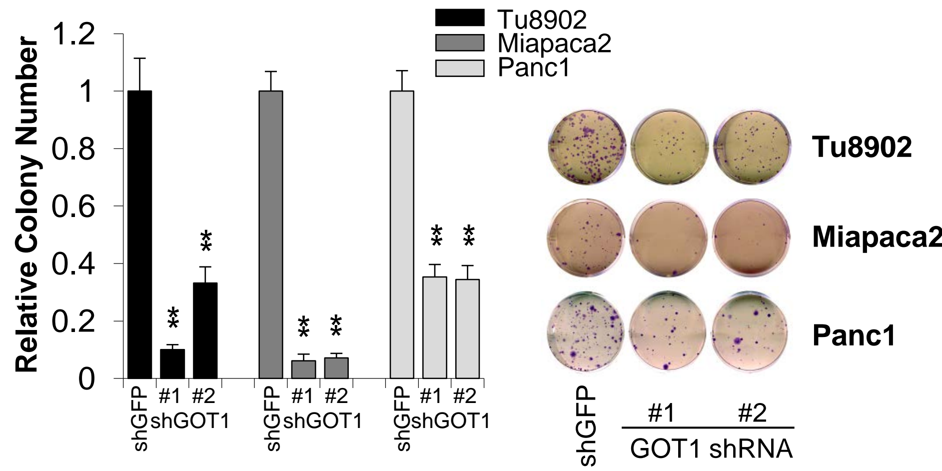
b



Supplementary Figure 4. a, Relative proliferation of 8988T cells expressing a control shRNA (shGFP), shRNAs to GLUD1 (#1 and #2), GOT1 (#1 and #2), GPT2 (#1 and #2) or PSAT1 (#1 and #2). Quantitative RT-PCR confirmed the knockdown efficiency of the shRNAs. **b,** Representative wells of the clonogenic growth experiment depicted in Fig.2a. Error bars represent s.d. of triplicate wells from a representative experiment. $^{**}P < 0.01$.

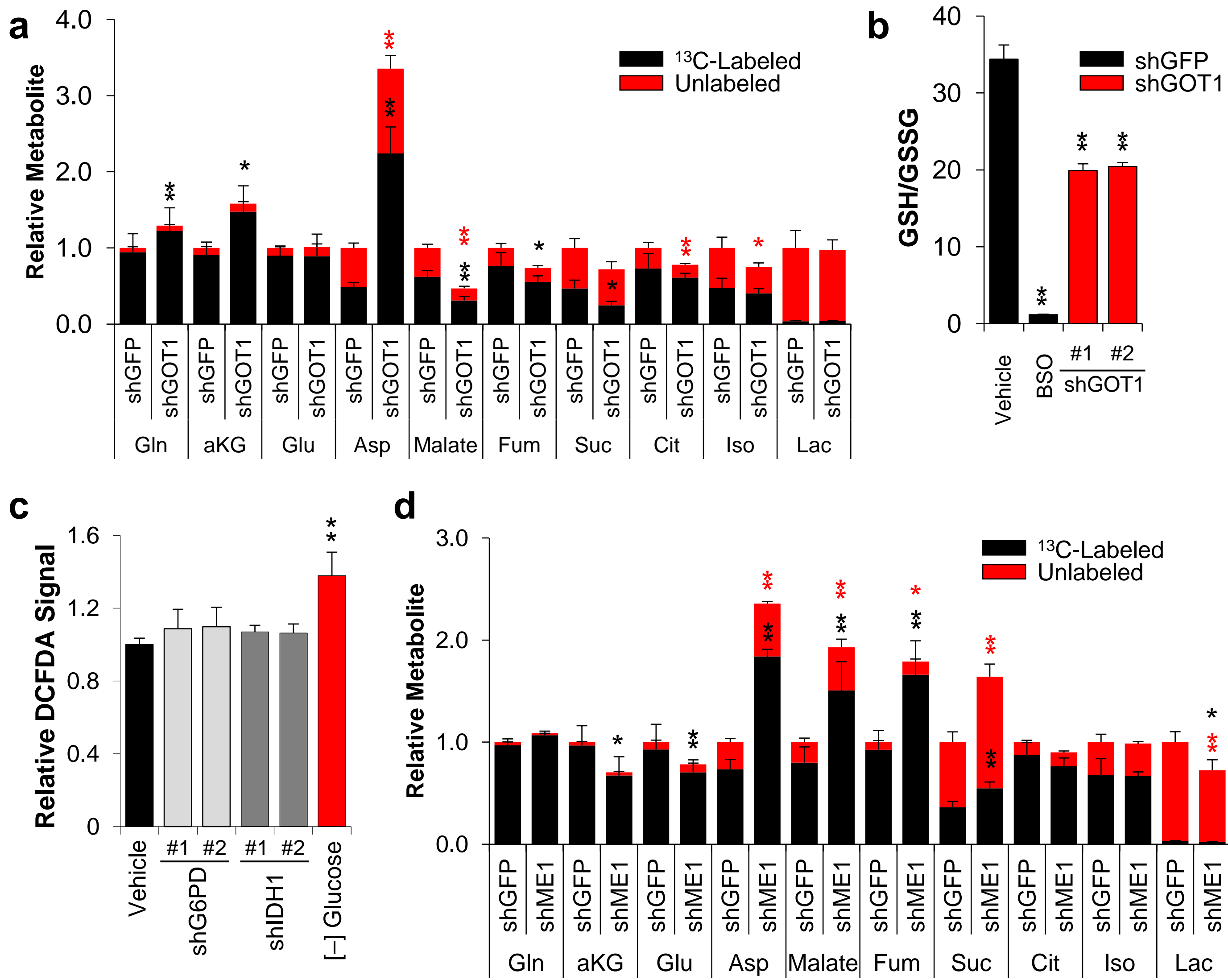
Supplementary Figure 5





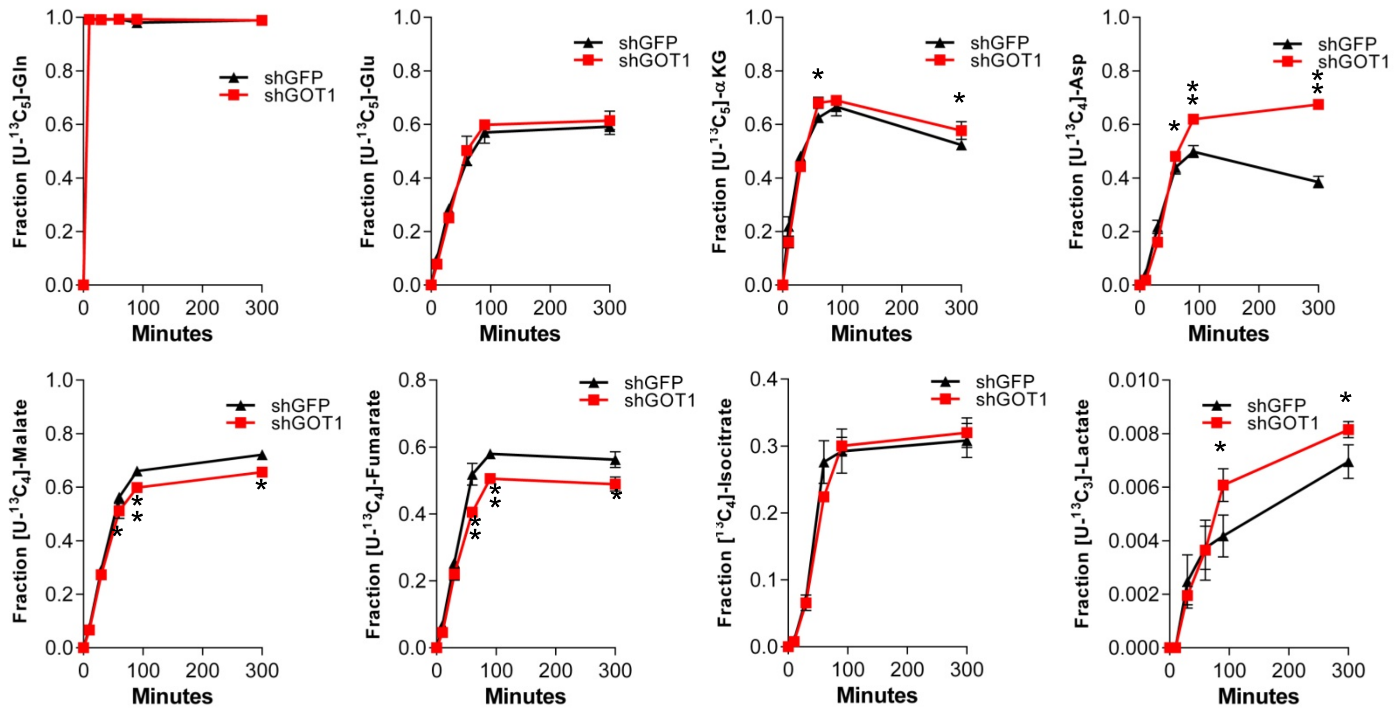
Supplementary Figure 5. a, Relative proliferation of PDAC cell lines (Tu8902, Panc1, Miapaca2, PL45 and MPanc96) and low passage primary human PDAC cell lines (#1 and #2) expressing a control shRNA (shGFP) or shRNAs to GOT1 (#1 and #2). **b,** Relative clonogenic growth of Tu8902, Miapaca2 and Panc1 cells expressing a control shRNA (shGFP) or shRNAs to GOT1 (#1 and #2). Error bars represent s.d. of triplicate wells from a representative experiment. $^{**}P < 0.01$.

Supplementary Figure 6



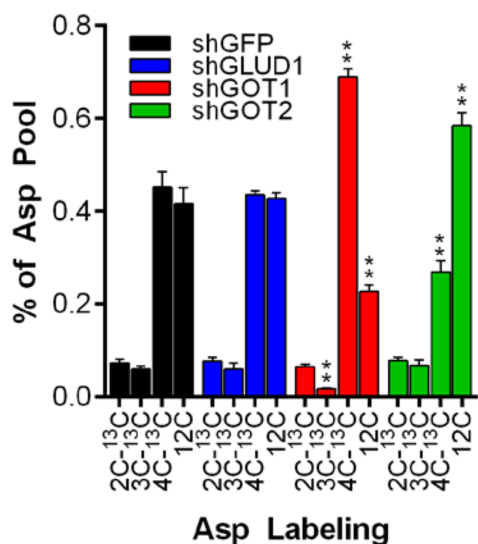
Supplementary Figure 6. a, Relative metabolite abundance in 8988T cells grown in [U-$^{13}C_5$]-Gln upon GOT1 knockdown, as compared to control (shGFP). Total metabolite pools are represented by the height of the bars; the fraction of the metabolite pool labeled and unlabeled are shown in black and red, respectively. Gln, aKG, Glu, Asp, Malate, Fum, Suc and Lac are [U-^{13}C]-labeled; Cit and Iso are [$^{13}C_4$]-labeled. The color of the asterisks correspond to the color of the metabolite bars. **b,** Knockdown of GOT1 in 8988T cells reduces the ratio of reduced to oxidized glutathione (GSH/GSSG). BSO (buthionine sulfoximine) was included as a positive control for GSH depletion. **c**, Relative ROS levels of 8988T cells expressing a control shRNA (shGFP), shRNAs to G6PD (#1 and #2) or IDH1 (#1 and #2). To deprive glucose, cells were plated in complete culture media (10mM glucose and 2mM Gln), which was replaced the following day with glucose-free medium supplemented with 10% dialyzed FBS. DCFDA assay was assessed 24hr after glucose-withdrawal. Each bar represents the mean of three independent experiments with error bars representing the s.d. **d**, Relative metabolite abundance in 8988T cells grown in [U-$^{13}C_5$]-Gln upon ME1 knockdown, as compared to control (shGFP). Total metabolite pools are represented by the height of the bars; the fraction of the metabolite pool labeled and unlabeled are shown in black and red, respectively. Gln, aKG, Glu, Asp, Malate, Fum, Suc and Lac are [U-^{13}C]-labeled; Cit and Iso are [$^{13}C_4$]-labeled. The color of the asterisks correspond to the color of the metabolite bars. Fum, fumarate; Suc, succinate; Cit, citrate; Iso, isocitrate; Lac, lactate. Error bars represent the s.d. of three independently prepared samples. *$P < 0.05$; **$P < 0.01$.

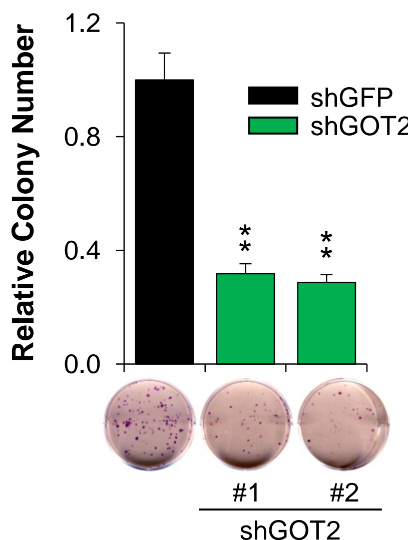
Supplementary Figure 7



Supplementary Figure 7. Flux of the Gln carbon skeleton into downstream metabolites as a function of time. Reads for the percentage of the metabolite pool that is ^{13}C-labeled is plotted for cells expressing the shGFP control or shGOT1. Error bars represent the s.d. of three independently prepared samples. *$P < 0.05$; **$P < 0.01$.

Supplementary Figure 8

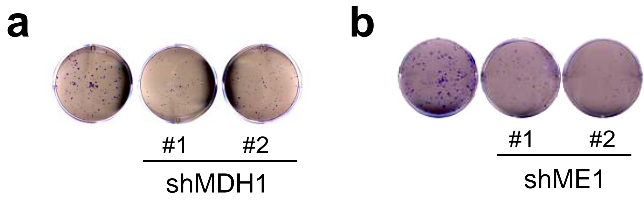




c

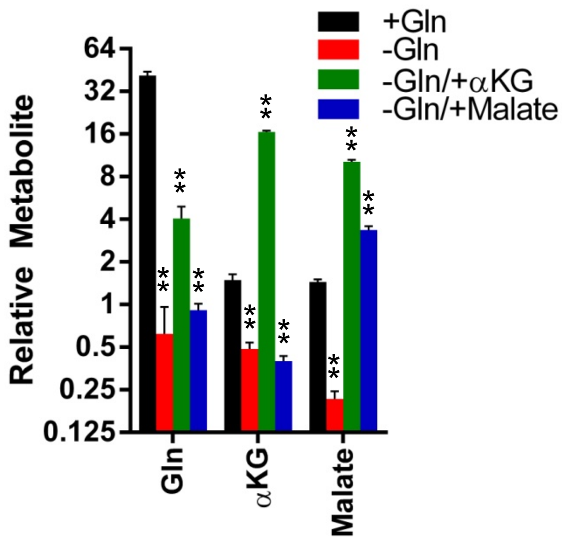
Supplementary Figure 8. a, Asp isotopomer analysis following GLUD1, GOT1 or GOT2 knockdown, as compared to control shRNA (shGFP). Asp data are presented as the fraction of the total metabolite pool that are unlabeled (12C), 13C-labeled on 2 carbons (2C-^{13}C), 3 carbons (3C-^{13}C) or uniformly labeled (4C-^{13}C). **b**, Relative clonogenic growth of 8988T expressing a control shRNA (shGFP) or shRNAs to GOT2 (#1 and #2). Error bars represent s.d. of triplicate wells from a representative experiment. **c**, Flux of the Gln carbon skeleton into downstream metabolites as a function of time. Reads for ion current for ^{13}C-labeled metabolites are plotted for cells expressing a control shRNA (shGFP) or shRNA to GOT2. Error bars represent the s.d. of three independently prepared samples. *$P < 0.05$; **$P < 0.01$.

Supplementary Figure 9

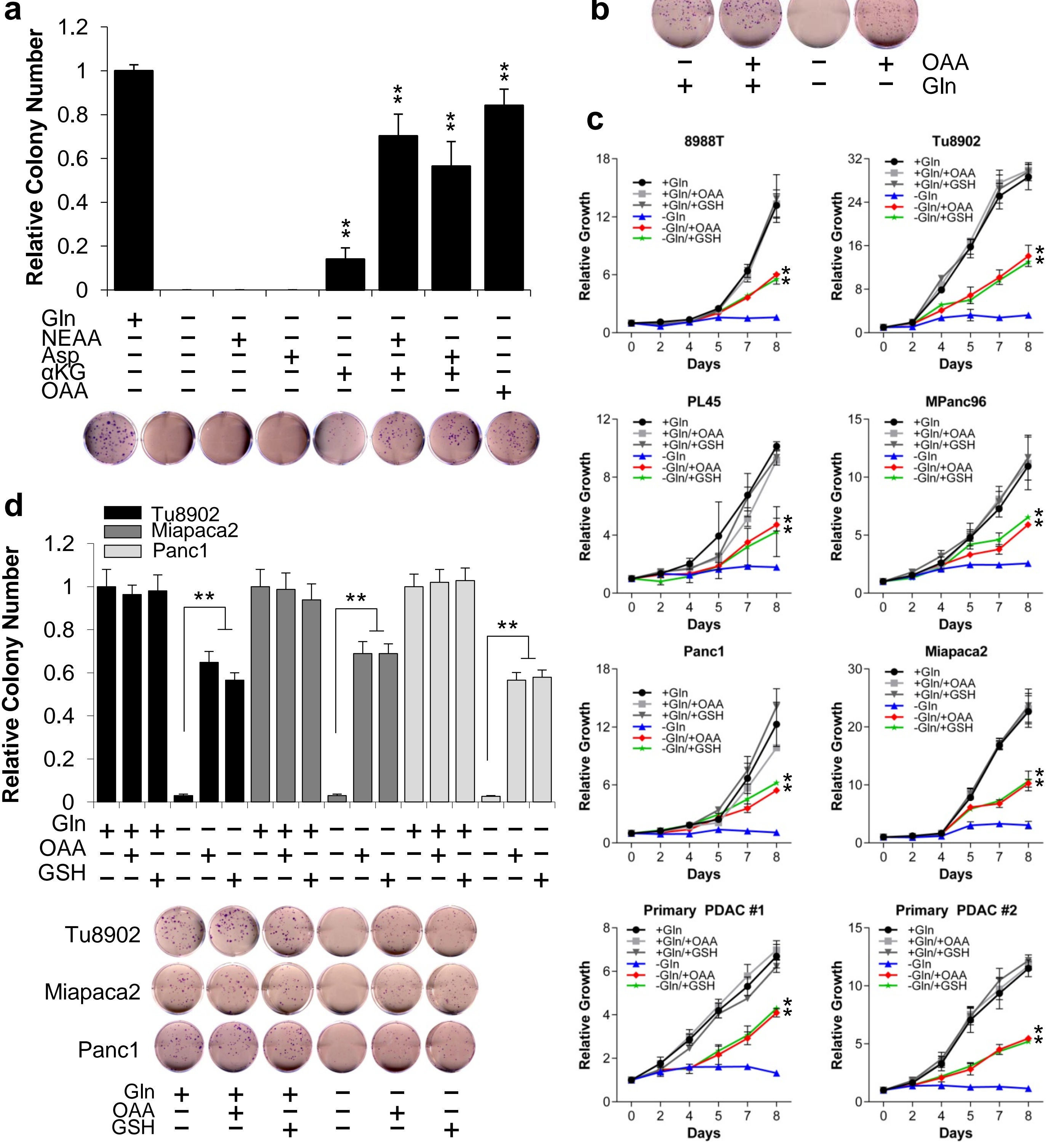


Supplementary Figure 9. a Representative wells of the clonogenic growth experiment depicted in Fig.3a. **b,** Representative wells of the clonogenic growth experiment depicted in Fig.3b.

Supplementary Figure 10

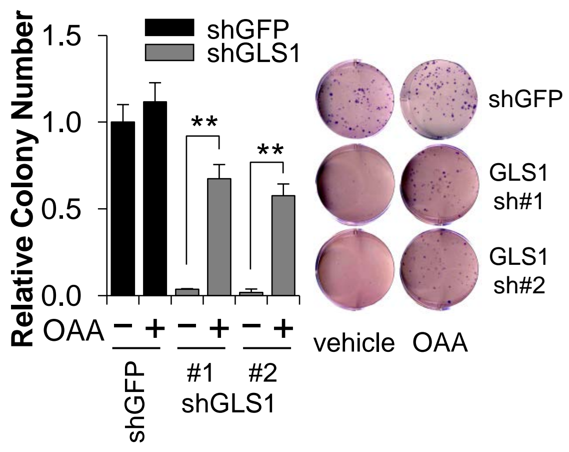


Supplementary Figure 10. Relative metabolite abundances in Gln deprived cells supplemented with dimethyl-αKG or dimethyl-malate, as compared to cells grown in complete media. Error bars represent the s.d. of three independently prepared samples. ** $P < 0.01$.



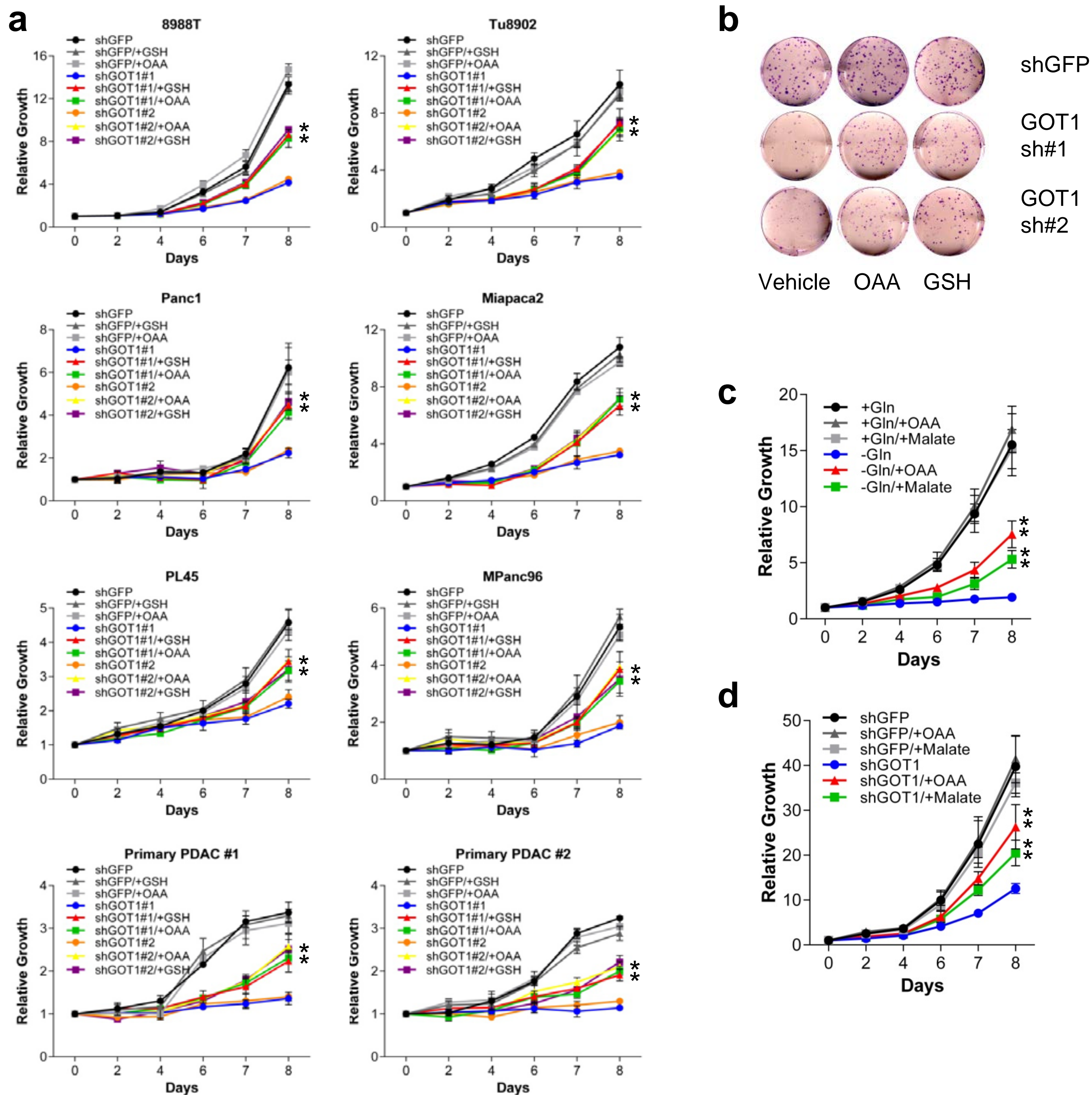
Supplementary Figure 11. a, 8988T cells were plated in complete culture media (10mM glucose and 2mM Gln) which was replaced the following day with Gln-free medium supplemented with NEAA mixture (0.1 mM), Asp (2mM), αKG (4mM) or OAA (4mM) and clonogenic growth was assessed. **b,** Representative wells of the clonogenic growth experiment depicted in Fig.3c. This data and that shown in Supplementary Fig. 14b were derived from the same representative experiment showing rescue with the indicated metabolites. **c,** PDAC cell lines and low passage primary human PDAC lines (#1 and #2) were plated in complete culture media (10mM glucose, 2mM Gln) which was replaced the following day with Gln-free medium supplemented with OAA (4mM) or GSH (4mM) and assayed for proliferation. **d,** Relative clonogenic growth of Tu8902, Miapaca2 and Panc1 cells. OAA (4mM) or GSH (4mM) were added to media after Gln-withdrawal. Error bars represent s.d. of triplicate wells from a representative experiment. ** $P < 0.01$.

Supplementary Figure 12



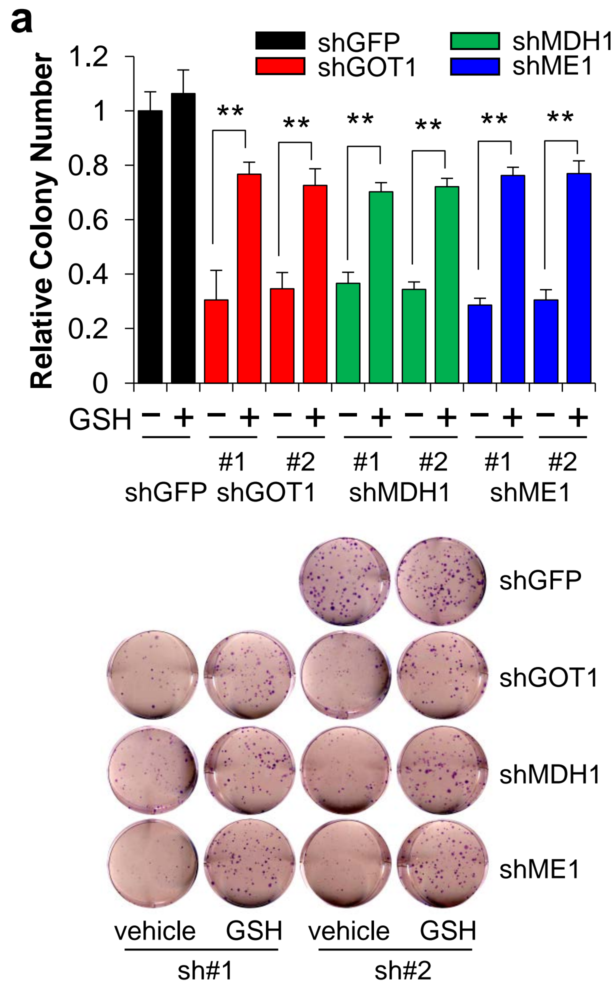
Supplementary Figure 12. 8988T cells expressing a control shRNA (shGFP) or shRNAs targeting GLS1 (#1 and #2) were plated in complete culture media (10mM glucose and 2mM Gln) supplemented with or without OAA (4mM) and clonogenic growth was assessed. Error bars represent s.d. of triplicate wells from a representative experiment. **$P < 0.01$.

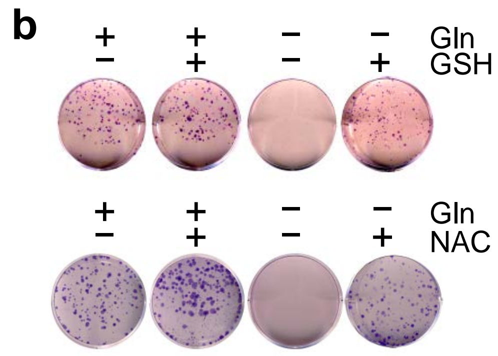
Supplementary Figure 13

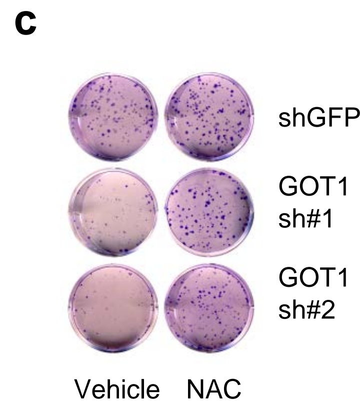


Supplementary Figure 13. a, PDAC cell lines (8988T, Tu8902, Panc1, Miapaca2, PL45 and MPanc96) and low passage primary human PDAC cell lines (#1 and #2) expressing a control shRNA (shGFP) or shRNAs targeting GOT1 (#1 and #2) were plated in the complete culture media (10mM glucose and 2mM Gln) supplemented with or without OAA (4mM) or GSH (4mM) and assayed for proliferation. **b,** Representative wells of the clonogenic growth experiment depicted in Fig.3d. **c,** 8988T cells were plated in complete culture media, which was replaced the following day with Gln-free medium containing dimethyl-malate (4mM) or OAA (4mM) and assayed for proliferation. **d,** 8988T cells expressing a control shRNA (shGFP) or shRNAs targeting GOT1 were plated in the complete culture media with or without dimethyl-malate (4mM) or OAA (4mM) and assayed for proliferation. Error bars represent s.d. of triplicate wells from a representative experiment. ***$P < 0.01$.

Supplementary Figure 14

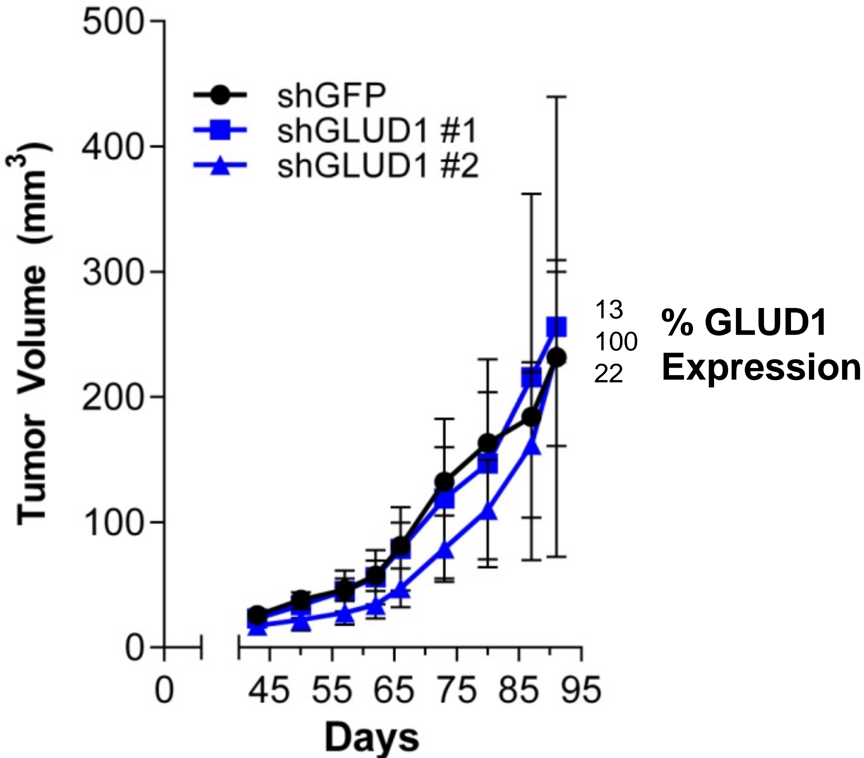






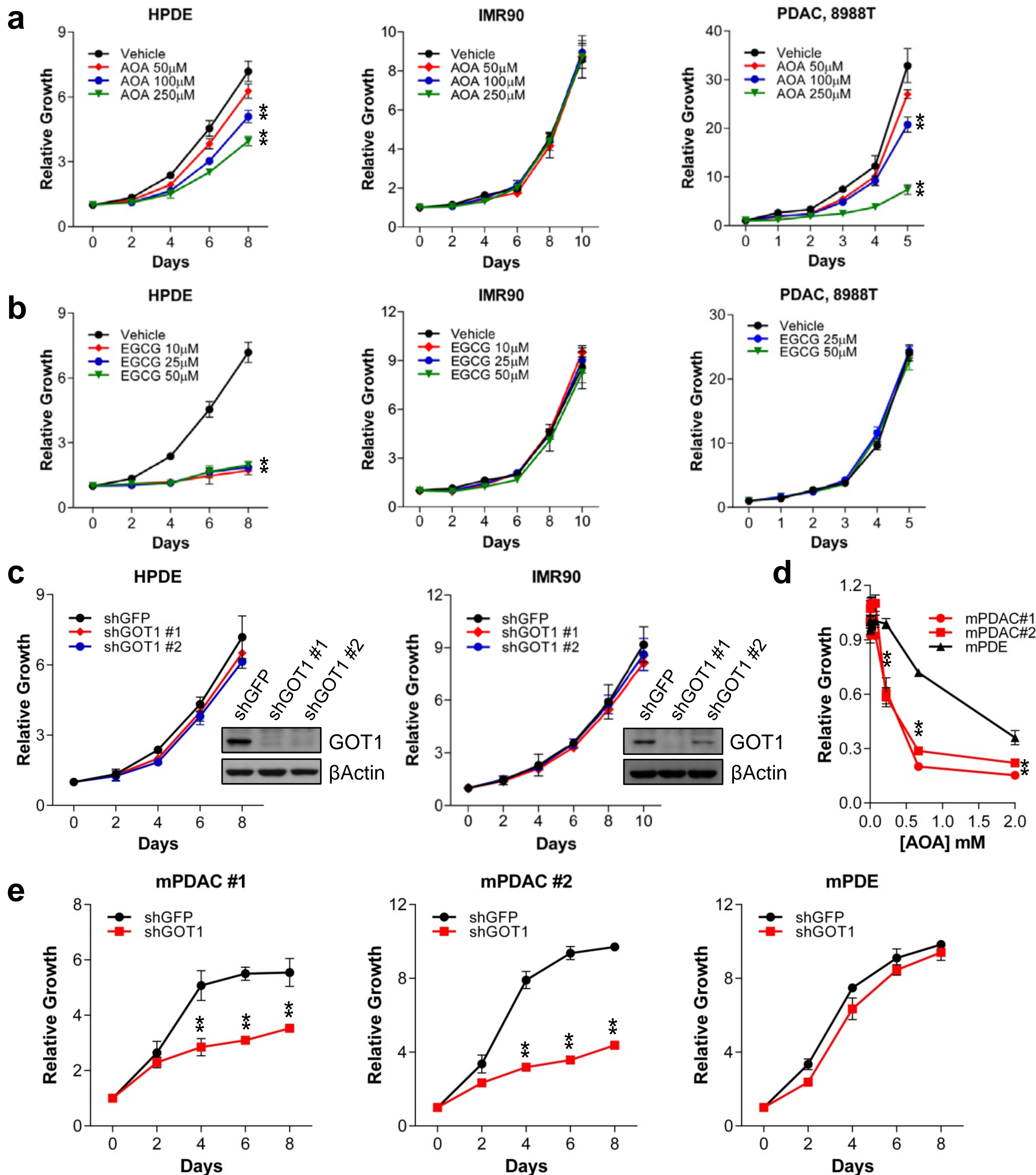
Supplementary Figure 14. a, 8988T cells expressing a control shRNA (shGFP), shRNAs to GOT1 (#1 and #2), MDH1 (#1 and #2) or ME1 (#1 and #2) were plated in the complete culture media with or without GSH (4mM) and clonogenic growth was assessed. The shGOT1 data (also depicted in Supplementary Fig. 13b) is included here for comparison as it was derived from the same representative experiment as shMDH1 and shME1. **b and c,** Representative wells of the clonogenic growth experiment depicted in Fig. 3e and Fig.3f, respectively. The data from **b**, along with that shown in supplemental figure 11b were derived from the same representative experiment showing rescue with the indicated metabolites. Error bars represent s.d. of triplicate wells from a representative experiment. **, $P < 0.01$.

Supplementary Figure 15



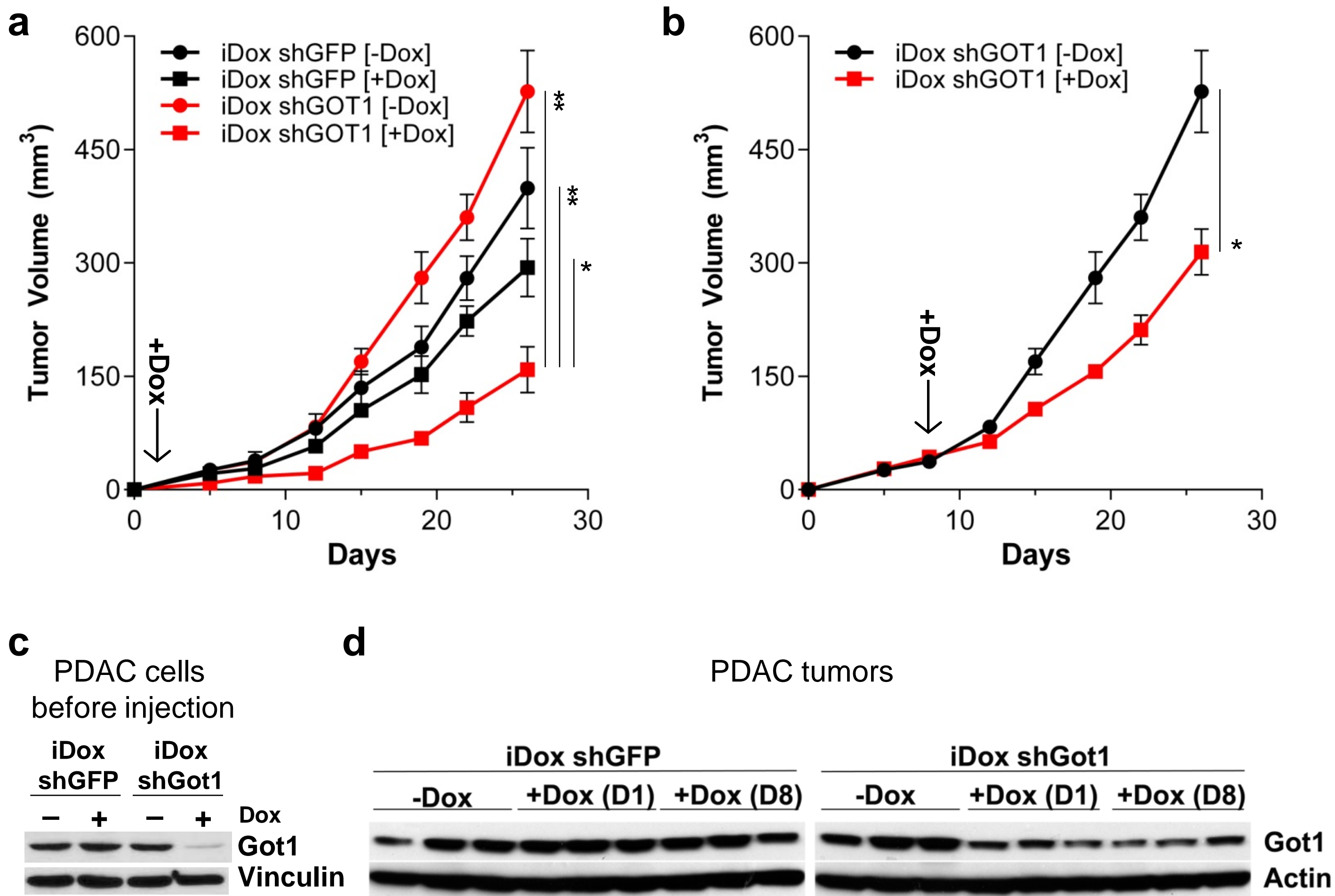
Supplementary Figure 15. Xenograft growth of 8988T cells expressing a control shRNA (shGFP) or shRNAs targeting GLUD1 (#1 and #2) in mice (n=10). The numbers to right of graph represent the knockdown efficiency of shRNAs measured before injecting the cells into mice. Error bars represent s.e.m. (n=10).

Supplementary Figure 16



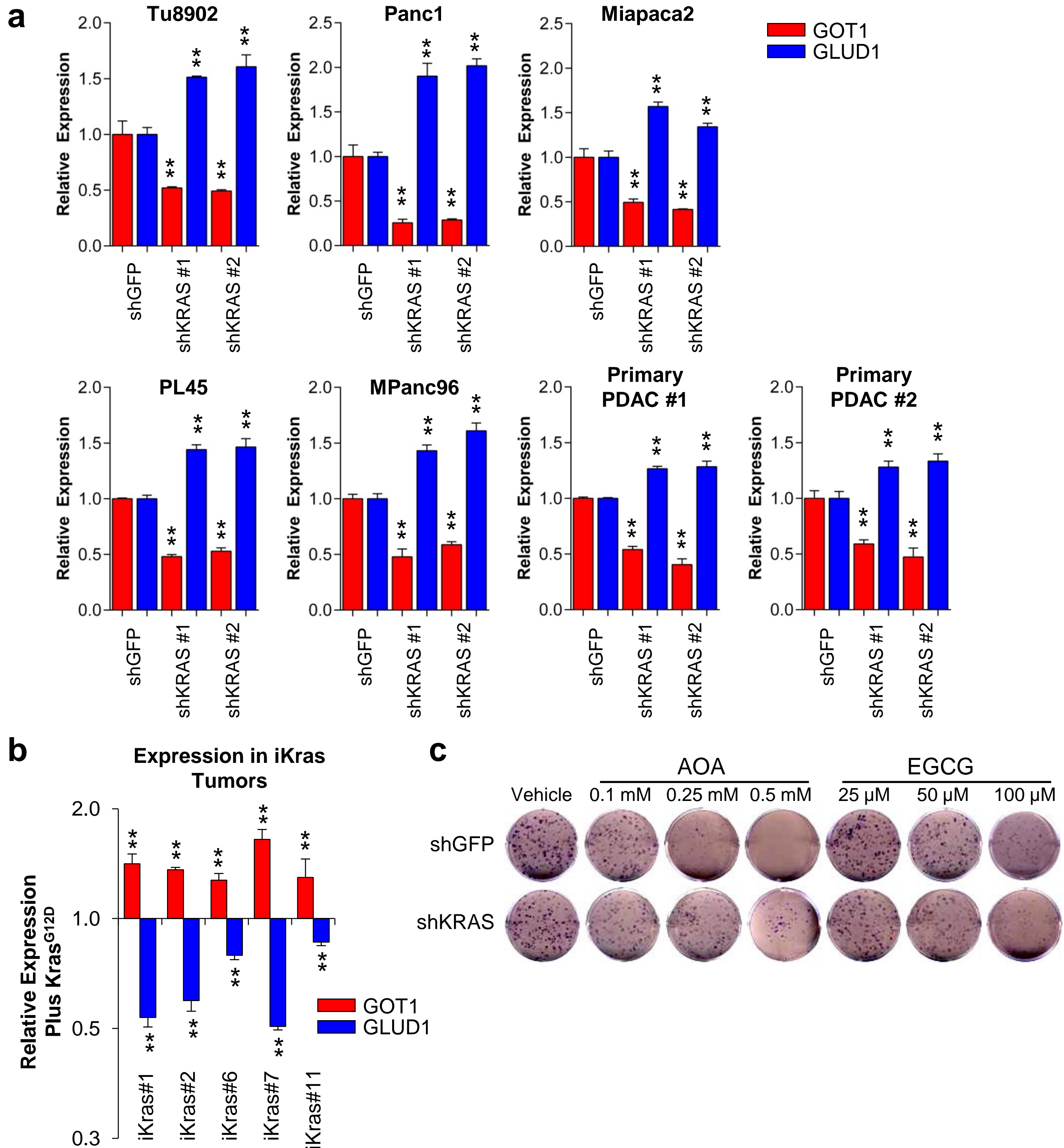
Supplementary Figure 16. a and b, HPDE (non-transformed human pancreatic ductal epithelial cells), IMR90 (human diploid fibroblasts) and 8988T were treated with AOA or EGCG and assayed for proliferation. **c,** Relative proliferation of HPDE and IMR90 expressing a control shRNA (shGFP) or shRNAs targeting GOT1 (#1 and #2). Western blot demonstrating GOT1 knockdown; full blots in Supplementary Fig. 21. **d,** Mouse normal ductal epithelial cells (mPDE) and two mouse PDAC (#1 and #2) lines were treated with AOA and assayed for proliferation. **e,** Relative proliferation of mPDE and two mouse PDAC lines expressing a control shRNA (shGFP) or shRNA targeting GOT1. Error bars represent s.d. of triplicate wells from a representative experiment. ** $P < 0.01$.

Supplementary Figure 17



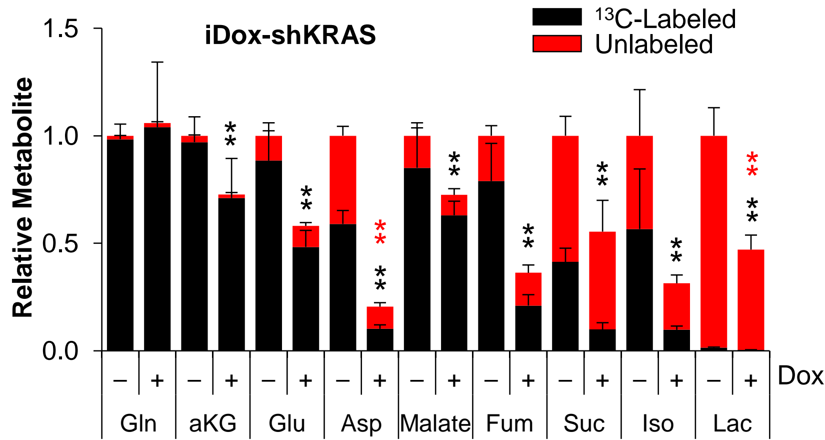
Supplementary Figure 17. a and b, LSL-Kras^{G12D}; p53$^{L/+}$ cell line expressing a doxycycline-inducible mouse shGOT1 in the presence or absence of doxycycline in mice (n=5). Doxycycline treatment was not initiated until (**a**) after the cells were implanted subcutaneously or (**b**) when the average tumor size of the cohort reached ~50 mm^3. **c,** Western blot demonstrating the knockdown efficiency of a doxycycline-inducible mouse shGOT1 in the presence of doxycycline prior to injection of the cells into mice. **d**, Xenograft tumors generated from mouse PDAC infected with a doxycycline-inducible shRNA against GOT1 were collected and tissue lysates were blotted for GOT1; full blots can be found in Supplementary Fig. 21. D1 represents tumors from panel (**a**) and D8 represents tumors from panel (**b**) at the termination of the experiment. Error bars represent s.e.m. (n=5). $^{*}P < 0.05$, $^{**}P < 0.01$.

Supplementary Figure 18



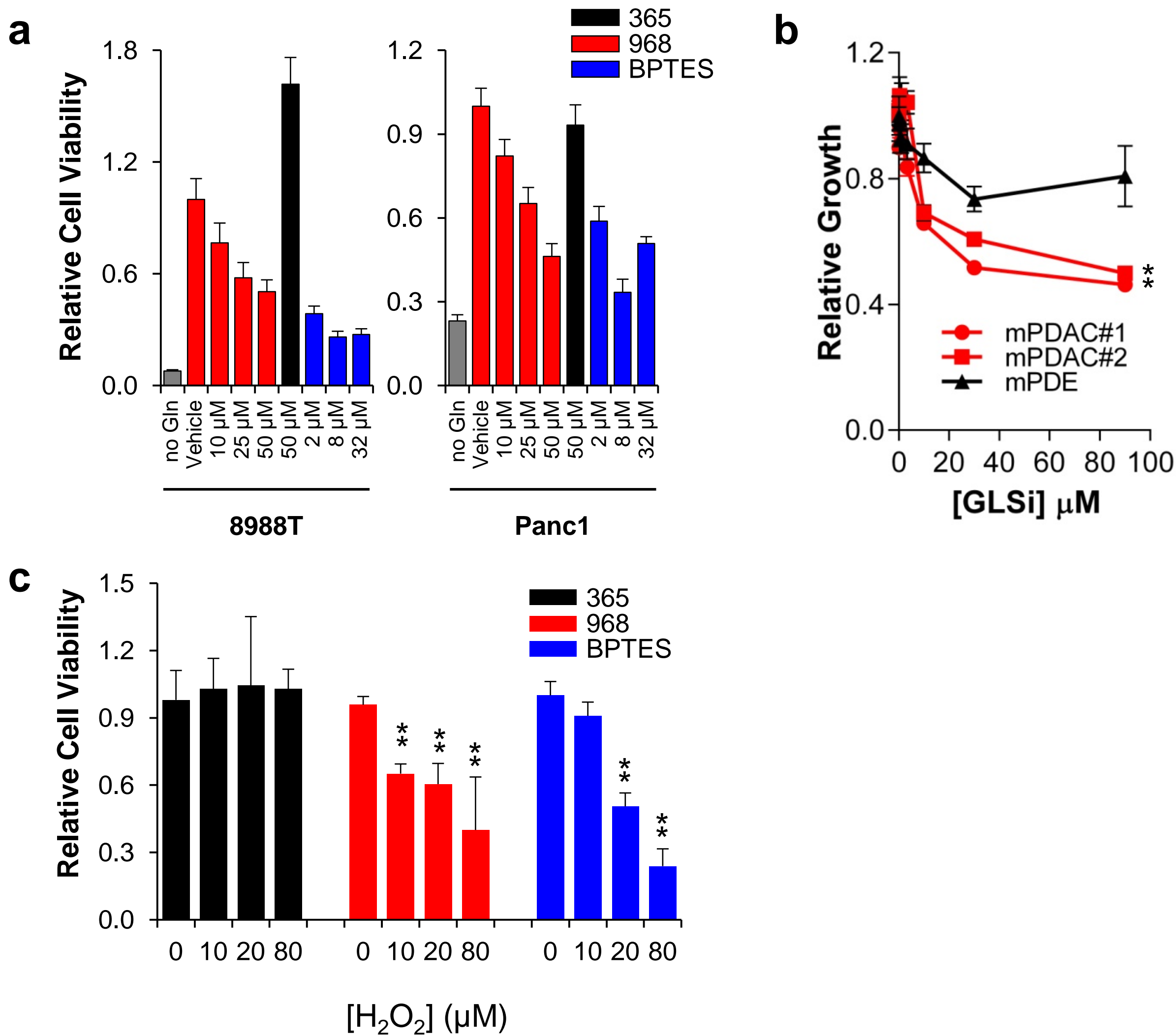
Supplementary Figure 18. a, Expression of GLUD1 and GOT1 as determined by quantitative RT-PCR in PDAC cell lines (8988T, Tu8902, Panc1, Miapaca2, PL45 and MPanc96) and low passage primary human PDAC cell lines (#1 and #2) expressing a control shRNA (shGFP) or two independent shRNAs to KRAS (#1 and #2). **b,** Expression of GLUD1 and GOT1 was determined by quantitative RT-PCR in five independent orthotopic tumors derived from inducible Kras mice (Ying et al., *Cell*, **2012**. *149*, 656–670). **c,** Representative wells of the clonogenic growth experiment depicted in Fig.4c. Error bars represent s.d. of triplicate samples from a representative experiments. ***P* < 0.01.

Supplementary Figure 19



Supplementary Figure 19. Relative metabolite abundance in 8988T cells expressing a doxycycline-inducible shKRAS grown in [U-$^{13}C_5$]-Gln following doxycycline administration (48h), as compared to non-treated cells. Total metabolite pools are represented by the height of the bars; the fraction of the metabolite pool labeled and unlabeled are shown in black and red, respectively. Gln, aKG, Glu, Asp, Malate, Fum, Suc and Lac are [U-^{13}C]-labeled; Iso is [$^{13}C_4$]-labeled. The color of the asterisks correspond to the color of the metabolite bars. Fum, fumarate; Suc, succinate; Iso, isocitrate; Lac, lactate. Error bars represent the s.d. of three independently prepared samples. **$P < 0.01$.

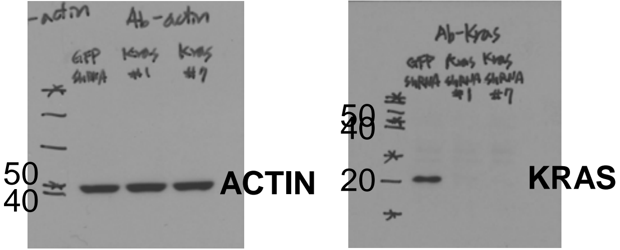
Supplementary Figure 20



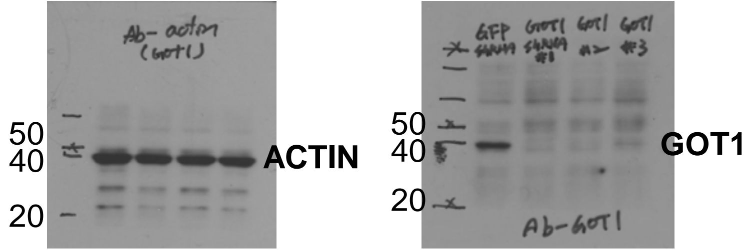
Supplementary Figure 20. a, Relative cell viability of 8988T or Panc1 cells treated with the indicated concentrations of GLS inhibitors 968 (active), 365 (inactive analog), or BPTES (Wang J-B, Cerione R, et al., *Cancer Cell* **2010**. *18*, 207–219; DeLaBarre B, Hurov J, et al. *Biochemistry* **2011**. *50*, 10764–10770). **b**, Relative cell viability of mPDE or two mouse PDAC (#1 and #2) cells treated with the indicated concentrations of the GLS inhibitor 968. **c,** Relative cell viability of Panc1 cells treated with GLS inhibitors 968 (active; 10 μM), 365 (inactive analog; 50 μM), or BPTES (100nM) with increasing concentrations of H_2O_2. Error bars represent s.d. of triplicate wells from a representative experiment. ***P* < 0.01.

Supplementary Figure 21

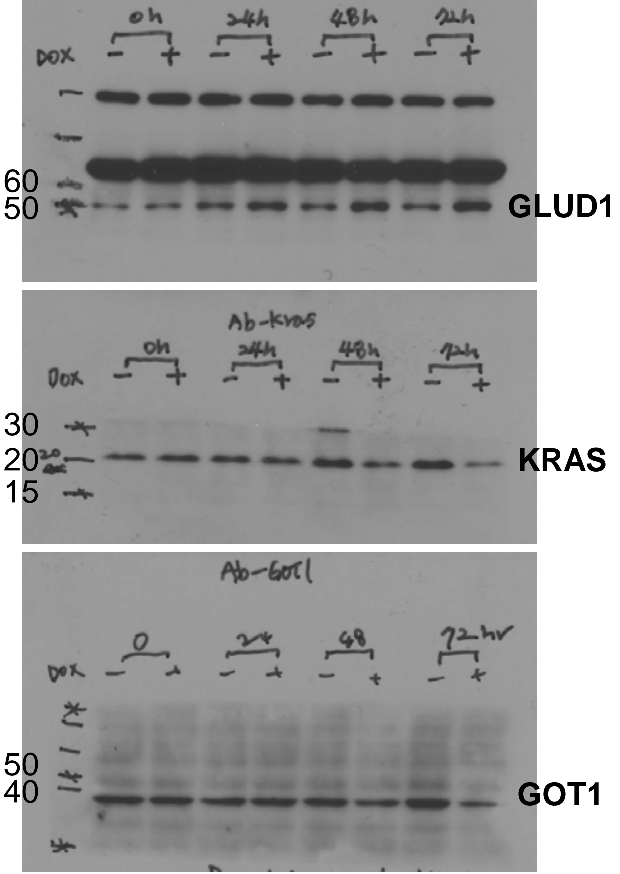
Full blots for *Figure 4a*



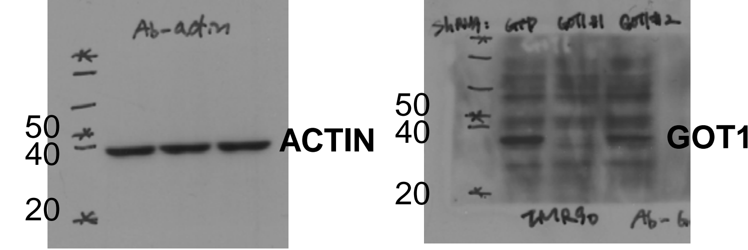
Full blots for *Supplementary Figure 16c, HPDE*



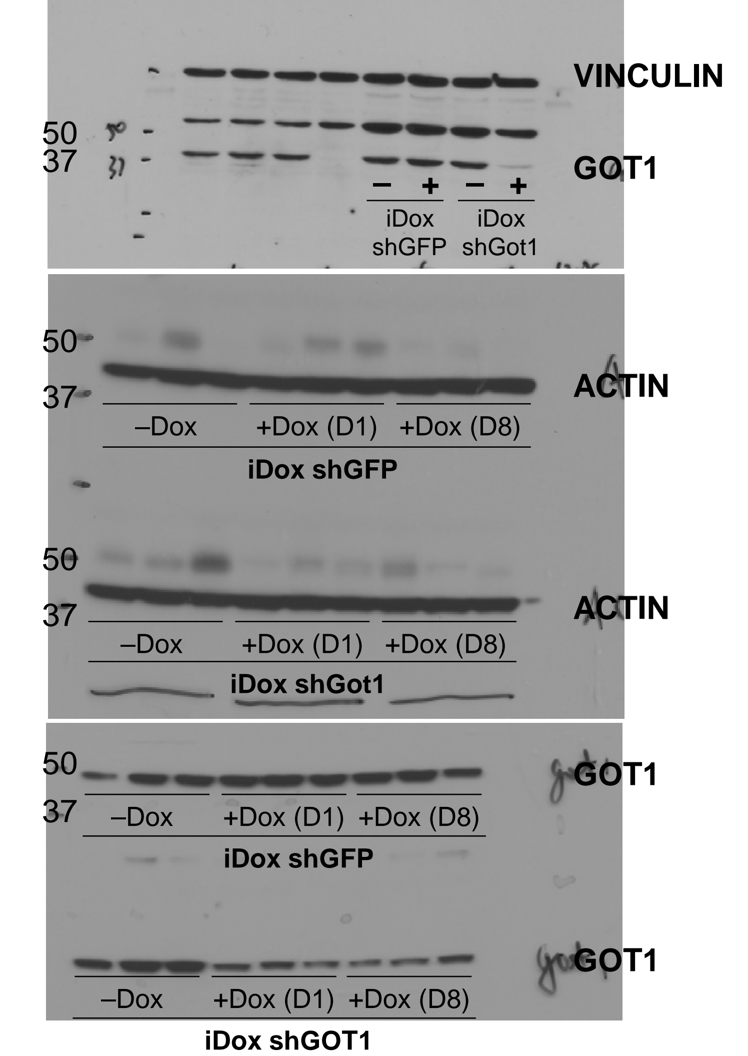
Full blots for *Figure 4b*

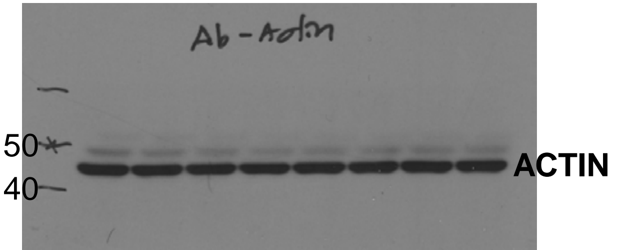


Full blots for *Supplementary Figure 16c, IMR90*



Full blots for *Supplementary Figure 17c*





Supplementary Figure 21. Full images of the western blots presented in the manuscript and supplementary information.

Primer Sequences for qRT-PCR

human	KRAS	forward	ACAGAGAGTGGAGGATGCTTT
human	KRAS	reverse	TTTCACACAGCCAGGAGTCTT
human	GOT1	forward	CAACTGGGATTGACCCAACT
human	GOT1	reverse	GGAACAGAAACCGGTGCTT
human	GLUD1	forward	GGGATTCTAACTACCACTTGCTCA
human	GLUD1	reverse	AACTCTGCCGTGGGTACAAT
human	GPT2	forward	CATGGACATTGTCGTGAACC
human	GPT2	reverse	TTACCCAGGACCGACTCCTT
human	PSAT1	forward	CGGTCCTGGAATACAAGGTG
human	PSAT1	reverse	AACCAAGCCCATGACGTAGA
mouse	Glud1	forward	CCTGCAACCATGTGTTGAGC
mouse	Glud1	reverse	CGGTAGCCTTCGATGACCTC
mouse	Got1	forward	GCGCCTCCATCAGTCTTTG
mouse	Got1	reverse	ATTCATCTGTGCGGTACGCTC