Metabolic Requirement for GOT2 in Pancreatic Cancer Depends on Environmental Context


1Doctoral Program in Cancer Biology, University of Michigan, Ann Arbor, MI 48109
2Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI 48109
3Department of Surgery, University of Michigan, Ann Arbor, MI 48109
4Department of Cell & Developmental Biology, University of Michigan, Ann Arbor, MI 48109
5Molecular and Cellular Pathology Graduate Program, University of Michigan, Ann Arbor, MI 48109
6Program in Chemical Biology, University of Michigan, Ann Arbor, MI 48109
7Department of Radiology, University of Michigan, Ann Arbor, MI 48109
8Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI 48109
9Department of Pathology and Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109
10Rogel Cancer Center, University of Michigan, Ann Arbor, MI 48109
11Department of Cancer Systems Imaging, University of Texas MD Anderson Cancer Center, Houston, TX, 77054
12Department of Molecular and Cellular Oncology, University of Texas MD Anderson Cancer Center, Houston, TX 77054
13‡Present address: Department of Pathology & Laboratory Medicine and Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami FL 33136

†Correspondence: clyssiot@med.umich.edu, shahy@umich.edu

ABSTRACT

Mitochondrial glutamate-oxaloacetate (GOT2) is part of the malate-aspartate shuttle (MAS), a mechanism by which cells transfer reducing equivalents from the cytosol to the mitochondria. GOT2 is a key component of mutant KRAS (KRAS*)-mediated rewiring of glutamine metabolism in pancreatic ductal adenocarcinoma (PDA). Here, we demonstrate that the loss of GOT2 disturbs redox homeostasis and halts proliferation of PDA cells in vitro. GOT2 knockdown (KD) in PDA cell lines in vitro induced NADH accumulation, decreased Asp and α-ketoglutarate (αKG) production, stalled glycolysis, disrupted the TCA cycle, and impaired proliferation. Oxidizing NADH through chemical or genetic means resolved the redox imbalance induced by GOT2 KD, permitting sustained proliferation. Despite a strong in vitro inhibitory phenotype, loss of GOT2 had no effect on tumor growth in xenograft PDA or autochthonous mouse models. We show that cancer-associated fibroblasts (CAF), a major component of the pancreatic tumor microenvironment (TME), release the redox active metabolite pyruvate, and culturing GOT2 KD cells in CAF conditioned media (CM) rescued proliferation in vitro. Furthermore, blocking pyruvate import or pyruvate-to-lactate reduction prevented rescue of GOT2 KD in vitro by exogenous pyruvate or CAF CM. However, these interventions failed to sensitize xenografts to GOT2 KD in vivo, demonstrating the remarkable plasticity and differential metabolism deployed by PDA cells in vitro and in vivo. This emphasizes how the environmental context of distinct pre-clinical models impacts both cell-intrinsic metabolic rewiring and metabolic crosstalk with the tumor microenvironment (TME).
INTRODUCTION

Cancer cells depend on deregulated metabolic programs to meet energetic and biosynthetic demands\(^1\)-\(^3\). Metabolic therapies aim to preferentially target these dependencies\(^4\). This approach has shown promise in preclinical models of pancreatic ductal adenocarcinoma (PDA) – one of the deadliest major cancers, notoriously resistant to anti-cancer therapies\(^5\),\(^6\). Pancreatic tumors are poorly vascularized and nutrient dysregulated\(^7\). Therefore, cancer cells commandeer metabolic pathways to scavenge and utilize nutrients\(^6\),\(^8\). A wealth of recent literature has identified that this is mediated predominantly by mutant KRAS (KRAS*), the oncogenic driver in most pancreatic tumors\(^9\)-\(^14\). KRAS* has also been implicated in shaping the pancreatic tumor microenvironment (TME)\(^15\),\(^16\). PDA tumors exhibit a complex tumor microenvironment\(^17\),\(^18\) with metabolic interactions between malignant, stromal, and immune cells enabling and facilitating tumor progression\(^19\). Recent successes in drug development have provided KRAS* selective inhibitors, and these are in various stages of preclinical and clinical testing. However, consistent with other targeted therapies, resistance inevitably occurs\(^20\),\(^21\). Therefore, disrupting downstream metabolic crosstalk mechanisms in PDA is a compelling combinatorial or alternative approach\(^22\).

In support of this idea, previous work from our lab described that PDA cells are uniquely dependent on KRAS*-mediated rewiring of glutamine metabolism for protection against oxidative stress\(^11\). Mitochondrial glutamate oxaloacetate transaminase 2 (GOT2) is implicated in this rewired metabolism in PDA. In normal physiology, GOT2 functions in the malate-aspartate shuttle (MAS), a mechanism by which cells transfer reducing equivalents between the cytosol and mitochondria to balance the two independent NADH pools and maintain redox balance (Figure 1A). PDA cells driven by KRAS* divert metabolites from the MAS and increase flux through malic enzyme 1 (ME1) to produce NADPH\(^11\). Since this pathway is critical for PDA, we set out to evaluate GOT2 as a potential therapeutic target. Using metabolomics analyses and manipulation of the redox state in PDA cells, we discovered that loss of GOT2 in vitro induces intracellular NADH accumulation and reductive stress. These metabolic changes impair cellular growth, which can be rescued with chemical or genetic interventions that oxidize NADH. However, loss of GOT2 had no effect on tumor growth or initiation in immunocompromised or immunocompetent mouse models of PDA. Cancer cells utilize a complex cell-intrinsic rewiring and crosstalk with the TME to maintain redox homeostasis in vivo. These data emphasize an under-appreciated role for GOT2 in pancreatic tumor redox homeostasis and illustrate the differential biochemical pathways and metabolic plasticity deployed by cancer cells in vivo.

RESULTS

Loss of GOT2 impairs PDA cell proliferation in vitro

To expand on our previous work studying GOT2 in PDA\(^11\), and to evaluate GOT2 as a potential therapeutic target, we generated a panel of PDA cell lines with doxycycline-inducible expression of either a control non-targeting shRNA (shNT) or two independent shRNAs (sh1, sh2) targeting the GOT2 transcript. Cells cultured in media containing doxycycline (+Dox) exhibited a marked decrease in GOT2 protein expression compared to cells cultured in media without doxycycline (-Dox) (Figure 1B; Figure 1-figure supplement 1A). This knockdown was specific for GOT2, relative to the cytosolic aspartate aminotransaminase GOT1 (Figure 1-figure supplement 1B). Having validated GOT2 knockdown (KD), we tested the importance of GOT2 for cellular proliferation. In general, GOT2 KD in PDA cells impaired colony formation (Figure 1C,D; Figure 1-figure supplement 1C) and proliferation (Figure 1-figure supplement 1D). Consistent with
our previous report, GOT2 was not required for the proliferation of non-transformed pancreatic cell types (Figure 1-figure supplement 1E,F).

Since GOT2 has several vital metabolic roles in a cell (Figure 1A), the changes caused by decreased GOT2 expression in PDA cells were examined using liquid chromatography coupled tandem mass spectroscopy (LC-MS/MS). Numerous changes in the intracellular metabolome of GOT2 KD cells were observed (Figure 1-figure supplement 2A,B). Of note, the products of the GOT2-catalyzed reaction, aspartate (Asp) and α-ketoglutarate (αKG), were decreased (Figure 1E), and supplementation of these metabolites rescued growth of GOT2 KD (Figure 1F). While these PDAC cell lines do not express Asp transporters, we confirmed that supraphysiological levels of Asp (20-fold excess) led to an increase in intracellular Asp (Figure 1-figure supplement 2C). In addition to reduced αKG, there was a disruption in TCA cycle intermediates, consistent with a role for GOT2 in facilitating glutamine anaplerosis (Figure 1-figure supplement 2D).

GOT2 KD perturbs redox homeostasis in PDA cells

Aside from the expected decrease in Asp and αKG, and the perturbation of the TCA cycle, closer examination of the GOT2 KD metabolomics dataset revealed an impairment in glycolysis with a node at glyceraldehyde 3-phosphate dehydrogenase (GAPDH), indicative of NADH reductive stress (Figure 2A). Examination of glycolytic rate via Seahorse Flux Analysis confirmed that glycolysis was indeed impaired in GOT2 KD cells (Figure 2-figure supplement 1A). GAPDH reduces NAD+ to produce NADH, where a build-up of NADH product-inhibits GAPDH activity. Indeed, metabolite pools in upstream glycolysis and branch pathways like the pentose phosphate pathway are increased, and those in downstream glycolysis are decreased (Figure 2B). In cultured PDA cells, the MAS transfers glycolytic reducing potential to drive the electron transport chain (ETC) and support the maintenance of cytosolic redox balance (Figure 1A). We thus hypothesized that GOT2 KD interrupted this shuttle, preventing the proper transfer of electron potential in the form of NADH between these two compartments. In support of this, GOT2 KD increased the intracellular ratio of NADH to NAD+ (Figure 2C).

NADH accumulation leads to reductive stress, which can be relieved if the cell has access to electron acceptors. Pyruvate is a notable metabolite in this regard, as it can accept electrons from NADH, producing lactate and regenerating NAD+, in a reaction catalyzed by lactate dehydrogenase (LDH). Therefore, we hypothesized that pyruvate could rescue the defect in cellular proliferation mediated by GOT2 KD. Indeed, culturing GOT2 KD cells in pyruvate rescued proliferation in a dose dependent manner (Figure 2D,E; Figure 2-figure supplement 1B). Additionally, cells expressing a genetically-encoded, fluorescent ATP sensor indicated that ATP levels dropped with GOT2 KD and were restored with pyruvate supplementation (Figure 2-figure supplement 1C), reflecting the link between TCA cycle activity, respiration, and oxidative phosphorylation. Furthermore, having identified a metabolite that permits in vitro proliferation of PDA cells without GOT2, we engineered CRISPR-Cas9 Got2 knock out (KO) cells for further investigation (Figure 2-figure supplement 1D). In support of the data generated using the doxycycline-inducible shRNA, Got2 KO impaired colony formation of PDA cells, which was similarly restored through extracellular pyruvate supplementation (Figure 2-figure supplement 1E,F).

α-ketobutyrate (αKB) is another electron acceptor that turns over NADH in a mechanism analogous to pyruvate but without entering downstream metabolism in the same fashion as pyruvate. αKB also rescued proliferation after GOT2 KD (Figure 2F; Figure 2-figure...
This mechanism is dependent on NADH turnover, and not NAD+ synthesis, as the NAD+ precursor nicotinamide mononucleotide (NMN) failed to rescue GOT2 KD (Figure supplement 1G).

To test this further, GOT2 KD cells were engineered to express either doxycycline-inducible cytosolic or mitochondrial *Lactobacillus brevis* NADH oxidase (LbNOX), which uses molecular oxygen to oxidize NADH and produce water and NAD+ (Figure 2-figure supplement 2A)\(^{25,26}\). Cytosolic LbNOX, but not mitochondrial LbNOX (mLbNOX), rescued proliferation of GOT2 KD cells (Figure 2G; Figure 2-figure supplement 2B). We confirmed the mLbNOX construct encoded a functional enzyme as defects in proliferation incurred by treatment with complex I inhibitor Piericidin could be rescued with mLbNOX as reported previously (Figure 2-figure supplement 2C)\(^{26}\). LbNOX reversed the increased NADH/NAD+ ratio induced by GOT2 KD via an overall decrease in NADH levels (Figure 2H; Figure 2-figure supplement 2D). Further, the secreted pyruvate/lactate ratios in the media of LbNOX-expressing cells dramatically increased, indicating a resolution of the cytosolic NADH stress induced by GOT2 KD (Figure 2-figure supplement 2E,F). Moreover, the metabolic defects observed following GOT2 KD were ameliorated by cytosolic LbNOX activity, including an increase in Asp and αKG, normalization of TCA cycle metabolites, and the release of the glycolytic block at GAPDH (Figure 2-figure supplement 2G). The spatial control of the LbNOX system indicated that KD of mitochondrial GOT2 could be rescued by balancing the cytosolic NADH/NAD+ pool.

Next, we traced the metabolic fate of U\(^{13}C\)-pyruvate in cells with GOT2 KD. Importantly, we also assessed the impact and metabolism of pyruvate in this system following inhibition of the mitochondrial pyruvate carrier (MPC) inhibitor UK5099, which blocks entry of pyruvate into the mitochondria (Figure 2I). Media containing equimolar unlabelled glucose and U\(^{13}C\)-pyruvate was used to prevent dilution of the pyruvate label by unlabelled pyruvate generated via glycolysis from the high glucose concentration used in normal media. This media formulation had no impact on the pyruvate GOT2 KD rescue phenotype (Figure 2-figure supplement 3A). In this experiment, roughly 50% of the intracellular pyruvate was labelled (Figure 2J), in line with the other half of the unlabelled pyruvate coming from glucose, and most of the labelled pyruvate was converted to lactate (Figure 2K). We observed modest labelling of citrate, aspartate, and alanine from pyruvate (Figure 2-figure supplement 3B-D). While the labelled TCA cycle and branching pathway intermediates decreased dramatically with UK5099, this MPC inhibition had no effect on the pyruvate GOT2 KD rescue phenotype (Figure 2-figure supplement 3E).

Both lactate and alanine can fuel oxidation via the TCA cycle\(^{27,28}\), and Asp is critical for nucleotide production. Pyruvate can be converted into all three of these metabolites, yet supplementation with exogenous lactate, alanine, or nucleoside bases failed to rescue GOT2 KD (Figure 2-figure supplement 3F-H). These data collectively suggest a redox role for pyruvate-mediated rescue of GOT2 KD, as opposed to entering the mitochondria for ATP production or biosynthesis. These findings provide clear evidence using several orthogonal strategies that GOT2 KD results in accumulation of NADH pools and reductive stress in PDA cells.

**GOT2 is not required for PDA tumor growth in vivo**

To test the effect of GOT2 KD on in vivo tumor growth, PDA cell lines were injected subcutaneously into the flanks of immunocompromised (NOD scid gamma; NSG) mice. We allowed the tumors to establish for 7 days, after which the mice were fed normal chow or
doxycycline chow ad libitum. Surprisingly, despite the inhibitory in vitro phenotype, and robust suppression of GOT2 expression in vivo, PDA tumors from five different cell lines grew unimpeded with GOT2 KD (**Figure 3A,B; Figure 3-figure supplement 1A,B**). Nuclear Ki67 staining confirmed that tumors lacking GOT2 were proliferative, and actually displayed a modest, but significant, increase in Ki67-positive nuclei (**Figure 3C,D**). To further examine the role of GOT2 in the proper tissue context, PDA cells were injected orthotopically into the pancreas of NSG mice and tumors were allowed to establish for 7 days before feeding the mice regular or DOX chow. Similar to the flank model, GOT2 KD had no effect on the growth of orthotopic tumors (**Figure 3-figure supplement 1C**).

Having observed a discrepancy between in vitro and in vivo dependence on GOT2 for proliferation, the relative abundances of intracellular metabolites from flank tumors were analyzed via LC-MS/MS to compare the metabolic changes between cell lines and tumors following loss of GOT2. While GOT2 KD induced some changes in tumor metabolite levels, the affected metabolic pathways were distinct from those observed in vitro, bearing in mind we were comparing homogenous cell lines with heterocellular xenografts (**Figure 3E,F; Figure 3-figure supplement 1D-F**). Asp abundance was significantly decreased, yet αKG levels remained constant (**Figure 3E**), and TCA cycle intermediates were unaffected (**Figure 3-figure supplement 1F**). This led us to initially hypothesize that PDA cells rewire their metabolism in vivo to maintain αKG levels when GOT2 is knocked down. However, upon examination of the expression of other αKG-producing enzymes in GOT2 KD tumors, we did not observe a compensatory increase in expression (**Figure 3-figure supplement 1G**). Certainly, expression does not always dictate metabolic flux, but these data led us to adopt an alternative, cell-extrinsic hypothesis to explain the different in vitro and in vivo GOT2 KD phenotypes. Finally, the glycolytic signature indicative of NADH stress was not observed in the metabolomics analysis from flank GOT2 KD tumors, further illustrating the differential dependence on GOT2 in PDA in vitro and in vivo (**Figure 3F**).

To evaluate the role of GOT2 in an immunocompetent model, we crossed the LSL-Kras<sup>G12D</sup>;Ptf1a-Cre (KC) mouse with Got2<sup>f/f</sup> mice to generate a LSL-Kras<sup>G12D</sup>;Got2<sup>f/f</sup>;Ptf1a-Cre (KC-Got2) (**Figure 4A, Figure 4-figure supplement 1A**). Loss of Got2 had no observable effect on the architecture of the healthy, non-transformed pancreas (**Figure 4-figure supplement 1B,C**). This is in support of our data demonstrating loss of GOT2 was not deleterious in human, non-malignant pancreatic cell types (**Figure 1-figure supplement 1F**). KC-Got2 and KC controls were aged to 3, 6, and 12 months, at which point pancreata were harvested. We confirmed loss of GOT2 in the epithelial compartment via IHC (**Figure 4B**). No differences were observed in the weights of pancreata between 3 month KC-Got2 and KC mice (**Figure 4C**). Scoring the H&E stained tissues from these groups by a blinded pathologist revealed that KC-Got2 mice had a significantly greater percentage of healthy acinar cells compared to KC controls (**Figure 4D,E**). However, no differences were observed in the percentages of acinar-ductal metaplasia or PanIN grade between KC-Got2 and KC mice (**Figure 4E**). Additionally, we aged KC-Got2 mice to 6 months and compared the pancreata to matched 6 month KC historic controls, observing a slight decrease in weight for KC-Got2 pancreatic (**Figure 4F,G**). A histological analysis by a blinded pathologist did not identify a difference in number or severity of lesions (**Figure 4H**). In addition, both KC and KC-Got2 mice had progressed to carcinoma after aging for 1 year (**Figure 4I,J**). This suggests that loss of Got2 does not affect the progression of PDA following transformation by oncogenic Kras.
Cancer-associated fibroblast conditioned media supports colony formation in GOT2 KD cells in vitro

Human PDA tumors develop a complex microenvironment composed of a tumor-promoting immune compartment, a robust fibrotic response consisting of diverse stromal cell types, and a dense extracellular matrix (ECM)\(^1\). While the flank tumor milieu in immunocompromised mice is less complex than that of a human PDA tumor, α-smooth muscle actin (αSMA) staining revealed that activated mouse fibroblasts comprised a substantial portion of the microenvironment in tumors regardless of GOT2 status (Figure 5-figure supplement 1A). Additionally, we and others have previously reported mechanisms by which CAFs in the stroma engage in cooperative metabolic crosstalk with pancreatic cancer cells\(^2\)\(^-\)\(^3\)\(^0\). So, we hypothesized that CAFs supported PDA metabolism following GOT2 KD. To investigate potential metabolic crosstalk in a simplified setting, PDA cells were cultured in vitro with conditioned media (CM) from human CAFs (hCAFs). In support of our hypothesis, hCAF CM promoted colony formation in PDA cells with GOT2 KD in a dose-dependent manner (Figure 5A,B; Figure 5-figure supplement 1B). Furthermore, hCAF CM displayed a more pronounced colony formation rescue phenotype compared to CM from tumor-educated macrophages (TEMs) or from PDA cells (Figure 5-figure supplement 1C).

To begin to identify the factors in hCAF CM responsible for this effect, hCAF CM was boiled, filtered through a 3 kDa cut-off membrane, or subjected to cycles of freezing and thawing. In each of these conditions, hCAF CM supported colony formation in GOT2 KD cells, suggesting the relevant factor(s) was a metabolite (Figure 5C; Figure 5-figure supplement 1D). Therefore, the relative abundances of metabolites in hCAF CM were analyzed via LC-MS/MS (Figure 5-figure supplement 1E). Interestingly, when the metabolites were ranked in order of relative abundance, compared to the media control, pyruvate was one of the most differentially abundant metabolites released by hCAFs into the conditioned media (Figure 5D). Since we used pyruvate-free DMEM to culture hCAFs, to avoid overinterpreting this finding, we quantified the absolute concentration of pyruvate in hCAF CM at 250 µM, with some variability between batches (Figure 5E). This is a physiologically relevant concentration of pyruvate in serum collected from mice harboring pancreatic tumors\(^3\)\(^1\), and 250 µM pyruvate rescued GOT2 KD in vitro (Figure 2E). Consistent with the idea of metabolite exchange, PDA cells cultured in hCAF CM had elevated levels of intracellular pyruvate (Figure 5-figure supplement 1F).

In the in vivo flank model, mouse fibroblasts infiltrate the pancreatic xenografts and engage in crosstalk with cancer cells. Therefore, we tested whether our findings with hCAFs were also applicable in mouse cancer-associated fibroblasts (mCAFs). mCAFs isolated from a pancreatic flank xenograft in an NSG mouse also secreted pyruvate at similar levels to hCAFs in vitro (Figure 5E). Further, mCAF CM promoted PDA colony formation following GOT2 KD (Figure 5F, Figure 5-figure supplement 1G). Since these mCAFs are the same CAFs encountered by PDA cells in our in vivo model, these data further support a mechanism by which CAFs compensate for loss of GOT2, by providing pyruvate to PDA cells lacking GOT2.

To better understand the production and release of pyruvate in CAFs, we traced glucose metabolism using uniformly carbon labeled (U13C)-Glucose and LC-MS metabolomics. We demonstrate that the pyruvate released by CAFs was produced from glucose (Figure 5G), and, in support of previous studies\(^3\)\(^2\)\(^,\)\(^3\)\(^0\), these CAFs displayed labelling patterns indicative of glycolytic metabolism (Figure 5H).
Aside from pyruvate, we also detected significantly elevated levels of Asp and αKG in CAF CM (Figure 5-figure supplement 1E). Since these are metabolites produced by GOT2, we next asked whether they were present at sufficient concentrations in CAF CM to compensate for loss of GOT2 in vitro. However, we quantified Asp at 15 µM and αKG at 50 µM in CAF CM (Figure 5-figure supplement 2A), well below the reported values from mouse serum31. Furthermore, millimolar levels of Asp are required to impact intracellular levels, as PDA cell lines do not express an Asp transporter34, and dimethyl-αKG must be used as αKG has limited membrane permeability35. Nevertheless, we tested the rescue activity of reported serum levels of Asp and αKG (50 µM and 500 µM, respectively)31, again utilizing the dimethyl-αKG, and compared this to serum levels of pyruvate (250 µM). Here we found that pyruvate rescued proliferation of GOT2 KD to a greater extent than the combination of Asp and αKG (Figure 5-figure supplement 2B). Therefore, we conclude that pyruvate, and not Asp or αKG, is responsible for the GOT2 KD rescue activity of CAF CM in vitro.

Like GOT2 inhibition, it is well-established that inhibiting the activity of complex I of the ETC also results in an increase in NADH, which can be counteracted with extracellular pyruvate23,24,26,34. Therefore, since pyruvate is highly abundant in hCAF CM, we hypothesized that PDA cells cultured in hCAF CM would be protected from complex I inhibitors. Indeed, both hCAF CM and extracellular pyruvate conferred resistance to PDA cells against the complex I inhibitors rotenone, phenformin, and IACS-010759 (Figure 5-figure supplement 2C)36. This points to a potential mechanism by which the TME could affect sensitivity to complex I inhibition, though more in depth studies are needed to test this finding further.

Inhibiting pyruvate uptake and metabolism blocks rescue of GOT2 KD in vitro

According to our model, PDA cells are more vulnerable to GOT2 KD or complex I inhibitors in a pyruvate-depleted environment or if pyruvate uptake were blocked. Pyruvate can be transported by four MCT isoforms37-39, and an analysis of the CCLE database suggests that PDA cell lines primarily express MCT1 and MCT4 (Figure 6-figure supplement 1A). Since MCT1 has a higher affinity for pyruvate than MCT438, we decided to focus on MCT1 as the transporter by which PDA cells import pyruvate40,41. Indeed, PDA cells express significantly higher levels of MCT1, as compared to hCAFs (Figure 6-figure supplement 1B). Similarly, examining expression of MCT1 from a recently published single cell analysis of a murine syngeneic orthotopic pancreatic tumor42 indicated that PDA cells express high levels of MCT1 (Figure 6-figure supplement 1C).

Import/export of pyruvate and lactate through MCT1, as well as the intracellular redox state, is affected by extracellular concentrations of pyruvate and lactate. The absolute concentrations of pyruvate and lactate were measured in hCAF and mCAF CM to calculate the relative pyruvate/lactate ratio from in vitro GOT2 KD rescue experiments (Figure 6A). In parallel, the absolute levels of pyruvate and lactate, and the relative ratio, were measured in tumor interstitial fluid (TIF) from flank xenografts and in serum from host mice (Figure 6B). When the measured pyruvate and lactate levels from each of these permutations were added back to regular media, the pyruvate/lactate ratios mimicking CAF CM, serum, or TIF promoted the growth of GOT2 KD cells in vitro (Figure 6C). These data indicate the pyruvate/lactate ratios in CAF CM in vitro and in the in vivo TME are favorable for pyruvate import, possibly through MCT1.

Therefore, we hypothesized that blocking pyruvate import through MCT1 would render cells vulnerable to GOT2 KD. The small molecule AZD3965 has specificity for MCT1 over MCT443-45, therefore GOT2 KD cells were cultured in pyruvate or hCAF CM in the presence of AZD3965. In
support of our hypothesis, MCT1 chemical inhibition reduced the pyruvate and hCAF CM rescue of Got2 KD (Figure 6D).

For a genetic approach, MCT1 was knocked down in doxycycline-inducible Got2 KD cells (Figure 6-figure supplement 1D,F). MCT1 KD modestly slowed the growth of Got2 KD cells cultured in pyruvate or hCAF CM (Figure 6-figure supplement 1E,G). We reasoned that partial knockdown could explain why MCT1 KD had a more modest effect than chemical inhibition of MCT1. Therefore, we next generated MCT1 KO clones in Got2 KD cells (Figure 6E). Indeed, MCT1 KO in Got2 KD cells blocked pyruvate rescue (Figure 6F). We also demonstrate that MCT1 inhibition was most effective at physiological levels of pyruvate (250 µM) (Figure 6F, Figure 6-figure supplement 1H). Furthermore, MCT1 KO cells cultured with U13C-pyruvate demonstrated reduced uptake of pyruvate, as measured by intracellular labelled pyruvate (Figure 6G). Since pyruvate is rapidly imported and converted to lactate, the reduced pyruvate uptake was observed more clearly in dramatically reduced levels of labelled lactate in MCT1 KO cells (Figure 6G). Lastly, MCT1 blockade was tested in combination with complex I inhibitors in PDA cells cultured in pyruvate or hCAF CM. AZD3965 also reversed the rescue activity of pyruvate or hCAF CM in PDA cells treated with IACS-010759 (Figure 6-figure supplement 1I).

The reduction of pyruvate to lactate by lactate dehydrogenase (LDH) is the central mechanism in our model by which NAD+ is regenerated to support proliferation in Got2 inhibited cells. Thus, we next asked whether inhibiting LDH activity could also prevent Got2 KD rescue by pyruvate or hCAF CM. Our PDA cell lines highly expressed the LDHA isoform of LDH, as determined by western blotting (Figure 6-figure supplement 1J). As such, we utilized the LDHA-specific chemical inhibitor FX11 in this study. In further support of our model, inhibiting LDHA with FX11 slowed the in vitro proliferation of Got2 KD cells cultured in pyruvate or hCAF CM, relative to single agent controls (Figure 6H, Figure 6-figure supplement 1K).

Cumulatively, these data support an in vitro model whereby perturbation of mitochondrial metabolism with Got2 KD or complex I inhibition disrupts redox balance in PDA cells. This can be restored through import of pyruvate from the extracellular environment and reduction to lactate to regenerate NAD+.

**Metabolic plasticity in vivo supports adaptation to combined Got2 KD and inhibition of pyruvate metabolism**

This proposed in vitro mechanism suggests that the unhampered growth of Got2 inhibited tumors (Figure 3A,B) could be explained by uptake of pyruvate from the TME. In vitro, MCT1 KD had a modest effect on the proliferation of Got2 KD cells cultured in pyruvate or hCAF CM. In contrast, we found that MCT1 KD had no effect on the growth of Got2 KD subcutaneous xenografts (Figure 6-figure supplement 2A, B). A caveat of this finding was the observed detectable levels of MCT1 at endpoint (Figure 6-figure supplement 2C,D). However, MCT1 KO similarly did not impact the growth of Got2 KD tumors in vivo, which is in stark contrast to the results observed in vitro whereby MCT1 KO blocked the rescue of colony formation in Got2 KD cells by pyruvate (Figure 6-figure supplement 2E). In these tumors, MCT1 was not detectable at endpoint and we did not observe a compensatory increase in MCT4 expression in either cell lines or xenografts with MCT1 KO (Figure 6-figure supplement 2F,G). Similarly, administration of AZD3965 did not impact the growth of Got2 KD xenografts, again contrasting our in vitro data showing strong blockade of Got2 KD rescue by pyruvate or hCAF CM with
MCT1 chemical inhibition (Figure 6-figure supplement 2E-G). Lastly, unlike in vitro, FX11 had no effect on GOT2 KD growth in vivo (Figure 6-figure supplement 3A,B).

**DISCUSSION**

GOT2 is an essential component of the MAS, which we now demonstrate is required for redox homeostasis in PDA cells in vitro. Knockdown of GOT2 in vitro disrupts the MAS and renders PDA cells incapable of transferring reducing equivalents between the cytosol and mitochondria, leading to a cytosolic accumulation of NADH. This predominantly impacts the rate of glycolysis, an NAD+-coupled pathway, with secondary impacts on mitochondrial metabolism, that together slow the proliferation of PDA cells in vitro. For instance, GOT2 feeds into the ME1 shunt, which we demonstrated previously also produces pyruvate and sustains intracellular NADPH levels.

Extracellular supplementation of electron acceptors like pyruvate and αKB, or the expression of a cytosolic NADH oxidase, relieves this NADH reductive stress and the associated pathway feedback inhibition.

In striking contrast to the in vitro data presented herein, we also illustrate that GOT2 KD does not affect the growth of PDA tumors in vivo, potentially because electron acceptors in the tumor microenvironment can restore redox homeostasis. Indeed, pyruvate is present in mouse serum at 250 µM, a concentration which is sufficient to compensate for GOT2 KD in vitro. Furthermore, we also demonstrate that pancreatic CAFs release pyruvate in vitro, which can be utilized by PDA cells. This is supported by previous findings in CAFs from other cancers. Therefore, a source of pyruvate is available to PDA tumors, either from circulation or potentially from the CAFs.

This led us to hypothesize that blocking pyruvate uptake and metabolism would deprive PDA cells of a critical means to relieve the NADH stress mediated by GOT2 KD. In vitro, this hypothesis was supported by data illustrating that either inhibition of MCT1 or LDHA blocked the GOT2 KD rescue activity of pyruvate and CAF CM. However, this proved to be more complicated in vivo, as neither approach successfully sensitized tumors to GOT2 KD. We believe this could be explained by several mechanisms. First, previous work in KRAS*-driven non-small cell lung cancer (NSCLC) reported differential dependencies on glutaminolysis in vitro in cells lines versus in vivo in lung tumors. Glutaminase inhibition was ineffective in vivo in these models as lung tumors primarily utilized glucose as a carbon source for the TCA cycle instead of glutamine. If this mechanism is active in our models, glucose, and not glutamine catabolism through GOT2, would fuel the TCA cycle. Second, while MCT4 has a lower affinity for pyruvate than MCT1, it can transport pyruvate and is also highly expressed in the cell lines used here (Figure 6-figure supplement 2F,G) and has been shown to confer resistance to cancer cells against MCT1 inhibition. Thus, dual inhibition of MCT1 and MCT4 may be required to effectively block pyruvate uptake. Third, even with sufficient MCT blockade, PDA cells could still obtain pyruvate through other processes, such as macropinocytosis. Fourth, while our study focuses on pyruvate, numerous circulating metabolites can function as electron acceptors and could relieve the intracellular accumulation of NADH if cancer cells are unable to import pyruvate. Fifth, reduction of pyruvate to lactate by LDH is not the only reaction by which NAD+ is regenerated. Recent studies have identified how serine pathway modulation, polyunsaturated fatty acid synthesis, and the glycerol-phosphate shunt all contribute to NADH turnover. Future work remains to assess these mechanisms in PDA cells in vivo and if they compensate for an impaired MAS. Nevertheless, our data emphasize that redox homeostasis is a vital
aspect of cancer cell metabolism and is maintained through a complex web of intracellular compensatory pathways and extracellular interactions.

Aside from its broader role in redox balance, GOT2 is also a prominent source of aspartate in PDA cells, and we demonstrate that GOT2 inhibition dramatically decreases aspartate levels both in vitro and in vivo. Previous studies have shown that aspartate availability is rate limiting in rapidly proliferating cells24,34,56,57. In our models, in contrast to physiological pyruvate concentrations simultaneous treatment with supraphysiological doses of both Asp and membrane-permeable dimethyl-αKG were required to provide a rescue of PDA cell proliferation in the absence of GOT2. Additionally, free Asp is available at low micromolar concentrations and has limited uptake capacity of PDA cells31. Supplementation of physiologically relevant concentrations of Asp and αKG afforded a modest rescue compared to physiological pyruvate. Furthermore, exogenous protein supplementation with bovine serum albumin (BSA), another potential source for aspartate, failed to rescue GOT2 KD in vitro (Figure 6-figure supplement 3C). While non-specific engulfment of extracellular matrix (ECM) via macropinocytosis in vivo could supply PDA cells with Asp 58, meeting a vital requirement for biosynthesis, we propose this does not address overall redox imbalance. Pyruvate, on the other hand, regenerates NAD+ allowing broader metabolic processes to resume, including Asp production and nucleotide biosynthesis. In support of this, recent work in myoblasts demonstrated that while complex I inhibition with piericidin increased the NADH/NAD+ ratio leading to depletion of Asp, adding Asp back to the system neither restored redox balance nor induced proliferation59.

Our work also highlights the emerging metabolic role of cancer-associated fibroblasts (CAFs) in PDA. Recent studies have shown that CAFs engage in cooperative metabolic crosstalk with cancer cells in many different tumor types19,28,29,60,61. We add to this body of literature by demonstrating that CAFs release pyruvate, which is taken up and utilized by PDA cells. However, much remains to be discovered about CAF metabolism how they contribute to redox homeostasis in tumors. Some types of activated fibroblasts are known to be highly glycolytic32,33, an observation supported by our data. Yet the advent of single-cell RNA sequencing in murine and human pancreatic tumor models has led to a recent appreciation for the heterogeneity of CAFs52-67. The newly identified iCAF, myCAF, and apCAF populations have distinct functions in a pancreatic tumor52 and likely employ distinct metabolism to carry out these functions. Much more remains to be uncovered regarding competitive or cooperative interactions between PDA cells and the various CAF subpopulations, including which subtype(s) are responsible for pyruvate release.

Lastly, this work suggests that the role of the tumor microenvironment should be considered when targeting cancer metabolism. Indeed, approaches like disrupting the MAS shuttle via GOT2 KD or blocking complex I with small molecule inhibitors were less effective in vitro when PDA cells were cultured in supplemental pyruvate or CAF CM. These data are relevant since numerous mitochondrial inhibitors are currently in clinical trials against solid tumor types (NCT03291938, NCT03026517, NCT03699319, NCT02071862). Previous studies have also shown that complex I inhibitors are more effective in combination with AZD396568, a selective inhibitor of MCT1, though our work and others indicate that the status of other MCT isoforms should also be considered. Furthermore, the abundance of CAFs present in a tumor, as well as the level of circulating pyruvate in the patient, could predict outcomes for treatment with metabolic therapies that lead to redox imbalance. Targeting pancreatic cancer metabolism is an alluring approach, and a more detailed understanding of the metabolic crosstalk occurring in a
pancreatic tumor can shed light on potential resistance mechanisms and inform more effective metabolic therapies.

**Acknowledgements:** This work was funded by T32AI007413, F31CA24745701, and 1F99CA264414-01 (SAK); CA148828 and CA245546 (YMS); a Pancreatic Cancer Action Network/AACR Pathway to Leadership award (13-70-25-LYSS), Junior Scholar Award from The V Foundation for Cancer Research (V2016-009), Kimmel Scholar Award from the Sidney Kimmel Foundation for Cancer Research (SKF-16-005), a 2017 AACR NextGen Grant for Transformative Cancer Research (17-20-01-LYSS), and NIH grants R37CA237421, R01CA248160, R01CA244931 (CAL).

Additional funding sources include F31CA254079 (JAJ); T32-DK094775 and T32-CA009676 (BSN); R50 CA232985 (YZ), T32-GM11390 and F31-CA247076 (SBK); T32-CA009676, the American Cancer Society Postdoctoral Award PF-19-096-01, and the Michigan Institute for Clinical and Healthy Research (MICHR) Postdoctoral Translational Scholar Program fellowship award (NGS); the Michigan Postdoctoral Pioneer Program (ZCN); K99CA241357 and P30DK034933 (CJH); R01GM101171 and CA253986 (DBL); and Cancer Center support grant (P30 CA046592).

Metabolomics studies performed at the University of Michigan were supported by NIH grant DK097153, the Charles Woodson Research Fund, and the UM Pediatric Brain Tumor Initiative.

Research reported in this publication was supported by the National Cancer Institutes of Health under Award Number P30CA046592 by the use of the following Cancer Center Shared Resource(s): Flow Cytometry Core, Tissue and Molecular Pathology Core, Center for Molecular Imaging (P30CA046592).

The University of Michigan Center for Gastrointestinal Research Core Director, Michael Mattea, provided assistance in tissue processing, sectioning, and staining.

**Author contributions:** SAK, YMS, and CAL conceived of and designed this study. SAK, YMS, and CAL planned and guided the research and wrote the manuscript. SAK, LL, ALM, DJS, AA, PS, LZ, YZ, JAJ, BSN, BC, AR, GT, SBK, NGS, MTH, HW, DL, SEA, JR, XG, ZCN, SG, CJH, DBL, DRP-W, HY, HCC, MPdM, YMS, and CAL provided key reagents, performed experiments, analyzed, and interpreted data. YMS and CAL supervised the work carried out in this study.

**Declaration of Interests:**


**MATERIALS AND METHODS**

**Cell culture:** MIAPaCa-2, BxPC-3, Capan-1, Panc03.27, Panc10.05, PL45, and HPNE cell lines were obtained from ATCC. PaTu-8902, PaTu-8988T, and YAPC cells lines were obtained from DSMZ. UM6, UM19, UM28, UM32, UM53, and UM76 were generated from primary patient tumors at the University of Michigan. Human pancreatic stellate cells (hPSCs, also described
here as hCAFs) were a generous gift from Rosa Hwang. Mouse cancer-associated fibroblasts (mCAFs) were isolated as described below. All cell lines were cultured in high-glucose Dulbecco’s Modified Eagle Medium (DMEM, Gibco) without pyruvate and supplemented with 10% fetal bovine serum (FBS, Corning). 0.25% Trypsin (Gibco) was used to detach and passage cells. Cell lines were tested regularly for mycoplasma contamination using MycoAlert (Lonza). All cell lines in this study were validated for authentication using STR profiling via the University of Michigan Advanced Genomics Core. L-Aspartic acid (Sigma), dimethyl-α-ketoglutarate (Sigma), adenine (Sigma), guanine (Sigma), thymine (Sigma), cytosine (Sigma), sodium pyruvate (Invitrogen), α-ketobutyrate (Sigma), nicotinamide mononucleotide (NMN, Sigma), L-alanine (Sigma), and sodium lactate (Sigma) were used at the indicated concentrations. UK5099, AZD3965, and phenformin were purchased from Cayman chemical, Rotenone from Sigma, FX11 from MedChem Express, and IACS-010759 was generously provided by Dr. Giulio Draetta, University of Texas MD Anderson Cancer Center.

Doxy-inducible shGOT2 cells: The tet-pLKO-puro plasmid was obtained from Dmitri Wiederschain via Addgene (#21915). Oligonucleotides encoding sense and antisense shRNAs (shGOT2.1- TRCN0000034824, shGOT2.2- TRCN0000034825) targeting got2 (NM_002080.4) were synthesized (Integrated DNA Technologies), annealed, and cloned at AgeI and EcoRI sites according to the Wiederschain Protocol. A tet-pLKO non-targeting control vector (shNT-CCGGCAACAAGATGAAAGACCAACTCGAGTTGGCTCTCATGTTTGTGTTTTTT) was constructed using the same strategy. Tet-pLKO-shGOT2 and tet-pLKO-shNT lentiviruses were produced by the University of Michigan Vector Core using purified plasmid DNA. Stable cell lines were generated through transduction with optimized viral titers and selection with 1.5 µg/mL puromycin for 7 days.

GOT2 or MCT1 knockout cells: GOT2 or SLC16A1 (MCT1) knockout PDA cell lines were generated using a CRISPR-Cas9 method described previously. sgRNA oligonucleotide pairs were obtained from the Human GeCKO Library (v2, 3/9/2015). For GOT2 KO (sg1 (Fwd) 5’-CACCgAACGCTCACCTGCGGAGCT-3’, (Rev) 5’-AAACAGCGTCCGCAAGGTGAGCTTc; sg2 (Fwd) 5’-CACCgCGTTCTGCCTAGGCTGCCGCA-3’, (Rev) 5’-AAACTGCGGACGCTAGGCCAGAACGc) were cloned into the pSpCas9(BB)-2A-Puro plasmid (PX459, v2.0; Addgene, #62988), transfected in PDA cell lines, and selected in puromycin for 7 days. Cells were then seeded into 96 well plates at a density of 1 cell per well, and individual clones were expanded. GOT2 KO cells were maintained in 1 mM pyruvate for this entire process. GOT2 knockout was verified via Western blot. Cells transfected with the empty PX459 vector were used as controls.

MCT1 KD cells: Cells were transduced with 8 µg/mL polybrene and lentivirus containing the pGFP-C-shLenti plasmid (Origine, #TR30023) containing an shCTR sequence, shMCT1.1 [TL309405A (5’-GAGGAAGAGACCAGTATAAGATTTGCTGG-3’)], or shMCT1.2 [TL309405B (5’-ATCCAGCTCTGACCATGATTGGAAGGT-3’)]. These plasmids were a generous gift from Dr. Sean Morrison. The cells were then centrifuged at 1000xg for 60 minutes at room temperature. Transduced cells were then expanded and sorted on the MoFlo Astrios (Beckman-
Coulter). GFP+ cells were collected and expanded before verification of MCT1 KD via Western blotting.

Transduction of LbNOX/mitoLbNOX: pINDUCER (Addgene, #44014) plasmids containing GFP, LbNOX, or mitoLbNOX were obtained from Dr. Haoqing Ying, MD Anderson. Plasmids were sequenced and transfected along with lentiviral packaging plasmids into HEK293FT cells with Lipofectamine 3000 (Thermo Fisher) per manufacturer’s instructions. Virus was collected after 48 hours and filtered through a 0.2 µm filter. PaTu-8902 and MIAPaCa-2 iDox-shGOT2.1 cells were seeded in 6 well plates at 250,000 cells/well, transduced with the indicated vectors, and selected in G418 at 500 µg/mL for 7 days. Expression of Flag-tagged LbNOX or mitoLbNOX was confirmed by Western blot with a Flag antibody after culturing cells in 1 µg/mL doxycycline for 3 days.

Luciferase-expressing cells: MIAPaCa-2 iDox-shGOT2.1 cells were transduced with the FUGW-FL (EF1a-luc-UBC6-EGFP) lentiviral vector constructed previously72 and GFP+ cells were selected via flow cytometry. Luciferase activity was confirmed following transduction and selection with an in vitro luciferase assay and detection on a SpectraMax M3 Microplate reader (Molecular Devices).

ATP fluorescent sensor/Incucyte growth assays: PaTu-8902 and MIAPaCa-2 iDox-GOT2.1 cells were transduced with CytoATP or CytoATP non-binding control vectors using the CytoATP Lentivirus Reagent Kit (Sartorious, #4772) and polybrene transfection reagent (Thermo Fisher) and selected for 7 days in 2 µg/mL puromycin. For proliferation and rescue experiments, cells were incubated in an Incucyte (Sartorious) equipped with a Metabolism Optical Module, where the ratio of ATP binding was detected and normalized to the non-binding control cells. Proliferation rate was determined by the percent confluence detected in the phase channel of the Incucyte normalized to Day 0 for each condition.

Isolating mouse CAFs: UM2 subcutaneous xenografts from NSG mice were isolated and prepared in the laboratory of Dr. Diane Simeone, as reported previously73, and single cell suspensions were plated and cultured in vitro. Mouse CAFs were separated from human pancreatic UM2 cancer cells using the Mouse Cell Depletion Kit (MACS Miltenyi Biotec) according to the manufacturer’s instructions.

Conditioned media: Conditioned media was generated by splitting cells at ~90% confluence in a 10 cm² plate into four 15 cm² plates containing a final volume of 27 mL of growth media, and incubating for 72 hours at 37°C, 5% CO₂. Afterward, the media was collected in 50 mL conical tubes, centrifuged at 1,000 rpm for 5 minutes to remove any detached cells or debris, and divided into fresh 15 mL conical tubes in 10 mL aliquots for long-term storage at -80°C. For all conditioned media experiments, unless indicated otherwise, growth media was mixed with conditioned media for a final ratio of 75% conditioned media to 25% fresh growth media. For the experiments in Figure 5C and Figure 5-figure supplement 1D, conditioned media were manipulated as follows. For boiling, the conditioned media tubes were placed in a water bath at 100°C for 10 minutes. To filter out factors >3 kDa, the conditioned media were transferred to a 3 kDa filter (Millipore) and centrifuged at 15,000 rpm in 30-minute increments until all the conditioned media had passed through the filter. To expose the conditioned media to freeze-thaw cycles, the tubes containing the conditioned media were thawed for 30 minutes in a 60°C water bath, and then frozen at -80°C for 30 minutes. This was repeated two more times for a total of three freeze-thaw cycles.
Colony formation assays: Cells were seeded in 6 well plates at 200-400 cells per well in 2 mL of growth media and incubated overnight at 37°C, 5% CO2. The next day, the growth media was aspirated and fresh media containing the indicated compounds were added to the cells. Doxycycline was used at 1 µg/mL for all assays. For each assay, cells were incubated in the indicated conditions for 10 days, with the media and doxycycline changed every three days. After 10 days, the media was aspirated, the wells were washed once with PBS, and the cells were fixed in 100% methanol for 10 minutes. Next, the methanol was removed, and the cells were stained with 0.4% crystal violet for 10 minutes. Finally, the crystal violet was removed, the plates were washed under running water and dried on the benchtop overnight. The next day, images were taken of the plates with a Chemidoc BioRad imager and quantified using the ColonyArea plugin in ImageJ, as described previously74.

Proliferation assays: Cells were pre-treated for 3 days with 1 µg/mL doxycycline before seeding in 96 well plates at 1,000 cells/well in 80 µL of media and incubated overnight at 37°C, 5% CO2. The next day, 150 µL of the indicated treatment media was added to the appropriate wells, and the cells were incubated for 6-7 more days, with a media change on day 3. Cell proliferation was determined by live cell imaging for the duration of the assay, or using CyQUANT (Invitrogen) at endpoint according to the manufacturer’s instructions, and detecting fluorescence on a SpectraMax M3 Microplate reader (Molecular Devices).

Glycolytic rate assay: PaTu-8902 iDox-shGOT2.1 cells that had been cultured in 1 µg/mL doxycycline for 3 days were seeded at 2x10^4 cells/well in 80 µL of normal growth media in an Agilent XF96 V3 PS Cell Culture Microplate (Agilent). To achieve an even distribution of cells within wells, plates were incubated on the bench top at room temperature for 1 hour before incubating at 37°C, 5% CO2 overnight. To hydrate the XF96 FluxPak (Agilent), 200 µL/well of sterile water was added and the entire cartridge was incubated at 37°C, CO2-free incubator overnight. The following day, one hour prior to running the assay, 60 µL of media was removed, and the cells were washed twice with 200 µL/well of assay medium (XF DMEM Base Medium, pH 7.4 containing 25 mM Glucose and 4 mM Glutamine; Agilent). After washing, 160 µL/well of assay medium was added to the cell culture plate for a final volume of 180 µL/well. Cells were then incubated at 37°C, in a CO2-free incubator until analysis. In parallel, one hour prior to the assay, water from the FluxPak hydration was exchanged for 200 µL/well of XF Calibrant 670 (Agilent) and the cartridge was returned to 37°C, CO2-free incubator until analysis. Rotenone/Antimycin (50 µM, Agilent) and 2DG (500 mM, Agilent) were re-constituted in assay medium to make the indicated stock concentrations. 20 µL of rotenone/antimycin was loaded into Port A for each well of the FluxPak and 22 µL of 2DG into Port B, for a final concentration of 0.5 µM and 50 mM, respectively. The Glycolytic Rate Assay was conducted on an XF96 Extracellular Flux Analyzer (Agilent) and PER was calculated using Wave 2.6 software (Agilent). Following the assay, PER was normalized to cell number with the CyQUANT NF Cell Proliferation Assay (Invitrogen) according to manufacturer’s instructions.

Protein lysates: Cell lines cultured in 6 well plates in vitro were washed with ice-cold PBS on ice and incubated in 250 µL of RIPA buffer (Sigma) containing protease (Roche) and phosphatase (Sigma) inhibitors on ice for 10 minutes. Next, cells were scraped with a pipet tip, and the resulting lysate was transferred to a 1.5 mL tube also on ice. The lysate was centrifuged at 15,000 rpm for 10 minutes at 4°C. After, the supernatant was transferred to a fresh 1.5 mL tube and stored at -80°C.
In vivo tumor tissue was placed in a 1.5 mL tube containing a metal ball and 300 µL RIPA buffer with protease and phosphatase inhibitors. The tissue was homogenized using a tissue lyser machine. Then, the resulting lysate was centrifuged at 15,000 rpm for 10 minutes at 4°C. After, the supernatant was transferred to a fresh 1.5 mL tube and stored at -80°C.

Western blotting: Protein levels were determined using a BCA assay (Thermo Fisher), according to manufacturer's instructions. Following quantification, the necessary volume of lysate containing 30 µg of protein was added to a mixture of loading dye (Invitrogen) and reducing agent (Invitrogen) and incubated at 90°C for 5 minutes. Next, the lysate was separated on a 4-12% Bis-Tris gradient gel (Invitrogen) along with a protein ladder (Invitrogen) at 150 V until the dye reached the bottom of the gel (about 90 minutes). Then, the protein was transferred to a methanol-activated PVDF membrane (Millipore) at 25 V for 1 hour. After that, the membrane was blocked in 5% blocking reagent (Biorad) dissolved in TBS-T on a plate rocked for >1 hour. The membrane was then incubated overnight at 4°C rocking in the indicated primary antibody diluted in blocking buffer. The next day, the primary antibody was removed, and the membrane was washed 3 times in TBS-T rocking for 5 minutes. Then, the membrane was incubated for 1 hour rocking at room temperature in the appropriate secondary antibody diluted in TBS-T. Finally, the membrane was washed as before, and incubated in Clarity ECL reagent (Biorad) according to manufacturer's instructions before imaging on a Biorad Chemidoc.

The following primary antibodies were used in this study: GOT2 (Atlas, HPA018139), GOT1 (Abcam, ab171939), GLUD1 (Abcam, ab166618), IDH1 (Cell Signaling, 3997S), MCT1 (Abcam, ab85021), MCT4 (Sigma, AB3316P), anti-Flag (Sigma, F3165), Vinculin (Cell Signaling, 13901S), LDHA (Cell Signaling, 3582), LDHB (Abcam, ab53292), and the anti-rabbit-HRP secondary antibody (Cell Signaling, 7074S).

Isolating polar metabolites: For intracellular metabolome analyses, cells were seeded at 10,000 cells in 2 mL of growth media per well of a 6 well plate and incubated overnight. The next day, the growth media was removed, and cells were incubated in media containing the indicated compounds for 6 days, with the media being changed every 3 days. On day 6, the media was removed, and the cells were fixed and metabolites extracted into 1 mL/well of ice-cold 80% methanol on dry ice for 10 minutes. Following the incubation, the wells were scraped with a pipet tip and transferred to a 1.5 mL tube on dry ice.

To analyze extracellular metabolomes, 0.8 mL of ice-cold 100% methanol was added to 0.2 mL of media, mixed well, and incubated on dry ice for 10 minutes.

Mouse serum and tumor interstitial fluid (TIF) were isolated and analyzed as described previously31. The tubes were then centrifuged at 15,000 rpm for 10 minutes at 4°C to pellet insoluble material, and the resulting metabolite supernatant was transferred to a fresh 1.5 mL tube. The metabolites were then dried on a SpeedVac until the methanol/water had evaporated, and the resulting pellet was re-suspended in a 50:50 mixture of methanol and water.

Snapshot metabolomics: Samples were run on an Agilent 1290 Infinity II LC -6470 Triple Quadrupole (QqQ) tandem mass spectrometer (MS/MS) system with the following parameters: Agilent Technologies Triple Quad 6470 LC-MS/MS system consists of the 1290 Infinity II LC Flexible Pump (Quaternary Pump), the 1290 Infinity II Multisampler, the 1290 Infinity II Multicolumn Thermostat with 6 port valve and the 6470 triple quad mass spectrometer. Agilent
Masshunter Workstation Software LC/MS Data Acquisition for 6400 Series Triple Quadrupole MS with Version B.08.02 is used for compound optimization, calibration, and data acquisition.

Solvent A is 97% water and 3% methanol 15 mM acetic acid and 10 mM tributylamine at pH of 5. Solvent C is 15 mM acetic acid and 10 mM tributylamine in methanol. Washing Solvent D is acetonitrile. LC system seal washing solvent 90% water and 10% isopropanol, needle wash solvent 75% methanol, 25% water. GC-grade Tributylamine 99% (ACROS ORGANICS), LC/MS grade acetic acid Optima (Fisher Chemical), InfinityLab Deactivator additive, ESI–L Low concentration Tuning mix (Agilent Technologies), LC-MS grade solvents of water, and acetonitrile, methanol (Millipore), isopropanol (Fisher Chemical).

An Agilent ZORBAX RRHD Extend-C18, 2.1 × 150 mm and a 1.8 um and ZORBAX Extend Fast Guards for UHPLC are used in the separation. LC gradient profile is: at 0.25 ml/min, 0-2.5 min, 100% A; 7.5 min, 80% A and 20% C; 13 min 55% A and 45% C; 20 min, 1% A and 99% C; 24 min, 1% A and 99% C; 24.05 min, 1% A and 99% D; at 0.8 ml/min, 27.5-31.35 min, 1% A and 99% D; at 0.6 ml/min, 31.50 min, 1% A and 99% D; at 0.4 ml/min, 32.25-39.9 min, 100% A; at 0.25 ml/min, 40 min, 100% A. Column temp is kept at 35 ºC, samples are at 4 ºC, injection volume is 2 µl.

6470 Triple Quad MS is calibrated with the Agilent ESI-L Low concentration Tuning mix. Source parameters: Gas temp 150 ºC, Gas flow 10 l/min, Nebulizer 45 psi, Sheath gas temp 325 ºC, Sheath gas flow 12 l/min, Capillary -2000 V, Delta EMV -200 V. Dynamic MRM scan type is used with 0.07 min peak width, acquisition time is 24 min. dMRM transitions and other parameters for each compounds are list in a separate sheets. Delta retention time of plus and minus 1 min, fragmentor of 40 eV and cell accelerator of 5 eV are incorporated in the method.

The MassHunter Metabolomics Dynamic MRM Database and Method was used for target identification. The QqQ data were pre-processed with Agilent MassHunter Workstation QqQ Quantitative Analysis Software (B0700). Each metabolite abundance level in each sample was divided by the median of all abundance levels across all samples for proper comparisons, statistical analyses, and visualizations among metabolites. Metabolites with values >1 are higher in the experimental conditions and metabolites with values <1 are lower in the experimental condition. The statistical significance test was done by a two-tailed t-test with a significance threshold level of 0.05.

Heatmaps were generated and data clustered using Morpheus Matrix Visualization and analysis tool (https://software.broadinstitute.org/morpheus).

Pathway analyses were conducted using MetaboAnalyst (https://www.metaboanalyst.ca).

U13C-Glucose, U13C-Pyruvate isotope tracing: For glucose tracing, CAFs were seeded in 6 well plates at 2x10^5 cells/well and incubated for 72 hours in growth media containing U13C-Glucose (Cambridge Isotope Laboratories).

For pyruvate tracing, PaTu-8902 and MIAPaCa-2 iDox-shGOT2.1 cells were cultured in media containing 1 mM unlabelled glucose and 1 mM U13C-Pyruvate (Cambridge Isotope Laboratories) for 16 hours.

Polar metabolites were extracted from the media and cells according to the method described above. Isotope tracing experiments utilized the same chromatography as described in the
Snapshot Metabolomics section, and were conducted on two instruments with the following parameters:

Agilent Technologies Q-TOF 6530 LC/MS system consists of a 1290 Infinity II LC Flexible Pump (Quaternary Pump), 1290 Infinity II Multisampler, 1290 Infinity II Multicolumn Thermostat with 6 port valve and a 6530 Q-TOF mass spectrometer with a dual Assisted Jet Stream (AJJ) ESI source. Agilent MassHunter Workstation Software LC/MS Data Acquisition for 6200 series TOF/6500 series Q-TOF Version B.09.00 Build 9.0.9044.a SP1 is used for calibration and data acquisition.

Agilent 6530 Q-TOF MS is calibrated with ESI-L Low Concentration Tuning mix. Source parameters: Gas temp 250 °C, Gas flow 13 l/min, Nebulizer 35 psi, Sheath gas temp 325 °C, Sheath gas flow 12 l/min, Vcap 3500 V, Nozzle Voltage (V) 1500, Fragmentor 140, Skimmer1 65, OctopoleRFPeak 750. The MS acquisition mode is set in MS1 with mass range between 50-1200 da with collision energy of zero. The scan rate (spectra/sec) is set at 1 Hz. The LC-MS acquisition time is 18 min and total run time is 30 min. Reference masses are enabled with reference masses in negative mode of 112.9856 and 1033.9881 da.

Agilent Technologies 6545B Accurate-Mass Quadrupole Time of Flight (MS Q-TOF) LC/MS coupled with an Agilent 1290 Infinity II UHPLC. Agilent Masshunter Workstation Software LC/MS Data Acquisition for 6500 Series QTOF MS with Version 09.00, Build9.0.9044.0 was used for tuning, calibration, and data acquisition.

In negative mode, the UHPLC was configured with 1290 Infinity II LC Flexible Pump (Quaternary Pump),1290 Infinity II Multisampler, 1290 Infinity II Multicolumn Thermostat with 6 port valves. In negative scan mode, the Agilent G6545B Q-TOF MS with Dual AJD ESI Sources in centroid mode was configured with following parameters: Acquisition range: 50-1200 da at scan rate of 1 spectra/sec, Gas temp 250 °C, Gas Flow 13 L/min, Nebulizer at 40 psi, Sheath Gas Heater 325 °C, Sheath Gas Flow 12L/min, Capillary 3500 V, Nozzle Voltage 1000 V, Fragmentor 130 V, Skimmer1 60 V, Octopole RFPeak 750V, Collision 0 V, Auto Recalibration limit of detection 150 ppm with min height 1000 counts, Reference ions of two at 59.0139 and 980.0164 da.

Data processing was performed in Agilent MassHunter Workstation Profinder 10.0 Build 10.0.10062.0. Isotopologue distributions were derived from a compound standard library built in Agilent MassHunter PCDL (Personal Compound and Database Library) v7.0.

Xenograft studies: Animal experiments were conducted in accordance with the Office of Laboratory Animal Welfare and approved by the Institutional Animal Care and Use Committees (IACUC) of the University of Michigan. NOD scid gamma (NSG) mice (Jackson Laboratory) 6-10 weeks old of both sexes were maintained in the facilities of the Unit for Laboratory Animal Medicine (ULAM) under specific pathogen-free conditions.

Cells expressing doxycycline-inducible shNT or doxycycline-inducible shGOT2 were injected subcutaneously into both the left and right flanks of male and female NSG mice, with 1-4x10^6 cells in a mixture of 50 µL media and 50 µL Matrigel (Corning) per injection. Tumors were established for 7 days before mice were fed either normal chow or chow containing doxycycline (BioServ). Tumors were measured with calipers two times per week, and mice were euthanized once the tumors reached a diameter of 2 cm^3. Subcutaneous tumor volume (V) was calculated as V=1/2(length x width^2). At endpoint, the tumors were removed, and fragments were either
Cells expressing doxycycline-inducible shGOT2 and luciferase were injected into the pancreas tail of NSG mice, with 200,000 cells in a mixture of 50 µL media and 50 µL Matrigel (Corning) per injection. Tumors were established for 7 days before mice were fed either normal chow or chow containing doxycycline (BioServ). Tumor progression was monitored by weekly intraperitoneal injections of luciferin (Promega) and bioluminescence imaging (BLI) on an IVIS SpectrumCT (Perkin Elmer). BLI was analyzed with Living Image software (PerkinElmer). At endpoint, the tumors were removed, and fragments were either snap frozen in liquid nitrogen and stored at -80°C or fixed in ZFix (Anatech) solution for histology.

AZD3965 or FX11 was dissolved in DMSO and stored at -80 ºC in aliquots. Each day, one aliquot was thaw and mixed with a 0.5% Hypromellose (Sigma), 0.2% tween80 (Sigma) solution such that the final DMSO concentration was 5%. Vehicle or AZD3965 was administered at 100 mg/kg by daily oral gavage. FX11 was administered at 2 mg/kg by daily intraperitoneal injection.

**Histology:** Tissues were processed using a Leica ASP300S tissue processor (Leica Microsystems). Paraffin-embedded tissues were sectioned at 4 µm and stained for specific target proteins using the Discovery Ultra XT autostainer (Ventana Medical Systems), with listed antibodies, and counterstained with Mayer's hematoxylin (Sigma). Hematoxylin and eosin (H&E) staining was performed using Mayer's hematoxylin solution and Eosin Y (Thermo Fisher). IHC slides were then scanned on a Pannoramic SCAN scanner (Perkin Elmer). Scanned images were quantified using algorithms provided from Halo software version 2.0 (Indica Labs). The following antibodies were used for IHC: Ki67 (1:1,000; Abcam, ab15580), αSMA (1:20,000; Abcam, ab5694), Got2 (1:500; Atlas, HPA018139).

**Histology:** Tissues were processed using a Leica ASP300S tissue processor (Leica Microsystems). Paraffin-embedded tissues were sectioned at 4 µm and stained for specific target proteins using the Discovery Ultra XT autostainer (Ventana Medical Systems), with listed antibodies, and counterstained with Mayer's hematoxylin (Sigma). Hematoxylin and eosin (H&E) staining was performed using Mayer's hematoxylin solution and Eosin Y (Thermo Fisher). IHC slides were then scanned on a Pannoramic SCAN scanner (Perkin Elmer). Scanned images were quantified using algorithms provided from Halo software version 2.0 (Indica Labs). The following antibodies were used for IHC: Ki67 (1:1,000; Abcam, ab15580), αSMA (1:20,000; Abcam, ab5694), Got2 (1:500; Atlas, HPA018139).

**Statistics:** Statistics were performed using Graph Pad Prism 8. Groups of 2 were analyzed with two-tailed students t test, groups greater than 2 were compared using one-way ANOVA analysis with Tukey post hoc test or two-way ANOVA with Dunnett’s correction for multiple independent variables. All error bars represent mean with standard deviation, all group numbers and explanation of significant values are presented within the figure legends. Experiments were repeated at least twice to verify results.

**Data availability:** The mass spectrometry-based metabolomics data are available in Supplementary Table 1. Annotated raw blots are included for all westerns as Source Data Files. All schematics and models were created using Biorender.com.
REFERENCES


https://www.nature.com/articles/nature12040#supplementary-information (2013).


Borst, P. The malate—aspartate shuttle (Borst cycle): How it started and developed into a major metabolic pathway. IUBMB Life 72, 2241-2259, doi:https://doi.org/10.1002/iub.2367 (2020).


**FIGURE LEGENDS**

**Figure 1: GOT2 KD impairs in vitro PDA proliferation.** A) The metabolic roles of mitochondrial glutamate-oxaloacetate transaminase 2 (GOT2). OAA=oxaloacetate, Glu=glutamate, Mal=malate, Asp=Aspartate, αKG=α-ketoglutarate, ME1=malic enzyme 1, TCA=tricarboxylic acid. B) Immunoblot of GOT2 and VINCULIN loading control in PaTu-8902 cells after 1 µg/mL doxycycline (DOX) induction of two independent GOT2 (sh1, sh2) and non-targeting (NT) shRNAs for 3 days. C) Representative images from colony formation assays in ishRNA PaTu-8902 cells -Dox (n=3) or +Dox (n=3). D) Heatmap summarizing the relative colony formation ishRNA PDA cell lines -Dox (n=3) or +Dox (n=3), normalized to -Dox for each indicated shRNA. Representative images from colony formation assays and western blots presented in Figure 1-figure supplement 1. E) Relative abundances of Asp and αKG in PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3). F) Relative proliferation of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or supplemented with 20 mM Asp and 1 mM dimethyl-αKG (dmαKG). For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 1-source data 1.** Full western blot images for Figure 1B.

**Figure 1-figure supplement 1.** A) Immunoblots of GOT2 and VINCULIN loading control in PDA cell lines after 1 µg/mL doxycycline (Dox) induction of the indicated shRNAs (ishRNA) for 3 days. NT=non-targeting shRNA. Westerns for data in Figure 1D. B) Immunoblot of GOT1, GOT2, and VINCULIN in PaTu-8902 ishRNA -Dox or +Dox after 3 days. C) Representative images from colony formation assays in PDA ishRNA cell lines -Dox (n=3) or +Dox (n=3) for the data in Figure 1D. D) Relative proliferation of PDA ishRNA cell lines -Dox (n=3) or +Dox (n=3), normalized to Day 0 cell number for each condition. E) Immunoblots of GOT2 and VINCULIN in human cancer associated fibroblast cell line (hPSC) and human pancreatic nestin expressing cells (HPNE) ishRNA cells. F) Relative proliferation of hPSC (left) and HPNE (right) ishRNA -Dox (n=3) or +Dox (n=3). For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 1-figure supplement 1-source data 1.** Full western blot images for Figure 1-figure supplement 1B,E.

**Figure 1-figure supplement 2.** A) Heatmaps of the relative abundances of metabolites significantly (p<0.05) different between -Dox (n=3) and +Dox (n=3) PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 cells. B) Metabolic pathway analysis of the data presented in A). C) Ion abundances of aspartate (Asp) in normal media or media with 10 mM Asp (left) and intracellular abundances in PaTu-8902 and MIAPaCa-2 cultured in normal media or media with 10 mM Asp (right). D) Relative abundances of TCA cycle metabolites in PaTu-8902 (top) or MIAPaCa-2 (bottom) ishGOT2.1 -Dox (n=3) or +Dox (n=3). For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 2: GOT2 KD induces reductive stress, which can be ameliorated by NADH turnover.** A) Schematic of glycolytic signature induced by GOT2 KD-mediated NADH build-up and reductive stress. G6P=glucose-6-phosphate, F6P=fructose-6-phosphate, FBP=fructose-1,6-bisphosphate, DHAP=dihydroxyacetone phosphate, GA3P=glyceraldehyde-3-phosphate, PEP=phosphoenol pyruvate, oxPPP=oxidative pentose phosphate pathway, Non-oxPPP=non-oxidative pentose phosphate pathway, X5P=xylulose-5-phosphate, R5P=ribose-5-phosphate, S7P=sedoheptulose-7-phosphate, HBP=hexosamine biosynthesis pathway, GlcNAc-1P=N-
acetylglucosamine 1-phosphate. B) Relative fold changes in the indicated metabolites between PaTu-8902 ishGOT2.1 -Dox (n=3) and +Dox (n=3). 2PG=2-phosphoglycerate, Pyr=Pyruvate, Lac=Lactate. C) Relative NADH/NAD+ ratio in PaTu-8902 ishGOT2.1 -Dox (n=3) and +Dox (n=3). D) Relative colony formation of MIAPaCa-2 ishRNA -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or DMEM with 1 mM pyruvate, normalized to -Dox for each condition. E) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or DMEM with the indicated concentrations of pyruvate (mM), normalized to -Dox for each condition. F) Relative proliferation of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing doxycycline-inducible empty vector (EV), cytosolic Lactobacillus NADH oxidase (LbNOX), or mitochondrial LbNOX (mLbNOX), normalized to Day 0 for each condition. G) Relative NADH/NAD+ ratio of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing EV, LbNOX, or mLbNOX. H) Schematic of 13C3-pyruvate into relevant metabolic pathways. 13C-carbon labels in blue, non-labeled carbon in gray. I) Fractional labelling of intracellular pyruvate or lactate in PaTu-8902 and MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in 1 mM 13C3-Pyruvate and treated with DMSO vehicle control or 5 µM UK5099 (MPC inhibitor). Unlabeled controls presented at right. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 2-figure supplement 1. A) Glycolytic rate assay showing the proton efflux rate (PER) of PaTu-8902 ishGOT2.1 -Dox (n=4) or +Dox (n=4). B-C) Relative proliferation B) and ATP levels C) of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or DMEM with 1 mM pyruvate (Pyr). D) Immunoblots of GOT2 and VINCULIN loading control in PaTu-8902 (top) and MIAPaCa-2 (bottom) parental (P), empty vector (EV), or two sgRNAs targeting GOT2 (sg1, sg2). E) Relative colony formation of PaTu-8902 (left) and MIAPaCa-2 (right) P, EV, sgGOT2.1, or sgGOT2.2 cultured in normal DMEM (-Pyr, n=3) or DMEM with 1 mM pyruvate (+Pyr, n=3), normalized to +Pyr for each condition. F) Relative colony formation of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing EV, LbNOX, or mLbNOX. G) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or DMEM with 1 mM pyruvate, αKB, or nicotinamide mononucleotide (NMN), normalized to -Dox for each condition. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 2-figure supplement 1-source data 1. Full western blot images for Figure 2-figure supplement 1D.

Figure 2-figure supplement 2. A) Immunoblots of GOT2, FLAG, and VINCULIN loading controls from PaTu-8902 (left) or MIAPaCa-2 (right) ishNT or GOT2.1 cells expressing doxycycline-inducible expression of empty vector (EV) or FLAG-tagged cytosolic Lactobacillus NADH oxidase (LbNOX) or mitochondrial LbNOX (mLbNOX). B) Relative colony formation of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing EV, LbNOX, or mLbNOX, normalized to -Dox for each condition. C) Relative proliferation of PaTu-8902 (top) and MIAPaCa-2 (bottom) ishNT -Dox (n=3) or +Dox (n=3) expressing EV, LbNOX, or mLbNOX.
mLbNOX and treated with DMSO vehicle control (left) or 1 µM piericidin (right), normalized to Day 0 for each condition. D) Ion abundances of NAD+ (left) and NADH (right) in PaTu-8902 (top) and MIAPaCa-2 (bottom) ishGOT2.1 -Dox (n=3) and +Dox (n=3). E) Relative extracellular pyruvate/lactate ratios in PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) and +Dox (n=3). F) Ion abundances of extracellular pyruvate (left) and lactate (right) in PaTu-8902 (top) and MIAPaCa-2 (bottom) ishGOT2.1 -Dox (n=3) and +Dox (n=3). G) Heatmap of log2 fold-changes in metabolite abundances between PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) and +Dox (n=3) expressing EV, LbNOX, or mLbNOX. G6P=glucose-6-phosphate, F6P=fructose-6-phosphate, FBP=fructose-1,6-bisphosphate, DHAP=dihydroxyacetone phosphate, 2PG=2-phosphoglycerate, PEP=phosphoenol pyruvate, X5P=xylulose-5-phosphate, R5P=ribose-5-phosphate, S7P=sedoheptulose-7-phosphate, αKG=α-ketoglutarate, PPP=pentose phosphate pathway. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 2-figure supplement 2-source data 1. Full western blot images for Figure 2-figure supplement 2A.

Figure 2-figure supplement 3. A) Relative proliferation of PaTu-8902 (left) and MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) cultured in 1 mM Glucose DMEM with 1 mM pyruvate (Pyr) normalized to Day 0 for each condition. B-D) Fractional labelling of intracellular citrate B), aspartate C), or alanine D) from 13C3-Pyruvate (1 mM) in PaTu-8902 and MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) treated with DMSO vehicle control or 5 µM UK5099 (MPC inhibitor). Unlabeled controls presented at right. E) Relative cell number of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or DMEM with 1 mM Pyr, treated with DMSO vehicle control or 5 µM UK5099, normalized to DMSO for each condition. F) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or DMEM with 1 mM Pyr or lactate (Lac), normalized to -Dox for each condition. G) Relative colony formation of PaTu-8902 ishGOT2.1 -Dox (n=3) or MIAPaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or DMEM with 1 mM alanine, normalized to -Dox for each condition. H) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or DMEM with 1 mM Pyr or 100 µM of the indicated combinations of adenine (A), guanine (G), thymidine (T), and cytidine (C), normalized to -Dox for each condition. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3: GOT2 is not required for in vivo growth of PDA xenografts. A) Tumor volumes of PaTu-8902 ishRNA flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6). Arrows indicate administration of Dox chow 1 week after PDA cell injection. B) Tumor volumes of four additional PDA cell line ishGOT2.1 flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6). Arrows indicate administration of Dox chow 1 week after PDA cell injection. C) Immunohistochemistry (IHC) for Ki67 in flank xenograft tissue from PaTu-8902 ishRNA -Dox (n=6) or +Dox (n=6). D) Quantification of Ki67+ cells in tissue depicted in C). E) Relative abundances of Asp and αKG in PaTu-8902 ishGOT2.1 -Dox (n=6) or +Dox (n=6) flank xenografts. F) Relative fold changes in the indicated metabolites between PaTu-8902 ishGOT2.1 -Dox (n=6) and +Dox (n=6) flank xenografts. G6P=glucose-6-phosphate, F6P=fructose-6-phosphate, FBP=fructose-1,6-bisphosphate, DHAP=dihydroxyacetone phosphate, GA3P=glyceraldehyde-3-phosphate, 2PG=2-phosphoglycerate, PEP=phosphoenol pyruvate, Pyr=Pyruvate, Lac=Lactate, X5P=xylulose-5-
phosphate, R5P=ribose-5-phosphate, S7P=sedoheptulose-7-phosphate, PPP=pentose phosphate pathway. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 3-figure supplement 1.** A) Tumor volumes of PDA cell line ishNT flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6). Arrows indicate administration of Dox chow 1 week after PDA cell injection. B) Immunoblots for GOT2 and VINCULIN loading control in PDA ishRNA -Dox or +Dox xenografts at endpoint. Each lane represents an independent xenograft. C) Bioluminescence of MIAPaCa-2 GFP-Luc ishGOT2.1 -Dox (n=3) or +Dox (n=3) orthotopic xenografts. D) Heatmaps of the relative abundances of metabolites significantly (p<0.05) different between PaTu-8902 ishGOT2.1 -Dox (n=6) and +Dox (n=6) flank xenografts. E) Metabolic pathway analysis of the data presented in D). F) Relative abundances of TCA cycle metabolites in PaTu-8902 ishGOT2.1 -Dox (n=6) or +Dox (n=6) flank xenografts. G) Immunoblots for GOT2, GOT1, GLUD1, IDH1, and VINCULIN in PaTu-8902 ishGOT2.1 -Dox or +Dox flank xenografts at endpoint. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 3-figure supplement 1-source data 1.** Full western blot images for Figure 3-figure supplement 1B,G.

**Figure 4: GOT2 deletion does not impact on PDA tumorigenesis in an autochthonous model.** A) Got2 deletion (floxed exon 2) with expression of mutant Kras (LSL-KrasG12D) driven by epithelial pancreas-specific Cre recombinase (p48-Cre) on an immunocompetent (C57B/6) background (KC-Got2). B) Representative immunohistochemistry (IHC) for Got2 in pancreata from 3-month old KC-Got2 or age-matched KC historic controls. C) Pancreas weights of 3-month KC (n=4) or KC-Got2 (n=6) mice. D) Representative H&E staining of pancreata from 3-month KC (n=4) or KC-Got2 (n=6) mice. E) Quantitation of H&E staining from C) of tissue area with healthy acinar cells, acinar-ductal metaplasia (ADM), or pancreatic intraepithelial (PanIN) lesion severity. F) Representative H&E staining of pancreata from 6-month KC (n=5) or KC-Got2 (n=4) mice. G) Pancreas weights of 6-month KC (n=4) or KC-Got2 (n=4) mice. H) Quantitation of H&E staining from F) of tissue area with healthy acinar cells, ADM, or PanIN lesion severity. I) Representative H&E staining of pancreata from 1-year KC (n=3) or KC-Got2 (n=7) mice. J) Number of KC or KC-Got2 tissue that had progressed to carcinoma at 1 year. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 4-figure supplement 1.** A) DNA gel showing representative genotyping of DNA isolated from tails of Got2f/f mice or wild type (WT) control. B) Representative H&E staining of pancreata from 3-month WT (Got2 f/f, n=2) or p48-Got2 f/f (n=2) mice. C) Pancreas weights of 3-month Got2 f/f (n=2) or p48-Got2 f/f (n=2) mice. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 5: Pancreatic CAFs release pyruvate and compensate for loss of GOT2 in vitro.** A) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or human CAF (hCAF) CM generated after 72 hours of conditioning, normalized to -Dox for each condition. B) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in DMEM or indicated dilutions of hCAF CM, normalized to -Dox for each condition. C) Relative colony formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in DMEM. Mock-treated hCAF CM, boiled, 3 kDa filtered, or that subjected to freeze/thaw cycles. Data normalized to -Dox for each condition. D) Ranked log2 fold changes
quantitation of pyruvate concentrations (mM) in three independently generated batches of hCAF
of metabolite abundances in hCAF CM compared to pyruvate-free normal DMEM. E) Absolute
or mouse CAF (mCAF) CM, including a pyruvate-free DMEM control. F) Relative colony
formation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM)
or mCAF CM, normalized to -Dox for each condition. G) Ion abundance of extracellular (EX) or
intracellular (IN) pyruvate (left) or lactate (right) isotopologues from hCAFs cultured with 25 mM
13C6-Glucose. H) Fractional labelling of intracellular glycolysis (left), TCA cycle (middle), and
pentose phosphate pathway (PPP, right) metabolites in hCAFs cultured with 25 mM 13C6-
Glucose. F6P=fructose-6-phosphate, FBP=fructose-1,6-bisphosphate, DHAP=dihydroxyacetone
phosphate, G3P=glyceraldehyde-3-phosphate, 2PG=2-phosphoglycerate, 3PG=3-
phosphoglycerate, PEP=phosphoenol pyruvate, Cit=citrate, dKG=α-ketoglutarate,
Suc=succinate, Fum=fumarate, Mal=malate, Gln=glutamine, Asp=aspartate,
S7P=seduhoptulose-7-phosphate, R5P=ribose-5-phosphate. For all panels, data represent
mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 5-figure supplement 1.** A) Immunohistochemistry (IHC) for α-smooth muscle actin
(αSMA) in PaTu-8902 ishGOT2.1 -Dox or +Dox flank xenografts. B) Relative colony formation
of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or human
CAF (hCAF) CM, normalized to -Dox for each condition. C) Relative colony formation of PaTu-
8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in DMEM, hCAF CM, PDA CM, or tumor-
eduicated macrophage (TEM) CM, normalized to -Dox for each condition. D) Relative colony
formation of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in DMEM, hCAF CM, or
hCAF CM boiled, 3 kDa filtered, or subjected to freeze/thaw cycles, normalized to -Dox for each
condition. E) Heatmap of the relative abundances of metabolites significantly (p<0.05) different
between normal pyruvate-free DMEM and three independently generated batches of hCAF CM.
Black arrows highlight Asp and dKG. F) Ion abundances of intracellular pyruvate in MIAPaCa-2
(left) or PaTu-8902 (right) cultured in normal DMEM media or hCAF CM. G) Relative colony
formation of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM)
or mCAF CM, normalized to -Dox for each condition. For all panels, data represent mean ± SD.
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 5-figure supplement 2.** A) Absolute quantitation of aspartate (left) and α-ketoglutarate
concentrations (µM) in hCAF or mCAF CM, relative to DMEM control. B) Relative proliferation of
MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM, 250 µM pyruvate,
or a combination of 50 µM aspartate (Asp) and 500 µM dimethyl-α-ketoglutarate (dmαKG). C) Relative cell number of PaTu-8902 treated with the indicated concentrations of rotenone (left),
phenformin (middle), or IACS-010759 (right), and cultured in normal DMEM, hCAF CM, or 1 mM
pyruvate. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 6: MCT1 inhibition prevents pyruvate-mediated restoration of redox balance in
vitro after loss of GOT2.** A) Absolute quantitation of pyruvate (left) and lactate (middle), and
the relative pyruvate/lactate ratio (right) in normal DMEM, hCAF CM, and mCAF CM. B) Absolute quantitation of pyruvate (left) and lactate (middle), and the relative pyruvate/lactate ratio (right) in serum or the tumor interstitial fluid (TIF) from NSG mice harboring PaTu-8902
ishGOT2.1 -Dox (n=8 tumors, 4 mice) or +Dox (n=8 tumors, 4 mice) flank xenografts. C) Relative proliferation of MIAPaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured with the
absolute levels and relative ratios of pyruvate/lactate in hCAF and mCAF CM (left) or serum/TIF.
(right) from A,B), normalized to Day 0 for each condition. D) Relative colony formation of MIA PaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM), 1 mM pyruvate, or human CAF (hCAF) CM, and treated with DMSO control or 100 nM AZD3965, normalized to -Dox for each condition. E) Immunobots of GOT2, MCT1, and VINCULIN loading control in PaTu-8902 ishGOT2.1 expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2). F) Relative colony formation of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2) and cultured in the indicated doses of pyruvate, normalized to -Dox for each condition. G) Ion counts of intracellular m+3 pyruvate (top) and lactate (bottom) in PaTu-8902 expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2) and cultured in 1 mM 13C3-Pyruvate for the indicated time points. H) Relative proliferation of PaTu-8902 (top) and MIA PaCa-2 (bottom) ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or 0.25 mM pyruvate, and treated with DMSO vehicle control or 25 µM FX11, normalized to Day 0 for each condition. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 6-source data 1. Full western blot images for Figure 6E.

Figure 6-figure supplement 1. A) Relative CCLE mRNA expression of the indicated SLC16 family members in PaTu-8902 and MIA PaCa-2. B) Immunoblot for MCT1 and VINCULIN loading control the indicated cell lines. C) Single-cell RNA sequencing data from murine KPC cell line syngeneic orthotopic tumors showing expression of Slc16a1 (MCT1) and Slc16a3 (MCT4) in CAF (marked by CDH11 expression) and epithelial (marked by Krt18 expression) populations. D,F) Immunobots for GOT2, MCT1, and VINCULIN in PaTu-8902 D) and MIA PaCa-2 F) ishGOT2.1 -Dox or +Dox expressing the indicated control (CTR) or constitutive MCT1-targeting shRNAs (sh1, sh2). E,G) Relative proliferation of PaTu-8902 E) and MIA PaCa-2 G) ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing the indicated shRNAs and cultured in normal DMEM (left), 0.25 mM pyruvate (middle), or hCAF CM (right), normalized to Day 0 for each condition. H) Relative colony formation of MIA PaCa-2 ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal media (DMEM) or the indicated doses of pyruvate, and treated with DMSO control or 100 nM AZD3965, normalized to -Dox for each condition. I) Relative viability of MIA PaCa-2 cultured in normal media (DMEM), 0.25 mM pyruvate (left), or hCAF CM (right) and treated with DMSO control or 100 nM IACS-010759, alone or in combination with 100 mM AZD3965, normalized to -Dox for each condition. J) Immunobots for GOT2, LDHA, LDHB, and VINCULIN in PaTu-8902 and MIA PaCa-2 ishRNA. K) Relative number of PaTu-8902 (left) and MIA PaCa-2 (right) ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM, 0.25 mM pyruvate, or hCAF CM, and treated with DMSO vehicle control or 25 µM FX11, normalized to -Dox for each condition. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 6-figure supplement 1-source data 1. Full western blot images for Figure 6E-supplement 1B,D,F,J.

Figure 6-figure supplement 2. A,B) Tumor volume (left) and weight (right) of PaTu-8902 A) and tumor volume of MIA PaCa-2 B) ishGOT2.1 flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6) expressing control (shNT) or shMCT1.1. C,D) Immunobots for GOT2, MCT1, and VINCULIN in representative tumors from PaTu-8902 C) or MIA PaCa-2 D) ishGOT2.1 -Dox (n=3) or +Dox (n=3) flank xenografts with shNT or shMCT1.1. Each lane represents an individual tumor. E) Tumor volume (left) and weight (right) of PaTu-8902 flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6) expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2). F) Relative colony formation of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2) and cultured in the indicated doses of pyruvate, normalized to -Dox for each condition. G) Ion counts of intracellular m+3 pyruvate (top) and lactate (bottom) in PaTu-8902 expressing empty vector (EV), or two sgRNAs targeting MCT1 (sg1, sg2) and cultured in 1 mM 13C3-Pyruvate for the indicated time points. H) Relative proliferation of PaTu-8902 (top) and MIA PaCa-2 (bottom) ishGOT2.1 -Dox (n=3) or +Dox (n=3) cultured in normal DMEM or 0.25 mM pyruvate and treated with DMSO vehicle control or 25 µM FX11, normalized to Day 0 for each condition. For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
n=6) expressing control (sgEV) or sgMCT1.1. F) Immunoblot for GOT2, MCT1, MCT4 and VINCULIN in representative, independent tumors from PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) flank xenografts with sgEV or sgMCT1.1. G) Immunoblot for GOT2, MCT1, MCT4 and VINCULIN in PaTu-8902 ishGOT2.1 -Dox or +Dox cell lines with sgEV or sgMCT1.1. For F,G) blots, arrow=MCT1 band, asterisk=non-specific band. H) Tumor volume (left) and weight (right) of PaTu-8902 flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6) treated with PBS control or 100 mg/kg AZD3965. I) Immunoblot for GOT2, MCT1, and VINCULIN in in representative, independent tumors of PaTu-8902 ishGOT2.1 -Dox (n=3) or +Dox (n=3) flank xenografts. J) Weights of mice treated in H). For all panels, data represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Figure 6-figure supplement 2-source data 1.** Full western blot images for Figure 6-figure supplement 2C,D,F,G,I.

**Figure 6-figure supplement 3. A,B) Tumor volume (left) and weight (right) of PaTu-8902 A) and MIAPaCa-2 B) ishGOT2.1 flank xenografts in NSG mice fed normal chow (-Dox, n=6) or doxycycline chow (+Dox, n=6) and treated with PBS control or 2 mg/kg FX11. C) Relative proliferation of PaTu-8902 (top) and MIAPaCa-2 (bottom) ishRNA -Dox (n=3) or +Dox (n=3) cultured in normal DMEM, 0.25 mM pyruvate, or 2% BSA, normalized to Day 0 for each condition.

**Supplemental File 1.** Metabolomics data from Figure 1-figure supplement 2A, Figure 3-figure supplement 1D, and Figure 5-figure supplement 1E.
Figure 1-figure supplement 2

A  PaTu-8902
Dox - +
Adipic acid
Methylglutaric acid
Dimethyl Succinate
α-Ketoglutarate
Aspartate
Phosphoenolpyruvate
2-Methyl-1-butanol
Pyruvate
Acetyleuraminate
Aminoacidic acid
Arabitol
Xylitol
Inosine
Xanthosine
Glucosamine-1-Phosphate
Fructose-1,6-Bisphosphate
Inosine Monophosphate
Hypoxanthine
Glucicolic acid
Glyceraldehyde-3-Phosphate
Dihydroxyacetone Phosphate
Sedoheptulose-7-Phosphate
Xylulose-5-Phosphate
Ribose-5-Phosphate
Arabinose-5-Phosphate
Hydroxy hippuric acid
Dihydroxyphosphoglucone
Guanosine
Orotic acid
Uridine
Deoxyuridine
Fructose-6-Phosphate

MIAPaCa-2
Dox - +
Threonine
Glutamine
Ketovulic acid
Citramalic acid
N-Carbamoyl aspartate
N-Acetylglutamate
Aspartate
N-Acetylglutamate
5-Deoxy-5-(methylthio)adenosine
Nicotinamide adenine dinucleotide
N-Acetylglutamic acid
2-Phosphoglyceric acid
2,3-Dimethyl Sulfoxinic acid
3-Methylglycine
Adipic acid
α-Ketoglutarate
Phosphoenolpyruvate
5,5-Adenosyl-L-homocysteine
Glucicolic acid
Galactonic acid
cis-Aconitic acid
trans-Aconitic acid
Sedoheptulose-7-phosphate
Glycerate
Uruxine 5'-diphosphogalactose
Uruxine 5'-diphosphoglucose
2-Dehydrouridine 5'-diphosphate
Adenosine 5'-diphosphate
Inosine 5'-diphosphate
N-Acetyl-alpha-glucosamine 1-phosphate
Creatine
Uridine 5'-monophosphate
Isopentyl acetate
2-Methyl-1-butanol
Pyruvic acid
Adenosine
Inosine
O-Phosphoryl ethanolamine
Hypoxanthine
Inosine 5'-monophosphate
Fructose 1,6-bisphosphate
Guanosine

C

Media
Intracellular Aspartate

D

PaTu-8902
-DOX
+DOX
Figure 3

A. PaTu-8902

<table>
<thead>
<tr>
<th>shNT</th>
<th>sh1</th>
<th>sh2</th>
</tr>
</thead>
<tbody>
<tr>
<td>-DOX</td>
<td>-DOX</td>
<td>-DOX</td>
</tr>
<tr>
<td>+DOX</td>
<td>+DOX</td>
<td>+DOX</td>
</tr>
</tbody>
</table>

Tumor volume (mm$^3$) vs. Days post-injection

B. MIAPaCa-2, BxPC-3, Capan-1, UM53

<table>
<thead>
<tr>
<th>shNT</th>
<th>sh1</th>
<th>sh2</th>
</tr>
</thead>
<tbody>
<tr>
<td>-DOX</td>
<td>-DOX</td>
<td>-DOX</td>
</tr>
<tr>
<td>+DOX</td>
<td>+DOX</td>
<td>+DOX</td>
</tr>
</tbody>
</table>

Tumor volume (mm$^3$) vs. Days post-injection

C. PaTu-8902

D. PaTu-8902

% Ki67+ cells

<table>
<thead>
<tr>
<th>shNT</th>
<th>sh1</th>
</tr>
</thead>
<tbody>
<tr>
<td>-DOX</td>
<td>-DOX</td>
</tr>
<tr>
<td>+DOX</td>
<td>+DOX</td>
</tr>
</tbody>
</table>

E. In vivo

Relative metabolite abundance (Median normalized)

Aspartate, αKG

F. PaTu-8902

Log2FC

Glycolysis, PPP
Figure 4

(A) Schematic representation of the genetic manipulation for Got2 floxed mice with LoxP sites at Exon 1 and Exon 3. The Cre recombinase drives the deletion of the floxed allele at 3 months of age.

(B) Immunostaining of KC and KC-Got2 floxed (Got2) at 3 months of age. Got2 expression is absent in KC-Got2 floxed (Got2).

(C) H&E staining of KC and KC-Got2 floxed (Got2) at 3 months of age. No significant differences in histological features are observed.

(D) Bar graph showing the pancreas weight (g) of KC and KC-Got2 floxed (Got2) at 3 months of age. No significant differences are observed.

(E) Graph showing the percentage of tissue area for different stages of pancreatic cancer (Acinar, ADM, PanIN1, PanIN2, PanIN3) in KC and KC-Got2 floxed (Got2) at 3 months of age. No significant differences are observed.

(F) H&E staining of KC and KC-Got2 floxed (Got2) at 6 months of age. No significant differences in histological features are observed.

(G) Bar graph showing the pancreas weight (g) of KC and KC-Got2 floxed (Got2) at 6 months of age. No significant differences are observed.

(H) Graph showing the percentage of tissue area for different stages of pancreatic cancer (Acinar, ADM, PanIN1, PanIN2, PanIN3) in KC and KC-Got2 floxed (Got2) at 6 months of age. No significant differences are observed.

(I) H&E staining of KC and KC-Got2 floxed (Got2) at 1 year of age. No significant differences in histological features are observed.

(J) Bar graph showing the number of mice with Acinar/ADM/PanIN and PDAC at 1 year of age. No significant differences are observed.
Figure 4-figure supplement 1

A  

B  Got2^{ff}  P48-Got2^{ff}  

C  

Pancreas Weight (g)
Figure 5

A

Relative colony formation (Normalized to -DOX)

MIAPaCa-2

**  ***

DMEM hCAF CM

shNT sh1 sh2

-DOX +DOX

B

Relative colony formation (Normalized to -DOX)

MIAPaCa-2

**

0% 25% 50% 75%

hCAF CM

-DOX +DOX

C

Relative colony formation (Normalized to -DOX)

MIAPaCa-2

**

p=0.06 p=0.16

-DOX +DOX

D

Pyruvate

p=0.000001

Log2FC (hCAF CM/DMEM)

E

[mM]

DMEM Batch1 Batch2 Batch3

hCAF CM Batch1 Batch2 Batch3

F

Relative colony formation (Normalized to -DOX)

MIAPaCa-2

****

p=0.06

hCAF CM mCAF CM

G

Pyruvate (hCAFs)

Lactate (hCAFs)

Ion Abundance

H

Glycolysis

TCA Cycle

PPP

% Fractional Labeling

FDP F6P DHAP G3P 2PG 3PG PEP Pyr Lac

S7P R5P

m+7 m+6 m+5 m+4 m+3 m+2 m+1 m+0

m+0