Tumour-reprogrammed stromal BCAT1 fuels branched-chain ketoacid dependency in stromal-rich PDAC tumours

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Branched-chain amino acids (BCAAs) supply both carbon and nitrogen in pancreatic cancers, and increased levels of BCAAs have been associated with increased risk of pancreatic ductal adenocarcinomas (PDACs). It remains unclear, however, how stromal cells regulate BCAA metabolism in PDAC cells and how mutualistic determinants control BCAA metabolism in the tumour milieu. Here, we show distinct catabolic, oxidative and protein turnover fluxes between cancer-associated fibroblasts (CAFs) and cancer cells, and a marked reliance on branched-chain α-ketoacid (BCKA) in PDAC cells in stroma-rich tumours. We report that cancer-induced stromal reprogramming fuels this BCKA demand. The TGF-β–SMAD5 axis directly targets BCAT1 in CAFs and dictates internalization of the extracellular matrix from the tumour microenvironment to supply amino-acid precursors for BCKA secretion by CAFs. The in vitro results were corroborated with circulating tumour cells (CTCs) and PDAC tissue slices derived from people with PDAC. Our findings reveal therapeutically actionable targets in pancreatic stromal and cancer cells.

Several studies have revealed the importance of BCAAs in cancer, including how they serve as requisite precursors for protein synthesis, maintain metabolite pools in the tricarboxylic acid (TCA) cycle and sustain production of nucleotides and lipids1–4. However, the role that stromal cells have in regulating BCAA metabolism in tumours is still poorly understood. In PDAC, the stromal cells identified as activated pancreatic stellate cells or CAFs account for up to 90% of tumour volume5. Furthermore, cancer cells are known to transform quiescent stromal cells into reactive stromal cells6. As such, the transformation entails the rewiring of metabolic pathways. Since most studies in pancreatic cancers have focused on systemic or cancer-cell-autonomous BCAA metabolism, understanding the cancer stromal ecosystem requires insight into the intersection of cancer-associated transformations in the stroma with reprogramming of their BCAA metabolism. Deciphering the precise role of various cellular components in the BCAA metabolism of tumours is complicated by conflicting evidence from past studies and the challenging nature of the intricate tumour microenvironment (TME). BCAA oxidation in the mouse pancreas has been found to be pronounced compared with that in other organs5. Conversely, decreased BCAA uptake has been reported in murine PDACs6. Neither systemic in vivo BCAA metabolism nor cancer cells’ BCAA metabolism alone is sufficient to dissect the role of the stroma. The difficulty in understanding BCAA metabolism in the tumour milieu is exacerbated by nutrient scarcity, exchange reactions and metabolite sharing between cancer and stromal cells7–11. Both the fibrotic environment and nutrient scarcity are difficult to mimic in murine models of aggressive PDAC models.

The metabolic fates of the BCAAs—leucine, valine and isoleucine—are cell- and tissue-dependent. BCAA transaminases (BCAT1 and BCAT2) first deaminate BCAAs to BCKAs (Fig. 1a). Where BCAT2 is expressed in most adult tissues, BCAT1 is restricted to the brain and spine, retina, ovaries, testes, pancreas and placenta, according to the Human Proteome Atlas11. Interestingly, in normal brain, prostate, testis and pancreas, stromal cells account for higher gene expression of BCAT1 than do epithelial cells, whereas normal ovaries show the opposite trend (Extended Data Fig. 1a). The second step in BCAA metabolism involves irreversible BCKA oxidation catalysed by the mitochondrial BCKA dehydrogenase (BCKDH) complex. Further, oxidation of BCKAs results in succinyl-CoA and acetyl-CoA that act as anaplerotic or ketogenic sources for the TCA cycle.

We revealed differential BCAA metabolism in cancer and stromal compartments of PDAC tumours. Our study identified a strikingly higher BCAA catabolic flux in CAFs, but increased BCKA oxidative flux in cancer cells. Further, CAF-secreted BCKAs were used for maintaining protein synthesis, augmenting TCA-cycle metabolite pools and increasing oxidative phosphorylation in...
cancer cells. To corroborate the mechanistic underpinnings discovered in our human CAF and cancer-cell-line model, we employed two models derived from people with PDAC: circulating tumour cells (CTCs) and tumour-slice cultures. Collectively, our results have elucidated an undiscovered metabolic-signalling cross-talk between PDACs and stromal cells and have demonstrated that targeting BCAA metabolism in PDAC tumours could mitigate PDAC aggression.

**Results**

**BCAA catabolism is upregulated in CAFs.** To determine the differences in BCAT catabolism between stromal and PDAC cells, we analysed protein and gene expression of BCAT1/2 (Fig. 1b,c). Notably, we found that CAFs had substantially higher BCAT1 expression than did PDAC cell lines. In contrast, BCAT2 expression was increased in PDAC cells relative to that in CAFs. Additionally, PDAC cells displayed higher expression of the BCKDH-complex enzymes BCKDHA, BCKDHB, and DBT than CAFs (Fig. 1d). Differential DBT expression was corroborated by immunoblotting (Fig. 1b). These results suggested that there was differential BCAA deamination and oxidation potential among stromal and cancer-cell lines. To corroborate this notion, we analysed transcriptomic data from The Cancer Genome Atlas (TCGA). Because TCGA tumour samples contain a heterogeneous cell population, we deconvoluted the expression of stromal and epithelial compartments from the bulk transcriptomic profile of tumour samples. Stromal-dominant samples showed higher expression of BCAT1, whereas epithelial- or malignant-cell-dominant samples showed higher expression of BCAT2, BCKDHA and BCKDHB (Fig. 1e and Supplementary Fig. 1a). Our results were further validated by analysis of ROBO1 expression, which was shown to be highly expressed only in stroma3. Transcriptomic data from independent studies corroborated these data (Extended Data Fig. 1b–d) [1,2,4].

PDAC tumours consist of a highly heterogeneous population of cancer, stromal and immune cells[5,6]. Bulk RNA-sequencing (RNA-seq) techniques are unable to dissect the true transcriptomic signature of these distinct compartments within the TME. Therefore, we used the high-resolution single-cell transcriptomic profiling[8] to analyse BCAA metabolism of people with PDAC (Fig. 1f,g and Supplementary Fig. 2a). Consistent with bulk expression data, single-cell expression analysis revealed that cells expressing epithelial or malignant markers also expressed BCAT2 but not BCAT1, whereas cells with fibroblasts markers expressed only BCAT1. Furthermore, analysis on another single-cell dataset from people with PDAC[9] showed a similar transcriptomic profile of BCAA genes (Supplementary Fig. 2b). Notably, single-cell data from normal pancreatic tissue identified that BCAT1 expression is a feature of CAFs rather than normal fibroblasts, whereas BCAT2 expression is similar in PDAC cancer cells and normal ductal cells (Fig. 1h and Supplementary Fig. 2b). We also found higher BCAT1 and lower BCAT2, BCKDHA and DLD expression in stromal compartments than in epithelial compartments of laser-microdissected tumours (Fig. 1i and Extended Data Fig. 1e). Immunohistochemistry (IHC) staining similarly revealed that the stromal component had substantially higher BCAT1 expression than did its epithelial counterpart (Fig. 1j and Extended Data Fig. 2a). We further compared BCAT1 and α-smooth muscle actin (α-SMA) expression in matched tumour and adjacent normal regions in people with PDAC and found that expression of both BCAT1 and α-SMA was high in CAFs compared with that in fibroblasts in normal stroma, thereby suggesting that enhanced BCAT1 expression in tumour stroma is associated with distinct desmoplasia differing from normal tissue (Extended Data Fig. 2b).

Since differential gene and protein expression does not always translate to metabolic phenotype, we measured BCAA catabolic flux using 13C-labelled BCAAs in PDAC cells and CAFs (Fig. 1k). We observed that CAFs had BCAA catabolic flux that was threefold higher than that in PDAC cell lines. To evaluate how essential BCAs are for the proliferation of CAFs and cancer cells, we cultured them under BCAA-deprived conditions. Expectedly, CAFs were resilient to BCAA deprivation in regard to proliferation, whereas cancer cells were BCAA dependent (Fig. 1l and Extended Data Fig. 2c,d). The finding that cancer cells not only have reduced BCAA catabolic flux compared to that in CAFs, but require BCAs for growth suggests that intermediates downstream of BCAs, such as BCKAs, have a major role in maintaining metabolic activity in the nutrient-starved pancreatic milieu. The reliance of healthy pancreatic ductal and PDAC cells on BCAA catabolism has been well-characterised[1], but PDACs rewire their metabolism to maintain their BCAA demand in the nutrient-deprived TME. This becomes important in stromal-rich tumours where BCAT1-expressing CAFs outcompete PDAC cells for BCAs, creating a uniquely nutrient-stressed environment of PDACs.

**BCAT2 regulates BCKA-mediated de novo protein synthesis in PDAC cells.** To address whether upregulated BCAA deamination in pancreatic CAFs fuels the BCAA demand of cancer cells, we measured the proliferation rate of green fluorescent protein-expressing PDAC cells co-cultured with CAFs.
(GFP)-labelled PDAC cell lines in direct coculture with CAFs or normal fibroblasts (NOFs) derived from people with PDAC. Notably, we observed that CAFs completely rescued the loss of proliferation of cancer cells under BCAA deprivation, whereas NOFs had no effect (Fig. 2a,b and Extended Data Fig. 3a–c). This suggests that CAFs may either be secreting BCAAs or BCKAs. We
measured BCKA secretion by CAFs to be around 200 pmol µg⁻¹ protein, which increased under BCAA deprivation to around 300 pmol µg⁻¹ protein (Fig. 2c). Interestingly, we found that under BCAA deprivation, BCKAs rescued proliferation at concentrations as low as 5–50 µM, whereas 100 µM BCAAs were needed to obtain a similar effect (Extended Data Fig. 3d). This suggested that BCKAs were a more effective nutrient at lower concentrations compared to BCAAs. Once the CAF-secreted BCKAs are consumed by cancer cells, they may be used directly for BCKA oxidation through the BCKDH complex to maintain oxidative TCA-cycle metabolism. BCKAs can also act as substrates for de novo synthesis of BCAAs through reamination by the reversible enzyme BCAT2 (Fig. 2d). Although not explicitly shown before, these newly synthesized BCAAs could maintain de novo protein synthesis in cancer. To confirm the fate of BCKAs, we cultured cancer cells with ¹³C-labelled BCKAs and estimated their contribution to the TCA cycle, and for de novo BCAA and subsequent protein synthesis (Fig. 2d). We observed that BCKAs are indeed oxidized and incorporated into the TCA cycle via acetyl-CoA and succinyl-CoA, evident from the ¹³C-labelled TCA intermediates citrate, malate and aspartate (Fig. 2d). Interestingly, BCKAs were also utilized for de novo protein synthesis, and contributed more than 60% of the intracellular BCAA pools under BCAA-deprived conditions (Fig. 2d). Further, ¹⁵N-labelled glutamine and serine were found to commensurately contribute the required nitrogen for BCAA synthesis. The constituent BCAAs in the protein achieved 40% enrichment from ¹³C-labelled BCKAs and 50% enrichment from ¹⁵N-labelled glutamine and serine, definitively proving that BCKA-derived BCAAs contributed substantially to de novo protein synthesis (Fig. 2d).

To substantiate the functional role of BCAT2 in the BCAA metabolism of PDAC cells, we silenced BCAT2 using both short hairpin RNA (shRNA) and CRISPR in PDAC cells. We found that BCAT2 knockdown significantly reduced their growth rate, implying that BCAT2 has an important role in PDAC cells (Fig. 2e,f). BCAT2 knockdown resulted in a decrease in ¹³C enrichment of BCAAs obtained after protein hydrolysis, confirming the BCAT2-mediated, anabolic, regulatory role of BCAT2 in PDAC (Fig. 2g). Having delineated that BCKAs can contribute to de novo protein synthesis, we next assessed whether CAFs could promote protein synthesis in cancer cells. Measurement of de novo protein synthesis revealed that PDAC cells indeed increased protein synthesis in CAF cocultures (Fig. 2h and Supplementary Fig. 3). In agreement with substantial enrichment of labelled BCKA-derived BCAAs in hydrolysed protein, BCKA supplementation restored the loss of newly synthesized protein levels under BCAA deprivation (Fig. 2i). To establish the essentiality of BCAT2, we cocultured BCAT2-knockdown PDAC cells with CAFs derived from people with PDAC (Fig. 2j and Extended Data Fig. 3e). Intriguingly, BCAT2 knockdown in cancer cells had no effect on CAF-mediated rescue of cancer-cell growth under conditions of BCAA deprivation. We further excluded the possibility that BCAAs are directly catabolized from autophagy-induced protein degradation by knocking down autophagy-related genes (ATG5 and ATG7) in CAFs and coculturing them with PDAC cells under BCAA deprivation. We found that ATG5 and ATG7 knockdown did not suppress CAF-mediated rescue of cancer-cell growth under BCAA deprivation (Extended Data Fig. 3f). Moreover, autophagy inhibitors did not inhibit the rescue effect of CAFs on cancer-cell growth under BCAA deprivation (Extended Data Fig. 3g). Taken together, these results provide strong evidence for the regulation of BCAA metabolism by BCAT2 in PDAC cells.

**BCKDH complex is essential for the growth and bioenergetics of PDAC cells.** Since BCAT2 knockdown did not result in a loss of cancer-cell growth in cocultures with CAFs under BCAA deprivation, we hypothesized that irreversible BCKA oxidative decarboxylation by the BCKDH complex could facilitate biosynthesis. To regulate BCKDH-complex activity, we targeted the E2 component encoded by dihydrolipoamide branched-chain transacylase E2 (DBT). DBT knockdown profoundly reduced proliferation and colony formation (Fig. 3a,b and Extended Data Fig. 4a,b). In contrast, when inhibited in PDAC cells, BCKDH kinase (BCKDK), known to suppress BCKDH activity, had no effect on growth rate of the cells (Extended Data Fig. 4c). In agreement with the essentiality of the BCKDH complex, the addition of BCAAs under conditions of BCAA deprivation rescued the loss of proliferation in PDAC cells (Fig. 3c and Extended Data Fig. 4d), but not in CAFs (Fig. 3d). To confirm that BCKA-mediated rescue of growth is conferred via the BCKDH complex and not BCAT2, we cultured shDBT cells under BCAA deprivation. As expected, BCKA-mediated rescue of cancer-cell growth was attenuated (Fig. 3e). We then cocultured DBT-knockdown PDAC cells with CAFs under BCAA deprivation to substantiate the BCKDH-dependent role of CAF-secreted BCKA in maintaining PDAC cells’ growth (Fig. 3f and Extended Data Fig. 4e). Notably, DBT knockdown in PDAC cells abrogated the rescue effect from CAFs, thereby validating the role of the BCKDH complex.

Given that complete oxidation of one molecule of L-α-ketoisovalerate (KIV), α-keto-β-methylbutyrate (KMB) or α-ketoisocaproate (KIC) can provide 6, 5 or 10 NADH molecules, respectively, BCKA-driven NADH production could provide a substantial measure for improving BCKA oxidative capacity in cells. To further validate the effect of BCKA oxidation on mitochondrial activity, we used a fluorescent, genetically encoded NADH sensor to measure mitochondrial activity.

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**Fig. 2 | PDAC cells are dependent on BCAT2 for growth and respiration.** a. Fluorescence microscopy images comparing the growth of GFP-labelled MIA PaCa-2 and Panc-1 cells in contact cocultures with CAFs or NOFs under BCAA deprivation. Experiments were repeated independently three times with similar results. b. Relative proliferation rates of MIA PaCa-2, PaTu 8988t and Panc-1 pancreatic cancer cells under BCAA deprivation. n = 3 biologically independent samples. c. BCKA secretion by CAFs, estimated by measuring extracellular concentrations of BCKAs, KIV and KMV, at 6, 12, 24 and 48 h by liquid chromatography–mass spectrometry. n = 3 biologically independent samples. d. Fate of [¹³C]BCKAs in PDAC cells, elucidated by measuring mole percent enrichment (MPE) of TCA-cycle intermediates that represent BCKA oxidation, and of intracellular BCAAs and BCAAs from acid-hydrolysed proteins that represent de novo protein synthesis. n = 7 biologically independent samples for intracellular metabolites and n = 4 biologically independent samples for protein–hydrolysed metabolites. e. Relative proliferation rates of MIA PaCa-2, Panc-1 and PaTu 8988t pancreatic cancer cells with BCAT2 knockdown by shRNA. n = 4 biologically independent samples. f. Relative proliferation rates of Panc-1 and PaTu 8988t pancreatic cancer cells with BCAT2 knockdown by CRISPR. n = 4 biologically independent samples. sg, single guide RNA. g. MPE of BCAAs in hydrolysed protein obtained from BCAT2-knockdown MIA PaCa-2 cells cultured with [¹³C]BCKA. n = 4 biologically independent samples. h. Fluorescence-activated cell sorting (FACS) analysis of GFP-labelled MIA PaCa-2 cells detected with antibodies against puromycin that were labelled with Alexa Fluor 647 (puro-A647). n = 3 biologically independent samples. MFI, mean fluorescence intensity; a.u., arbitrary units. i. Representative images of results from SUnSET assay of MIA PaCa-2 cells cultured in the indicated medium for 48 h. Whole-cell lysates were subjected to western blotting with puromycin antibodies. Experiments were repeated independently three times with similar results. j. CAF cocultures rescue the loss of growth in BCAT2-knockdown PDAC cells. n = 6 biologically independent samples. Data are presented as mean ± s.d. *P <0.0001. Two-way ANOVA with Dunnett’s multiple-comparison test (b,e,f,j); multiple, two-tailed, unpaired Student’s t-test (d,g); one-way ANOVA with Tukey’s post hoc comparison (h).
mitochondrial NADH/NAD⁺ in MIA PaCa-2 cells cultured in the absence or presence of either BCAA or BCKA (Fig. 3g,h and Extended Data Fig. 4f). Interestingly, BCKAs increased the mitochondrial NADH/NAD⁺ ratio and corroborated that BCKA oxidation enhances energy metabolism. BCKAs can be either reaminated into BCAAs via BCAT2 or oxidized through the

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**Figure a**: Images showing BCAA+ and BCAA- in PANC-1 and MIA PaCa-2 cells cultured in the absence or presence of either BCAA or BCKA. **Figure b**: Graphs showing relative growth rate for MIA PaCa-2, PaTu 8988t, and PANC-1 in monoculture and No BCAA coculture. **Figure c**: Graphs showing KIC secretion and KMV secretion over time in complete and without BCAA conditions. **Figure d**: Diagram of the metabolic pathways showing de novo protein synthesis and oxidative metabolism. **Figure e**: Graphs showing relative growth rate for MIA PaCa-2, PANC-1, and PaTu 8988t in control, shControl, shBCAT2 (1), and shBCAT2 (2) conditions. **Figure f**: Graphs showing BCAA MPE from hydrolysed protein in control, CRISPR, and shBCAT2 conditions. **Figure g**: Graphs showing BCAA MPE from hydrolysed protein for Leu, Isoleucine, and Valine. **Figure h**: Graphs showing Puromycin-AF647 MFI in control, shControl, and shBCAT2 conditions. **Figure i**: Electrophoretic analysis showing Antipuromycin and Pancreas S. **Figure j**: Graphs showing relative growth rate for Mia Paca-2 and Patu 8988t in control and shBCAT2 conditions.
BCKDH complex. To investigate both possible fates, we measured NADH and shDBT, shBCAT2 and PDAC cells under varying α-ketoglutarate (αKG)/glutamate ratios. DTT knockdown reduced the NADH/NAD⁺ ratio in PDAC cells, confirming the involvement of DTT and BCKA oxidation in maintaining this ratio (Fig. 3i and Supplementary Fig. 4a,b). We further increased the αKG/glutamate ratio by supplementing αKG to reduce reamination of BCKAs and favour oxidation. The increased αKG/glutamate ratio nudged BCKAs towards oxidation, confirmed with our observation of increased NADH/NAD⁺ ratio and PDAC cell growth (Fig. 3j,k and Supplementary Fig. 4c,d). Since the effect of modulating NADH should directly affect mitochondrial oxygen consumption rate (OCR), we measured OCR with different substrates and varying αKG/glutamate ratios. Increasing the αKG/glutamate ratio (by supplementing αKG) increased the OCR significantly, whereas the converse was true when decreasing the ratio (by glutamate supplementation) (Fig. 3i). Finally, we used BCAT2-knockdown cells and found that adding BCKAs increased NADH (Fig. 3m and Supplementary Fig. 4e,f), thus substantiating that the two BCKA fates affect NADH homeostasis differently. Cumulatively, these experiments show that both fates of BCKAs are relevant in the context of PDAC cells, and BCKAs contribute to both the proliferation and bioenergetic metabolism of PDAC cells.

To identify the dominant substrates contributing to cellular oxidative capacity, we measured OCR. Remarkably, the BCKA-driven OCR in PDAC cells was significantly higher than that of alternative substrates (Fig. 3n and Extended Data Fig. 4g). To dissect the role of BCAT2 and DTT in the oxidative capacity of PDAC cells, we measured the OCR of DBT- and BCAT2-knockdown PDAC cells, and found that it was substantially reduced (Fig. 3o and Extended Data Fig. 4h). To associate substrate specificity with BCAT2 and the BCKDH complex, we measured substrate-specific OCR in BCAT2- and DTT-knockdown cells. Notably, there was no change in BCKA-driven OCR in BCAT2-knockdown cells; however, BCAA-driven OCR was significantly reduced (Fig. 3p and Extended Data Fig. 4i). Strikingly, in DTT-knockdown cells, both BCAA- and BCKA-driven OCRs were significantly reduced (Fig. 3q and Extended Data Fig. 4j). Furthermore, the BCKA-driven OCR was higher than the BCAA-driven OCR, thereby suggesting that BCKAs are a better fuel source for PDAC cells (Fig. 3p–q). These results strongly suggest that PDAC cells are heavily dependent on BCKAs under BCAA-deprived conditions, and that DTT is a potential target for exploiting this dependency.

Stromal BCAT1 regulates BCKA synthesis in PDAC tumours. The mechanistic underpinnings of stromal BCKA secretion are necessary to elucidate its dynamics and targetable vulnerabilities. Because the synthesis of stromal BCKA is dependent on transamination by BCAT1, we first inhibited BCAT1 activity using the BCAT1 inhibitor gabapentin and measured BCKA secretion in CAFs. Indeed, gabapentin significantly reduced BCKA production and subsequent secretion by 40–50%, as indicated by extracellular BCKA concentrations (Fig. 4a). To conclusively associate BCAT1 with CAF-mediated rescue of cancer cells in BCAA-deprived conditions, we added gabapentin to cocultures (Fig. 4b and Extended Data Fig. 5a,b). Notably, inhibition of stromal BCAT1 abrogated the CAFs’ ability to rescue PDAC cell growth, whereas addition of BCKA markedly restored it. Gabapentin concentration for subsequent experiments was selected after assessment of its inhibition efficacy on BCAT1 activity (Extended Data Fig. 5a). Similarly, BCAT1 knockdown in CAFs, and consequently reduced BCKA secretion, significantly reduced the growth rate of PDAC cells (Fig. 4c and Extended Data Fig. 5c). Notably, knockdown of BCAT2 in CAFs had no effect on the growth rate of PDAC cells (Fig. 4c). These results suggest that BCKA synthesis in CAFs is severely impacted upon loss of BCAT1 expression or activity.

Activated CAFs in PDAC are known to secrete a vast array of extracellular matrix (ECM) proteins, such as collagen, enzymes and glycoproteins. Therefore, we surmised that under nutrient-scarce conditions of the pancreatic TME, ECM proteins in the milieu could be a source of amino acids for CAFs. It is well-established that stromal cells, including fibroblasts, internalize ECM proteins through uPAR-associated protein (uPARAP/Endo180) (encoded by the mannose receptor C type 2 (MRC2) gene), and its expression is maximal in fibroblasts. However, there is a gap in knowledge regarding the role of ECM uptake by stromal fibroblasts in cancer. To demonstrate that CAFs utilize ECM proteins, we added collagen I or collagen IV to coculture. As seen in Fig. 4d, both collagen I and IV enhanced PDAC cell growth rate in coculture under BCAA deprivation, but had no effect in monolayer. Further, gabapentin attenuated ECM-protein-mediated rescue of cancer-cell growth by CAFs. We also found that gabapentin markedly reduced de novo protein synthesis (Fig. 4e) and mitochondrial NADH/NAD⁺ ratio in PDAC cells (Fig. 4f). To characterize internalization and to confirm uptake and cleavage of ECM proteins by CAFs, we used fluorogenic DQ collagen. We found that collagen uptake in CAFs under BCAA deprivation was increased substantially compared with that in the BCAA-replete condition, and this increase was pronounced in the presence of transforming growth factor beta (TGF-β) (Fig. 4g and Extended Data Fig. 5d). Furthermore, we quantified markedly higher collagen uptake in CAFs than in PDAC cells (Extended Data Fig. 5e). In concurrence with our previous results, uPARAP/Endo180 expression was much higher in CAFs than in cancer cells.

Fig. 3 | BCKDH complex is essential for PDAC cell growth and cell biosynthesis. a, Absolute numbers of PDAC cells expressing control shRNA or one of two independent shRNAs targeting DTT. n = 3 biologically independent samples. b, Colony-formation assay of DTT-knockdown PDAC cell lines. n = 3 biologically independent samples. c, BCAA deprivation in the cancer monoculture can be rescued by BCKAs. n = 4 biologically independent samples. d, BCKA has no influence on CAF proliferation. n = 4 biologically independent samples. e, Relative proliferation rates of DTT-knockdown cells in BCAA-depleted medium under BCKA-replete conditions. n = 8 biologically independent samples. f, Relative proliferation rates of DTT-knockdown cells cocultured with CAFs. n = 3 biologically independent samples. g, h, NADH/NAD⁺ ratio measured using confocal fluorescence imaging of MIA PaCa-2 cells in BCAA-depleted medium under BCKA-replete conditions (color scale in arbitrary fluorescence units). n = 5 biologically independent samples. Experiments were repeated independently three times with similar results. i, NADH/NAD⁺ ratio measured using confocal fluorescence imaging of MIA PaCa-2 cells transfected with siControl or siDBT. n = 5 biologically independent samples. j, NADH/NAD⁺ ratio measured using confocal fluorescence imaging of MIA PaCa-2 cells in complete medium with 9 mM αKG and/or added BCKAs. n = 4 biologically independent samples. k, EdU uptake was measured in MIA PaCa-2 cells in the presence of αKG and/or BCKAs after 1 d. n = 3 biologically independent samples. l, Substrate-specific OCR in permeabilized pancreatic cancer cells, measured using Seahorse Analyzer. n = 6 biologically independent samples. M, malate. m, NADH/NAD⁺ ratio measured using confocal fluorescence imaging of MIA PaCa-2 cells transfected with siControl or siBCAT2 in complete medium or BCAA-depleted medium under BCKA-replete conditions. n = 6 biologically independent samples. n, Substrate-specific OCR in permeabilized cells. n = 4 biologically independent samples. o, OCR measurements in BTT- and BCAT2-knockdown cells. n = 6 biologically independent samples. p, Substrate-specific OCR measurements of BCAT2-knockdown cells. n = 4 biologically independent samples. q, Substrate-specific OCR measurements of BTT-knockdown cells. n = 4 biologically independent samples. Data are presented as mean ± s.d. *P < 0.0001. Multiple, two-tailed, unpaired Student’s t-test (b), two-way ANOVA with Dunnett’s multiple-comparison test (c–f,l,p,q); one-way ANOVA with Tukey’s post hoc comparison (h–k,m,n).
(Extended Data Fig. 5g). Moreover, inhibiting uPARAP/Endo180 in CAFs substantially affected the uptake of collagen (Extended Data Fig. 6a). Cumulatively, these results indicate that ECM internalization was indeed high in CAFs, but was undetectable in PDAC cells. Measuring intracellular BCAA levels and [13C]enrichment in CAFs cultured with [13C]BCAAs further confirmed this (Extended
Data Fig. 6b). Notably, intracellular BCAA levels increased gradually following deprivation (Extended Data Fig. 6c). In contrast, 13C enrichment of BCAAs gradually decreased in the same timeframe (Extended Data Fig. 6c), indicative of the introduction of unlabelled BCAAs in BCAA-deprived CAFs from ECM proteins.

We hypothesized that proteasomal proteolysis has a major role in degradation of ECM internalized by CAFs in nutrient-deprived conditions. Consistent with this notion, both BCAA deprivation and TGF-β increased the chymotrypsin-like proteasome activity in CAFs, but not trypsin-like and caspase-like protease activities (Fig. 4h and Extended Data Fig. 6d). We further investigated the role of the proteasome in collagen degradation (Fig. 4i) and found that collagen is localized with proteasomes in CAFs, thereby suggesting that ECM proteins are indeed degraded by proteasomes. We further tested our hypothesis by measuring BCKA secretion of CAFs and PDAC cell growth rate in coculture under BCAA-deprived conditions. We found that delanzomib, the chymotrypsin-like proteasome activity inhibitor, and MG-132, a proteasomal inhibitor, attenuated ECM–protein-mediated rescue of cancer-cell growth by CAFs (Fig. 4j and Extended Data Fig. 6e). These data confirm that collagen is indeed degraded by proteasomal proteolytic activity in CAFs. To further strengthen our findings, we measured CAF secretion of BCKAs in the presence of delanzomib, and found that delanzomib impeded their ability to secrete BCKAs by 40% (Fig. 4k).

To establish whether internalized ECM proteins are a carbon source for CAF-secreted BCKAs, we cultured CAFs on decellularized [13C]BCAA-labelled ECM proteins (Fig. 4l). We used scanning electron microscopy to characterize the ECM structure, and observed that the CAF-derived three-dimensional (3D) matrix was free of cellular debris and remained attached to the culture surface (Fig. 4m). Secreted ECM proteins were hydrolysed with acid, and their constituent BCAAs were found to be enriched 40–50% by [13C]BCAAs (Fig. 4n and Extended Data Fig. 6f,g). We then cultured CAFs with [13C]BCAA ECM under BCAA-deprived conditions and analysed the spent medium obtained after 48 h of culture. BCKAs secreted by the CAFs were found to be enriched with [13C] derived from the proteolysed ECM (Fig. 4o). This indicated that CAFs cultured with labelled ECM internalized and proteolysed it to maintain intracellular BCAA pools, and to produce and secrete BCKAs. Collectively, these results provide strong evidence that the ECM in the pancreatic milieu could serve as a storage pool of BCAAs for CAFs under nutrient-stressed conditions.

BCAT1 is a direct target of the TGF-β–SMAD5 pathway in CAFs.

To unravel the mechanism underlying the regulation of BCAT1 expression in CAFs, we postulated that cancer cells reprogram fibroblasts to upregulate their BCAT1 expression to meet the cancer cells’ demand for BCKAs under BCAA deprivation38. We first transformed NOFs and MSCs into CAFs by culturing them in PDAC-cell-conditioned medium (CM) and measured expression of genes involved in BCAA metabolism. This revealed that increased CM-mediated activation of NOFs and primary MSCs progressively increased expression of BCAT1 and activated CAF markers (Fig. 5a,b and Extended Data Fig. 7a–e). Consistent with previous results, there was no change in BCAT2 expression in MSCs and NOFs cultured in CM. These results were further corroborated when CAFs were exposed to CM and generated similar upregulation of BCAT1 and no significant changes in BCAT2 and BCKDHA/B (Fig. 5c and Extended Data Fig. 7f). We next asked whether these activated NOFs could acquire the PDAC-supporting characteristics of CAFs. Notably, we found that, like CAFs, activated NOFs completely rescued PDAC cell growth in BCAA-deprivation conditions (Fig. 5d). Having established that fibroblast activation specifically upregulates BCAT1 expression in CAFs, we sought to elucidate the congruence between NOF-activation pathways and BCAT1 expression. Many pathways of fibroblast activation converge towards TGF-β-based activation39. We assessed whether TGF-β could regulate BCAT1 expression in CAFs, and surprisingly found that induction of BCAT1 expression by TGF-β is pronounced in CAFs (Fig. 5e). By contrast, TGF-β could neither influence BCAT2 expression in CAFs nor induce changes in BCAT1 expression in PDAC cells (Extended Data Fig. 7g). Notably, depletion of TGF-β abrogated upregulation of stromal BCAT1 (Fig. 5f) and α-SMA expression mediated by cancer-cell-secreted TGF-β, whereas there was no change in BCAT2 expression (Extended Data Figs. 7h and 8a). We employed a genetic approach using αvβ3-integrin-knockout CAFs, which become activated upon preactivated (as opposed to immuno/latent) administration of TGF-β in a non-cell-autonomous way. BCAT1 expression of αvβ3-integrin-knockout CAFs was not activated by CM (Extended Data Fig. 8b). Additionally, we found that cancer cells secreted TGF-β at concentrations that were several fold higher than those secreted by CAFs (Extended Data Fig. 8c), thereby corroborating our claim that cancer-cell-secreted TGF-β regulates BCAT1 expression.

Previous studies have provided evidence that SMAD proteins are the effectors of TGF-β activation and, once activated, they regulate gene expression by translocating to the nucleus. NOF activation significantly upregulated SMAD2, SMAD4 and SMAD5 (Fig. 5g and Extended Data Fig. 8d–f). To establish which SMAD directly regulated BCAT1 expression, we performed quantitative chromatin

Fig. 4 | BCAT1 regulates stromal cells’ synthesis of ketoacids. a, BCKA secretion by CAFs treated with 10 mM gabapentin, n = 3 biologically independent samples. b, Effect of 10 mM gabapentin on CAF-mediated rescue of the MIA PaCa-2 growth rate under BCAA-deprived conditions, n = 6 biologically independent samples. c, Effect of BCAT1 and BCAT2 knockdown using shBCAT1 and shBCAT2, respectively, on CAF-mediated rescue of cell growth under BCAA deprivation, n = 3 biologically independent samples. d, Relative proliferation rates of MIA PaCa-2 cells cocultured with CAFs and collagen or 10 mM gabapentin under BCAA deprivation, n = 6 biologically independent samples. e, FACs analysis of GFP-labelled MIA PaCa-2 cells detected with puromycin antibodies in the coculture system with 10 mM gabapentin, n = 6 biologically independent samples. f, Effect of 10 mM gabapentin on NADH/NAD+ ratio of cancer cells cocultured with CAFs, n = 6 biologically independent samples. g, Uptake of DQ collagen by CAFs, assessed using confocal imaging after 24 h. Experiments were repeated independently three times with similar results. h, Proteasome activity in CAFs that were treated with TGF-β and were under BCAA deprivation, n = 6 biologically independent samples. i, Colocalization of collagen and proteasome analysed by immunofluorescence against 205 proteasome and FITC-collagen. Experiments were repeated independently three times with similar results. j, Relative proliferation rates of MIA PaCa-2 pancreatic cancer cells cocultured with CAFs in combination with collagen or 10 nM delanzomib under BCAA deprivation, n = 6 biologically independent samples. k, BCKA secretion by CAFs treated with 10 nM delanzomib, n = 3 biologically independent samples. l, Schematic of the protocol used to synthesize ECM labelled with [13C]BCAAAs and secretion of [13C]BCAAAs after culturing BCAA-deprived CAFs in ECM labelled with [13C]BCAAAs. m, Scanning electron microscopy image of CAF-derived 3D matrices. Experiments were repeated independently two times with similar results. LC–QTOF, quantitative liquid chromatography–quadrupole time of flight mass spectrometry; GC–MS, gas chromatography–mass spectrometry. n, Fractional enrichment of BCAAs after acid hydrolysis of decellularized ECM proteins produced by CAFs cultured with [13C]BCAAAs, n = 3 biologically independent samples. o, Fractional enrichment of BCAAs secreted by CAFs after 48 h of being cultured under BCAA deprivation on ECM labelled with [13C]BCAAAs, n = 3 biologically independent samples. Data are presented as mean ± s.d. *P < 0.0001. Multiple, two-tailed, unpaired Student’s t-test (e,f); two-way ANOVA with Dunnett’s multiple-comparison test (b,d,h,j).

872
immunoprecipitation–PCR (ChIP–qPCR) to elucidate the targets for the BCAT1-binding regions. Our analysis revealed the enrichment of SMAD5 for BCAT1-promoter-binding regions compared with the control regions (Fig. 5h and Extended Data Fig. 8g,h). Furthermore, only SMAD5 binding to the BCAT1-promoter regions was increased upon TGF-β activation; SMAD4 binding
was not affected. To further decipher whether TGF-β and SMAD5 activation increased BCAT1-promoter activity, we performed a dual-luciferase reporter assay and found that incubation with TGF-β strongly increased activity of the BCAT1 promoter in CAFs (Fig. 5i). Expectedly, the increase of TGF-β-mediated activity of the BCAT1 promoter is suppressed following either treatment with the TGF-β pathway inhibitor, RepSox, or by silencing SMAD5 expression (Fig. 5i). Furthermore, IHC staining confirmed that the stromal component had increased expression of SMAD5 compared with that in the epithelial compartment (Fig. 5j). SMAD5 inhibition significantly impacted BCAT1 expression at the messenger RNA and protein levels (Fig. 5k,l). In contrast, SMAD4 silencing did not induce any changes in BCAT1 expression. Together, these results provide strong evidence that cancer-cell-secreted TGF-β upregulates stromal BCAT1 activity through SMAD5 activation in stromal cells (Fig. 5m).

Validation using CTC and tissue-slice models derived from people with PDAC. We further expanded our findings in an alternative human preclinical model to allay any cell-intrinsic and cell-extrinsic abnormalities that are model specific. To replicate tumour stroma interactions in humans in the context of PDAC-cell BCAA metabolism, and to overcome inconsistencies observed in animal models, we have relied on two different patient-derived models: human CTCs and PDAC tissue slices. CTCs shed by the primary tumour are the seeds of metastasis and have been established as potential biomarkers of disease progression. CTCs freshly obtained from the blood of people with PDAC using the Labyrinth, a label-free size-based inertial microfluidic CTC isolation device, allowed us to compare their transcriptional profile with that of CAFs and cells obtained from a healthy person (Fig. 6a and Extended Data Fig. 9a). It is evident that gene expression of BCKDHA and DBT are higher in day 0 CTCs than in healthy controls and CAFs (Fig. 6b). In contrast, BCAT1 expression is much higher in CAFs than in CTCs (Fig. 6b). These data indicate the clinical relevance and corroborate our observations in the in vitro model. We then used expanded CTC lines derived from people with PDAC for downstream experiments. Similar to PDAC cell lines, CTC lines showed lower BCAT1 and higher DBT expression than did CAFs (Fig. 6c). Consistent with our findings in PDAC lines, CTC lines also had higher BCAT2 and lower BCAT1 expression at the protein level (Fig. 6d). Further, BCKAs could rescue the impaired proliferation of CTC lines under BCAA deprivation, thereby demonstrating that, similar to their cancer-cell-line counterparts, CTC lines are also BCKA dependent in stromal-rich conditions (Extended Data Fig. 9b). Interestingly, there is a marked increase in BCAAs secretion by CAFs when they are cocultured with CTC lines after 6 and 12 h (Fig. 6e and Extended Data Fig. 9c). Conversely, we did not find any secretion of ketoacids from cancer cells alone (Extended Data Fig. 9d). We next assessed whether anaplerotic substrates other than BCKAs could rescue the loss of growth rate of PDAC cells and CTC lines under BCAA deprivation. This could also reveal the role of BCKAs as opposed to other TCA-cycle substrates in the oxidative mitochondrial metabolism. Anaplerotic TCA substrates only partially rescued the reduction in growth rate; however, BCKAs could completely rescue the growth of PDAC cells and CTC lines under BCAA deprivation (Extended Data Fig. 9e). We further corroborated our hypothesis that stromal BCAT1 maintains BCAA metabolism in a CTC-organoid model (Fig. 6f and Extended Data Fig. 9f,g). Notably, targeting stromal BCAT1 reduced proliferation and de novo protein synthesis in cytokertatin-positive cancer cells of CTC organoids (Fig. 6g and Extended Data Fig. 9h,i). Our results substantiate the concept that CAFs maintain their ability to fuel the high BCKA demand of CTCs in organoids, a system that recapitulates tumour heterogeneity closely.

Having illustrated that CAF-derived BCKAs support BCKA dependence in CTC lines, we wanted to validate these findings in a setting that mimics the in vivo TME. Since tissue slices in ex vivo culture retain most components of the TME, they are believed to better recapitulate stromal-rich tumours than are other in vivo tumour models. We obtained fresh tissue slices from people with PDAC (Fig. 6h), validated their viability for 14 d in culture (Extended Data Fig. 10a), and examined whether simultaneously targeting stromal BCAT1 and cancer-cell DBT could result in an enhanced therapeutic effect. Remarkably, knocking down either BCAT1 or DBT significantly reduced PDAC cell viability (Fig. 6i and Supplementary Fig. 5). Both PCNA- and Ki67-positive cell populations had substantially reduced cytokeratin-positive cells when DBT or BCAT1 were knocked down in combination, compared with the number of those cells in populations with individual knockdowns. Furthermore, a reduction in DBT and BCAT1 expression validated our siDBT and siBCAT1 silencing (Extended Data Fig. 10b). Moreover, there were no changes in other BCAA-related genes. We then asked whether BCKAs were indeed consumed by PDAC cells in our tissue-slice model. Tissue slices were cultured in medium containing [13C]-labelled BCKAs, and after 48 h, the slices were collected to extract intracellular metabolites and intracellular proteins. Intracellular BCKAs were found to be enriched between 10% and 40%. BCKAs obtained by hydrolysing the intracellular tumour-slice protein had 2–15% 13C enrichment, supporting the concept that [13C]BCKA-derived BCAAs were utilized for de novo protein synthesis (Fig. 6j).

We tested whether suppressing stromal BCAT1 could reduce BCKA-mediated de novo protein synthesis in cancer cells within tissue slices using surface sensing of translation (SUnSET) immunofluorescence (IF). To specifically analyse cancer cells, we used areas of colocalization of puromycin with cytokeratin, a cancer-cell-specific marker. Notably, cancer-cell-specific de novo protein synthesis was pronouncedly reduced in slices treated with gabapentin (Fig. 6k and Supplementary Fig. 6). Overall, our results highlight that stromal-BCAT1 not only supports PDAC-cell BCKA
demand, but also exposes the synthetic lethal vulnerabilities in 
stromal-rich PDAC by cotargeting stromal BCAT1 and the cancer 
BCKDH complex (specifically DBT) as a clinically relevant therapy.

**Discussion**
Recent reports have concluded that stromal cells have a major role in 
mitigating nutrient deficiency in PDAC cells. Furthermore, it
has recently been shown that BCAAs contribute around 20% of the carbon in the TCA cycle of pancreatic cells7. In contrast, we investigated how stromal CAFs regulate BCAA metabolism in PDAC cells, and discovered that there exists a mutualistic relationship in regard to BCAA metabolism between CAFs and PDAC cells. We found that BCKAs play a major role in maintaining metabolic activity in the nutrient-starved pancreatic milieu. Unconventionally, CAF-secreted BCKAs were used as substrates for de novo BCAA synthesis by the reversible action of BCAT2, and newly synthesized BCAAs maintained de novo protein synthesis in cancer cells under BCAA-deprived conditions. Our results provide evidence for dependency on the BCKDH complex and are suggestive of its synergistic involvement with BCAT2 in regulating BCAA metabolism in PDAC cells. Recent studies have investigated BCAT2’s role...

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**Figure Descriptions**

**Figure a**: Schematic diagram of the workflow for Blood-draw CTC isolation, Labyrinth chip, CTC culture, CTC lines, and Functional and metabolic assays.

**Figure b**: Heatmap showing the enrichment of CAFs and PDAC cells.

**Figure c**: Relative expression of BCAT1, BCAT2, BCAT4, BCAT6, and CB7 in BCAA-deprived and + CTC1 coculture conditions.

**Figure d**: Western blot analysis showing the expression levels of HSP90, Vinculin, BCA1, and BCA2.

**Figure e**: Time course of Ki67 positive cells (% in pan-CK+ cells) in CTC1 and CTC2.

**Figure f**: Immunofluorescence staining for DAPI and pan-cytokeratin in CTC1 and CTC2.

**Figure g**: Mean enrichment of [13C]BCKA in PDAC tissue slice, hydrolysed protein and intracellular [13C]BCKA.

**Figure h**: Schematic diagram of the workflow for person with PDAC, Fresh tumour, Tissue embedding, Tissue slicing, Slice culture, 200-µm tumour slices, and Functional and metabolic assays.

**Figure i**: Comparison of Ki67 positive cells (% in pan-CK+ cells) and PCNA+ cells (% in pan-CK+ cells) between control and Gabapentin-treated CTC organoids.

**Figure j**: Flow cytometry analysis for EdU-positive cells in CTC organoids.

**Figure k**: Immunofluorescence staining for DAPI, Pan-cytokeratin, and Pan-cytokeratin in CTC organoids with control and Gabapentin treatment.
in PDAC development and found that BCAT2 is elevated in PDAC models\textsuperscript{42,43}. Our work develops further understanding of BCAA metabolism in the PDAC TME, especially in the context of the pancreatic cancer-cell centric observations of previous studies. To our knowledge, this is the first report that uncovers heavy reliance on BCKAs in stromal-rich tumours and reveals that DBT is a vulnerable target. Concurrently, we observed that BCKA-driven oxidation is dependent only on BCKDH expression, and not on BCAT2 expression. These results are in line with those from Neinast et al.\textsuperscript{7} that show that BCKDK inhibitors do not affect BCKDH-mediated oxidation, alluding to a high basal BCKD activity in the pancreas.

The population of CAFs we focus on herein is the myofibroblasts\textsuperscript{46}. Recently identified CAF subpopulations explain the heterogeneity seen in CAFs\textsuperscript{44–46}. These different CAFs may have unique metabolic profiles and therefore warrant future studies to assess the metabolic role of distinct CAF populations. Further, why slowly proliferating BCAT1-expressing CAFs have high BCAA consumption needs to be uncovered. BCAT1-mediated regulation of epigenetics via αKG\textsuperscript{2} could have a role in the αKG-mediated epigenetic regulation observed in stroma\textsuperscript{47,48}. Moreover, the effect of varying concentrations of gabapentin on LAT transporters in CAFs must be assessed in the future. Here, we used clinically relevant human PDAC-derived ex vivo models to corroborate and highlight the impact of the metabolic cross-talk discovered in vitro. Unfortunately, validating these observations in in vivo models is extremely challenging. One major divergence of mouse models from human PDAC tumours is the limitation of studying the interaction between human PDAC cancer cells and mouse CAFs. A second major challenge is the technological limitation of assessing compartmentalized metabolism in vivo, although improvements in mass-spectrometry imaging may allow spatial resolution of metabolites across tissue compartments. Third, most current mouse models suboptimally capture the complex phenotype of desmoplasia, so developments of models capturing desmoplasia in the tumour parenchyma are needed to support the mechanism described here.

Recently, PDAC cells have been shown to use ECM proteins for maintaining amino-acid levels\textsuperscript{49}. We found that CAFs uptake ECM under nutrient-limiting conditions, underscoring that ECM uptake through the uPARAP receptor is upregulated in fibroblast\textsuperscript{50}. CAFs were previously shown to secrete ECM and to induce a fibrotic environment in tumours. Our observations stress that this process is reversed under nutrient-deprived conditions, whereby internalized ECM is degraded through chymotrypsin-like proteasomal proteolysis. Further investigations are needed to ascertain whether ECM uptake through uPARAP or micropinocytosis is the dominant mechanism in PDAC tumours. Altogether, our results reveal vulnerabilities related to BCAA metabolism in desmoplastic PDAC that are synthetically lethal when cotargeting stromal BCAT1 and the cancer BCKDH complex, specifically DBT. This bridges the gap between knowledge of BCAA metabolism in stroma and BCKA utilization in cancer cells.

**Methods**

**Cell culture.** PDAC cell lines. All the cell lines used in this study were purchased from ATCC, used below passage 25 and continuously cultured in 100 µM penicillin and 100 µg ml\(^{-1}\) streptomycin. The MIA PaCa-2, Panc-1 and PaTu 8988T cell lines were routinely cultured in DMEM with 10% FBS (Atlanta Biologicals, S11150). AsPc1 and BxPC3 cell lines were routinely cultured in RPMI 1640 (Invitrogen) with 10% FBS. All cell lines were mycoplasma free as determined by PCR-based assays that were run every month in the laboratory. For metabolic and metabolomics assays, 10% dialysed FBS (Sigma-Aldrich, F0392) was used. For the rescue experiments, DMEM without BCAAs was used (United States Biological).

**Fibroblast cell culture.** Fibroblast cells derived from people with PDAC were kindly provided by E. Cukierman, A. Maitra and M. Sherman, and internal STR profiling was maintained and checked annually. CAFs were cultured at 37 °C under 5% CO\(_2\), using DMEM supplemented with 10% FBS, 100 µM penicillin and 100 µg ml\(^{-1}\) streptomycin. Normal fibroblast cell lines IMR-90 and MRC-5 were purchased from ATCC and cultured at 37 °C under 5% CO\(_2\), using DMEM supplemented with 10% FBS, 100 µM penicillin and 100 µg ml\(^{-1}\) streptomycin. MSCs were provided by M. Andreeff and cultured in α-MEM containing 10% FBS, 4% pooled human platelet lysate and 1% penicillin–streptomycin. Only third- or fourth-passage cells were used for experiments.

**CTC culture.** Cells were maintained at 37 °C, 5% CO\(_2\), under normoxic conditions. PDAC CTC-derived cell lines were grown in RPMI 1640 supplemented with 10% FBS and 1% antibiotic-antimycotic (Gibco).

**Tissue-slice culture.** Fresh pancreatic cancer tissue samples were procured immediately after surgical resection at the University of Michigan Hospital. Informed consent was obtained from all participants. The remaining connective, fibrotic or adipose tissue was removed with razor blades. Tumour specimens were embedded in 3% low-melting-point agarose/PBS and then were cut in the Leica VT1200 tissue slicer. The slice thickness ranged between 100 µm and 200 µm. Slices were then cultured in DMEM with penicillin (100 U ml\(^{-1}\)), streptomycin (100 U ml\(^{-1}\)) and amphotericin (Fungizone 2.5 µg ml\(^{-1}\)). All experiments were performed in triplicate and were repeated at least three times.

**CTC isolation from blood of people with PDAC and healthy controls.** The experimental protocol was approved by the University of Michigan Medicine Institutional Review Board, and all participants gave their informed consent to participate in the study. Participants were diagnosed with metastatic PDAC and were treatment-naïve at the time of the first sample collection. Blood was collected in EDTA tubes and processed within 2 h of sample collection. Red blood cells (RBCs) were depleted from the sample using RBC aggregation via HetaSep (STEMCELL Technologies) following the manufacturer's protocol. Briefly, blood
shRNA vectors were purchased from Sigma-Aldrich. The clone IDs for each shRNA are as follows: shBCAT1 (1), TRCN0000005907; shBCAT1 (2), TRCN0000010957; shBCAT2 (1), TRCN0000035115; shBCAT2 (2), TRCN0000028538. A non-targeting shRNA (shControl) was used as a control. Knockdown was confirmed by quantifying real-time qPCR (qRT–qPCR) or immunoblotting.

For CRISPR knockdown of BCAT2, sgRNA oligonucleotide pairs (pair 1, 5′-CACCGACCGATCATATGTGACG-3′, 5′-AAACCCGTCAG CATATGTCGTCG-3′; pair 2, 5′-CACCGGTTACGGATCATATGGTCG-3′, 5′-AAACCTGACATATGTCGTAAC-3′) were phosphorylated, annealed and cloned as previously described into the BbsI-linearized pSPCas9(2B)-2A-Puro (PX549) V2.0 (PX549) plasmid (Addgene, no. 62988).

qRT–PCR. Total RNA was isolated using TRIZol (Life Technologies) according to the manufacturer’s instructions. RNA concentration was determined using a purified RNA by NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific), and 1 μg of complementary DNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). qRT–PCR was performed using a QuantStudio 3 Real-Time PCR System (Applied Biosystems) with the Power SYBR Green PCR Master Mix (Invitrogen), per the manufacturer’s protocol and run on the QuantStudio 3. Detection thresholds were determined using the QuantStudio Design and Analysis Software.

Immunoblotting. Cells were washed twice in ice-cold PBS, scraped and collected in 700 μl trypsin. The cells and incubated at room temperature for 5 min, then further incubated for an additional 10 min at room temperature to improve cell dissociation. The cells were centrifuged at 300 g for 5 min. Supernatant was passed through a 0.45-μm filter. NOFs or MGCs were exposed to fresh medium for 30 min and washed 3 times with 5-min incubation PBS washes. Slides were incubated in the dark for 45 min at room temperature with secondary antibodies (against Alexa Fluor (AF) 488, 546 and 647). The slides were washed 3 times with 5-min incubation PBS washes and mounted using Prolong Gold Antifade Mountant with DAPI (Invitrogen). The slides were scanned using a Nikon T2 microscope at 20× magnification. The tiled images were individually analysed, and CTCs were identified on the basis of their fluorescent signature in each channel.

Cells were considered CTCs when they were DAPI+CD45 (AF 488) CK (AF 546)–. CTC phenotype was determined on the basis of vimentin expression. Cells were considered epithelial if they were DAPI+CD45 (AF 488) CK (AF 546)–Vim (AF 647)–, and epithelial to mesenchymal transition if they were DAPI+CD45 (AF 488) CK (AF 546)–Vim (AF 647)+.

Proliferation assay. Cells were cultured on a 96-well plate in the indicated conditions. For cancer cells, cell growth was measured thereafter as fluorescence intensity, using a plate reader (SpectraMax M5, Molecular Devices). For CAFs, a CyQUANT direct cell-proliferation assay was performed, according to the manufacturer's instructions.

Colony-formation assay. Cell growth of shRNA-treated cell lines was assayed for 16 h and was centrifuged at 3,000 r.p.m. for 5 min. Supernatant was passed through a 0.45-μm filter. NOFs or MGCs were fixed and processed with Simple ChIP Enzymatic Chromatin IP Kit (Magnetic Beads) (CST, 9003), according to the manufacturer's instructions. Chromatin IP was performed using the BCA assay. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting were performed in pre-cast bis–Tris 4–20% gradient gels (Bio-Rad). Blots were imaged using a ChemiDoc (Bio-Rad). The following antibodies were used: BCA1 (Novus Biologicals, NB2-01826), BAT2 (Cell Signaling Technologies, CST, 94325), DTT (Abcam, ab15191), HSP90 (CST, 4877) and Vinculin (Santa Cruz Biotechnology, sc-25336).

ChIP–qPCR. CAFs were treated with vehicle or 5 ng ml−1 TGF-β1, and then were cross-linked, fixed and processed with Simple ChIP Enzymatic Chromatin IP Kit (Magnetic Beads) (CST, 9003), according to the manufacturer's instructions. Chromatin IP was performed using the BCA assay. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting were performed in pre-cast bis–Tris 4–20% gradient gels (Bio-Rad). Blots were imaged using a ChemiDoc (Bio-Rad). The following antibodies were used: BCA1 (Novus Biologicals, NB2-01826), BAT2 (Cell Signaling Technologies, CST, 94325), DTT (Abcam, ab15191), HSP90 (CST, 4877) and Vinculin (Santa Cruz Biotechnology, sc-25336).

Dual-luciferase reporter assay. The Dual-luciferase reporter system was conjugated to the transcription start site of the NanoLuc gene in the pNL2.1 vector (Promega). CAFs were plated in 96-well plates 12 h before transfection. The NanoLuc reporter vectors were cotransfected with promoter firefly luciferase reporter vector using Lipofectamine 3000 Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. After 48 h of transfection, the luminescence was quantified and normalized using Nano-Glo Dual-Luciferase Reporter Assay (Promega).
Puromycin incorporation assay. SUNSET assay was performed as previously described1. Briefly, cells were incubated with 10 μg ml−1 puromycin (Thermo Fisher) for 10 min, followed by washing with ice-cold PBS and lysing with RIPA buffer. Cell lysates were loaded onto SDS-PAGE, and western blotting was performed with a mouse anti-puromycin monoclonal antibody (Millipore) and normalized against Ponceau S staining (Sigma).

Mitochondrial NADH/NAD+ measurement. Mitochondrial NADH/NAD+ sensor, RenRed, was prepared as previously described2. MIA PaCa-2 cells were transected using Lipofectamine 2000 Reagent (Thermo Fisher Scientific) according to the manufacturer’s protocol. Then cell medium was replaced by complete medium, BCAA-deprived medium or BCAA-deprived medium with BCKA. We used a Nikon A1Si Laser Scanning Confocal Microscope to visualize the fluorescence of transfected cells 24–48 h after transfection. Fluorescence detection was carried out with a 405 nm laser line for RenRed and 561 nm laser line for HyperRed-C1995. Imaging intensity was measured, and ratio imaging was generated by Nikon NIS-Elements AR.

Flow cytometry. In the mixed coculture, cancer-associated fibroblasts were seeded in a 6-well plate for 4 h, and the SUNSET assay was performed as described above, and cocultured for 3 d in the indicated medium. Puromycin intensity was analysed by FACS in tumour cells with GFP gating. For the NADH/NAD+ measurement, PKH26-labelled cells were added and cocultured for 3 d in the indicated medium. NADH/NAD+ ratio was analysed by FACS in tumour cells with PKH26 gating. All data were acquired with the Bio-Rad ZES flow cytometer analyser, and analyses were performed using FlowJo.

Measurements of OCR. Mitochondrial OCR was measured by XF96 Analyzer (Seahorse Biosciences). Cells were seeded in 96-well Seahorse plates and incubated at 37 °C with 5% CO2 overnight. Medium was replaced with 100 μl medium free of serum and sodium pyruvate. Plates were loaded onto the Seahorse and equilibrated for 1 h before they were placed in an analyser. The OCRs were measured with procedure of 3 min of mixing, 2 min of waiting and 3 min of measuring. Oligomycin, FCCP and antimycin were injected through port A, B and C, respectively, to calculate mitochondrial function under different stress. All data were normalized to total cell protein as measured by the BCA assay.

Substrate-specific OCR. OCR was measured in MAS medium supplemented with 0.2% (wt/vol) BSA, 4 mM ADP, 1 mM XF Plasma Membrane Permeabilizer (Seahorse Bioscience), 500 nM coenzyme B12 and biontin, and oligomycin, FCCP and antimycin were sequentially added. Permeabilized pancreatic cancer cells were offered 5 mM BCCAs. Substrate-specific respiration was calculated as the maximum respiration, and the data were normalized to total cell protein.

HIF and IF staining. Tissues were fixed in 10% formalin overnight and embedded in paraffin. PDAC sections were deparaffinized in xylene, dehydrated through sequential ethanol, and rinsed in PBS. Non-specific signals were blocked using 10% goat serum in 0.1% Triton X-100. Tumour samples were stained with primary antibodies against the following: α-SMA (Sigma, A5228, 1:500), BCAT1 (Sigma, HPA048592, 1:200), SMAD5 (Sigma, HPA058931, 1:200), Ki67 (Santa Cruz Biotechnology, sc-239001:500) and PCNA (Santa Cruz Biotechnology, sc-561:500). α-SMA (Sigma, A5228, 1:500), BCAT1 (Sigma, HPA048592, 1:200), SMAD5 (Sigma, HPA058931, 1:200), Ki67 (Santa Cruz Biotechnology, sc-239001:500) and PCNA (Santa Cruz Biotechnology, sc-561:500). After overnight incubation, the slides were washed and incubated with biotinylated secondary antibody (Vector Laboratories) for 30 min at room temperature. All slides were then incubated with avidin–biotin peroxidase complex for 30 min, and the signals were visualized by using DAB Substrate Kit (Vector Laboratories). The tissue sections were counterstained with VECTASTAIN (Vector). The slides were de-embedded and dehydrated with ethanol at concentrations of 50%, 70%, 90%, 95% and 100% at room temperature for 1 h and then rinsed with PBS, followed by sequential dehydration with ethanol at concentrations of 50%, 70%, 90%, 95% and 100% for 10 min each. The specimens were then immersed in a mixture of 3:1 dichloromethane: hexane for 10 min and then dried to transparency in a desiccator. The dehydrated specimen attached to a carbon double-sided tape are mounted on a SEM stub and coated with gold by sputtering. The SEM was examined by FEI Nova 200 Nanolab Dualbeam FIB scanning electron microscope under low beam energies (2.0–5.0 keV) at the Michigan Center for Materials Characterization (MC2) at University of Michigan.

Bioinformatics analysis of clinical data. Deconvolution and gene expression analysis. Data from real tumour samples available via the CBioPortal Database (most updated TCGA data for Pancreatic Ductal Adenocarcinoma patients) reflects the genomic and transcriptomic profile of a mixed population of cells in the TME. In order to compare the transcriptomic signatures of cancer cells and stromal cells (cancer-associated fibroblasts, CAFs) in the TCGA data we developed a method to classify tumour samples as stromal-dominant and epithelial-dominant to represent CAFs and cancer cells. We used putative stromal and epithelial markers to identify samples stromal- and epithelial-dominant. We performed k-means clustering using gene expression of 7 epithelial markers (CDH1, EPCAM, KR7/8/10/18/19) and 46 myofibroblast markers (ACTA2, IL1B, FAP, CCN2, ACTN4, CSF2RA, COL1A1, ITGB2, ITGAV, TIMP2, ADAM12, COL6A3, MMP9, FN1, PDGFC, COL1A2, COL6A2, FBN1, MMP2, MMP14, PLAU, PECAM1, CD93, CXCL12, SPRY1, ERG, ROBO4, T0XN, ACAN, CH131L1, COL1A2, COMP, EFEMP2, FBLN1, FBLN2, FBN1, FBN2, IMPG2, ANOS1, LAMA4, LAMBI, LAMCN1, MATN3, MMP26) and 8 myofibroblast markers (MGP, TIMP3, PREL1) to cluster samples into 3 or 4 clusters. Based on the tumour gene expression was performed to identify tumour samples that showed a dominant epithelial signature to represent cancer-cell populations, and a dominant fibroblast signature to represent CAF-cell populations. Since tumour samples are heterogeneous, containing cells other than malignant and CAF cells, we employ unsupervised k-means clustering based on epithelial and stromal markers alone. We account for ‘mixed’ clusters, which represent samples that do not fall into either of the epithelial or stromal clusters. We tune the number of clusters, k, for the algorithm to pick out samples with distinct epithelial and CAF signatures. Performance of the k-means clustering is verified by assessing the distribution of samples with respect to their average stromal gene expression and average epithelial gene expression. Finally, expression of genes involved in the anchorage- chain amino-acid metabolism (BCAT1/2, BCKDHA/B, DBT and DLD) were compared across the clustered samples using a two-tailed t-test. ROBO1 expression was also compared across the clusters to independently validate the performance of clustering, since the ROBO1 gene is known to be highly expressed in stromal cells. The same methodology is employed for microarray data for PDAC tumour samples.
available from GSE21501, GSE36924 and GSE26165 (refs. 17,18), as well as RNA-seq for healthy tissue (GTEx Analysis v8) from the GTEx Portal, accessed on 29 August 2019. These studies were performed to discover molecular subtypes in people with PDAC, which involved employing high-throughput transcriptomics analysis of tumour and normal tissue samples.

Differential expression from PDAC tumours microdissected by lasers. Raw counts of gene-expression data were downloaded from GSE93326 (ref. 19) and normalized using DESeq2. Normalized gene expression data were plotted to represent epithelial–stromal pairs obtained from the same tumour. A paired Wilcoxon t-test was used to estimate statistical significance of differential expression.

Single-cell gene-expression analysis. CTC expression clustering. Gene expressions of BCAAMetabolism genes (BCAT1/2, BCKDHA and DBT) were measured in day 0 CTCs and primary CAFs using qRT–PCR. Expression values of respective genes were normalized to gene expression measured in CAF1. Normalized expression values were converted to z-scores, followed by unsupervised hierarchical clustering.

PDAC single-cell RNA-seq clustering. Preprocessed single-cell RNA-seq data from 2 datasets published by Bernard et al.18 and Peng et al.19 were used in the analysis containing single-cell RNA-seq data from 2 and 24 people with PDAC, respectively. The data from Bernard et al.18 was first filtered to remove outlier cells, and only cells with total gene expression between 200 and 6,100 UMI were retained for the downstream analysis. The filtered gene-expression data for each cell were scaled and normalized. The average expression intensity for each gene was computed, and the z-score of the dispersion of expressions from the mean was computed for each cell to identify genes that are differentially expressed across all cells. Principal component analysis (PCA) was then used to identify the most variable genes across all cells. An elbow plot of the PCA suggested the first 20 PCs were sufficient to capture most variance needed for the unsupervised clustering of the cell data using a PCA-based approach. The clusters generated were assigned using k-distributed stochastic neighbour embedding (t-SNE) and identified using known cell-type markers. All analyses were performed in R (v3.5) following the Seurat (v2.3.4) pipeline20. The cluster information based on cell-type markers in the Peng et al.19 study was used to generate a dotplot (Seurat v2.3.4) for visualizing the differential gene expression of BCAAMetabolism genes.

Metabolic-flux analysis. Metabolite extraction for in vitro metabolomics and tracer studies. Cells cultured in 6-well plates were quenched with 800 µl of ice-cold methanol/water (1:1) solution containing 1 µg norvaline. Cells were scraped while plate was kept on ice, followed by addition of 800 µl chloroform. The cell extracts were transferred to microcentrifuge tubes and vortexed for 30 min at 4°C.

Metabolite extraction from tissue slices for metabolomics and tracer studies. Frozen tissue slices were transferred to Precellys CKMix Lysing tubes (Bertin 03961-1-009) and kept on dry ice; 200 µl ice-cold methanol/water (1:1) solution containing 1 µg norvaline was added to the tubes to barely submerge the beads and tissue. The samples were homogenized using a Precellys Evolution Homogenizer with the Cryolys attachment to maintain the temperature below 4°C in the homogenization chamber. Homogenization was achieved using two 30-s cycles at 6,000 r.p.m., with a pause of 60 s. Additional homogenization cycles were performed only when samples were not homogenized. Following homogenization, an additional 600 µl of ice-cold methanol/water (1:1) solution, as well as 800 µl chloroform, was added to the tubes. The homogenized extracts were transferred to microcentrifuge tubes and vortexed for 30 min at 4°C.

Sample processing for polar metabolites, and amino-acid composition of proteins and lipids. Metabolite extracts were centrifuged at 14,000g for 10 min to separate the polar phase, protein interphase and chloroform phase. The water/methanol phase containing polar metabolites was transferred to fresh microcentrifuge tubes and dried in a SpeedVac and stored at −80°C until GC–MS analysis. Chloroform-phase-containing lipids were transferred to microcentrifuge tubes and dried under nitrogen and stored at −80°C. The protein layer was rinsed gently with chloroform, then PBS, and the liquid was discarded. The rinsed protein fractions were transferred to glass tubes with sealable caps and subjected to acid hydrolysis with 6 M hydrochloric acid at 100°C for 18–24 h to obtain constituent amino acids. Hydrolysed samples were dried under nitrogen and stored at −80°C until GC–MS analysis.

GC–MS analysis for intracellular polar metabolites and amino acids from hydrolysed protein. Thirty microlitres of methoxyamine hydrochloride (MOX, Thermo Scientific) was added to dried samples, which were incubated at 30°C for 2 h with intermittent vortexing. Forty-five microlitres of MSTFA + 1% TBDMS was added to the samples, which were incubated at 55°C for 1 h. Derivatized samples were transferred to GC vials with glass inserts and added to the GC–MS autosampler queue. GC–MS analysis was performed using an Agilent 3900 GC equipped with a 30-m HP-5MSU capillary column connected to an Agilent 5977B MS. For polar metabolites, the following heating cycle was used for the GC oven: 100°C for 3 min, followed by a ramp of 5°C min−1 to 300°C and held at 300°C for a total run time of 48 min. Data were acquired in scan mode. The relative abundance of metabolites was calculated from the integrated signal of all potentially labelled ions for each metabolite fragment. Mass isotopologue distributions were corrected for natural abundance using IsoCor prior to analysis with the model. Metabolite levels were normalized to internal standard Norvaline’s signal and quantified using 6-point calibration with external standards for 19 polar metabolites.

LC–MS analysis to quantify BCKA secretion in media samples. Spent culture media samples were collected from culture plates, and 200 µl was transferred to fresh microcentrifuge tubes for metabolite analysis. Eight hundred microlitres of prechilled methanol was added to media samples and kept at −20°C for 2 h to deproteinize the samples. The samples were centrifuged at 14,000g for 10 min at 4°C, following which the supernatant was transferred to fresh tubes and dried in the SpeedVac. Media samples were derivatized with 500 µl of 12.5 mM O-phenylenediamine solution in 2 M hydrochloric acid. Samples were incubated at 80°C for 20 min and transferred to ice to cool to room temperature. The derivatized solution was transferred to microcentrifuge tubes containing 0.08 g of sodium sulfate. Five hundred microlitres of ethyl acetate was added to the samples, followed by vigorous vortexing and centrifuging at 1,000g for 10 min. The ethyl-acetate phase containing the derivatized ketocids was transferred to fresh tubes and dried under nitrogen.

The dried samples were reconstituted in 200 µl of 200 mM ammonium acetate solution and transferred to LC vials with glass inserts. The samples were analysed with an Agilent Infinity LC stack using an Agilent Eclipse Plus C18 column (2.1 mm × 100 mm × 1.8 µm) connected to an Agilent 6520 QTOF mass spectrometer. The following parameters were used for analysis: 5 M ammonium acetate as solvent A, 2 M ammonium acetate + 0.1% formic acid as solvent B, 0.4 µl injection volume, 55% B for 4.2 min, ramp B to 95% for 0.9 min, retain 95% B for 1.5 min, return to initial conditions for 2.5 min. The analysis was performed in full-scan mode with the MS in the positive ion mode.

MPE. MPE represents the fractional contribution of 13C from a substrate to intermediate metabolite. It is calculated as follows, where N is the number of carbons that can be labelled as 13C, and χ is the fraction of (M + 1)th isotopologue:

\[ MPE = \frac{\sum_{i=1}^{n} x_i N_i}{N} \]

Newly synthesized BCKA flux. To estimate de novo synthesized BCKA flux from 13-carbon labelled BCAAs, we measured 13C enrichment in respective BCKAs before cells reached the isotopic steady state, using the following equation, where t is time, and T is the time to achieve 13C enrichment:

\[ \text{BCKA catabolic flux} = \frac{(13C \text{ mean enrichment})_{t} - (13C \text{ mean enrichment})_{0}}{\text{Intracellular abundance of BCKA}}\]

Statistics and reproducibility. Data are presented as mean ± s.d. All experiments were repeated twice with similar results unless otherwise stated. GraphPad Prism software v8.4 was used to conduct the statistical analysis of all data. Comparison of the datasets of the experiments with the different experiment conditions was performed with the two-tailed Student’s t-test. Comparisons between multiple groups were done with one-way ANOVA with Tukey’s post hoc comparison, and two-way ANOVA with Dunnett’s post-testing for comparisons between multiple groups with independent variables. *p < 0.0001 unless otherwise stated.

Illustrations. Mitochondria used in the branched-chain amino-acid metabolism scheme (Fig. 1) was drawn using the PathWiz tool21. The human body figure used in experimental schematics (Fig. 6 and Extended Data Figure 9) was obtained from the https://www.freepik.com/ database of free vector images.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Code availability
R code used for stromal/epithelial deconvolution, heatmap generation and violin plots and bioinformatics analysis is available from the corresponding author upon request. Source data are provided with this paper.

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assays. J.F.-B. and E.C. provided CAFs and helped with stromal characterization, J.S. helped with patient tissue collection, pathological interpretation and IHC, V.G. and V.S. collected blood for CTC analysis, M.H.S. provided CAFs and helped with stromal characterization, A.M.P. helped with proteasomal analysis, A. Maitra analysed the data and helped in clinical correlations and M.A.M. and T.S.L. provided tissue slices and helped in designing various experiments. Z.Z., A.A. and D.N. wrote the manuscript with input from coauthors.

Competing interests
A. Maitra receives royalties from Cosmos Wisdom Biotechnologies for a license related to a biomarker test for pancreatic cancer early detection. V.S. is a consultant at Halozyme, QED, Ipsen and Incyte. V.S. receives funding from Celgene, Bristol-Myers Squibb, Agios, Incyte, Clovis Oncology, Debiopharm Group, FibroGen, Halozyme, MedImmune, Rafael Pharmaceuticals and Ipsen. M.A.M. receives honoraria from AstraZeneca. S.N. is the named inventor on a patent for Microfluidic Labyrinth Technology granted to the University of Michigan. S.N. is a co-founder of Labyrinth Biotech. The funders and the company had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Additional information
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Transcriptomic analysis of BCAA metabolic genes in PDAC tumors. 

**a.** Expression of BCAT1 healthy tissue samples from the GTEx database (Brain, n = 2642; Prostate, n = 245; Testis, n = 361; Pancreas, n = 328; Ovary, n = 180).  

**b.** Expression of genes involved in BCAA metabolism in samples from GSE21501 (n = 132). Tumor samples with dominant epithelial markers and dominant fibroblast markers are deconvolved to compare expression of metabolic genes between pancreatic cancer cells and stromal cells in the TME. ROBO1 is a marker for validation that has been found to be expressed in stromal cells but not in cancer cells in independent studies.  

**c.** Expression of genes involved in BCAA metabolism in samples from GSE36924 (n = 91).  

**d.** Expression of genes involved in BCAA metabolism in samples from GSE62165 (n = 118). Samples with dominant epithelial markers and dominant fibroblast markers are deconvolved to compare expression of metabolic genes between epithelial cells and stromal cells (a–d).  

**e.** Gene expression of BCAA pathway genes and ROBO1 in paired epithelial and stromal compartments obtained by laser microdissection (GSE 93326, n = 63 paired samples). Violin plot represents all data points in each group (a–d). Boxplot limits represent median and interquartile range (IQR), and notches represent 1.5*IQR (a–e). Data analyzed using multiple, two-tailed, unpaired, Student’s t-test (a–d); multiple, two-tailed, paired, Student’s t-test (e).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Characterization of BCAA metabolism. 

**a.** Representative IHC staining image comparing BCAT1 expression between stromal and tumor compartments. Experiments were repeated independently three times with similar results. 

**b.** Representative IF images showing protein expression of stromal αSMA, BCAT1 and Vimentin from paired healthy and PDAC tissue. Experiments were repeated independently twice with similar results.

**c.** Absolute cell numbers of PDAC CAFs were determined in the presence or absence of BCAA. $n = 3$ biologically independent samples.

**d.** Absolute cell numbers of PDAC cell were determined in the presence or absence of BCAAs. $n = 3$ biologically independent samples. Data are presented as mean ± s.d.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | PDAC cells are BCAT2 dependent for growth. a. Fluorescence microscopy images merged with brightfield images comparing growth of GFP-labeled Mia Paca-2 and Panc-1 cells in contact co-cultures with CAFs or NOFs under BCAA deprivation. Experiments were repeated independently three times with similar results. b. Relative growth rates of Mia Paca-2 cells co-cultured with CAFs or NOFs at different seeding ratios under BCAA deprivation. n = 3 biologically independent samples. c. Relative growth rates of AsPC1 and BxPC-3 cells co-cultured with CAFs or NOFs under BCAA deprivation. n = 3 biologically independent samples. d. Relative growth rates of Mia Paca-2 cells in various concentrations of BCAAs or BCKAs. n = 6 biologically independent samples. e. Model for the rescue of proliferation in BCAT2 KD cancer cells by BCKAs released from CAFs under BCAA deprivation. f. Relative growth rates of Mia Paca-2 and Panc-1 cells co-cultured with ATG-5/7 knockdown CAFs. n = 3 biologically independent samples. g. Relative growth rates of Mia Paca-2 and Patu 8988t cells cocultured with CAFs treated with autophagy inhibitors (chloroquine, Bafilomycin A1 and LY294002) under BCAA deprivation. n = 3 biologically independent samples. *P < 0.0001. Data are presented as mean ± s.d. Two-tailed, unpaired, Student’s t-test (c).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4  |  BCKDH complex is essential for PDAC cells growth and cell biosynthesis.  

**a.** Relative proliferation rates of Mia Paca-2, Panc-1 and Patu 8988t cells expressing control shRNA or two independent shRNAs to DBT.  

n = 8 biologically independent samples.  

**b.** Colony-formation assay of DBT knockdown pancreatic cell lines.  

n = 3 biologically independent samples.  

**c.** Relative growth rates of Patu 8988t, Mia Paca-2 cells, and CAFs treated with BCKDK inhibitor, 3,6- dichlorobenzo[b]thiophene-2-carboxylic acid (BT2).  

n = 3 biologically independent samples.  

**d.** Relative growth rates of MiaPaca-2 and Patu 8988t cells under BCAA deprivation and low glucose and low glutamine conditions after supplementation with BCKAs.  

n = 3 biologically independent samples.  

**e.** Schematic for the loss of rescue in DBT knockdown cancer cells by BCKAs released from CAFs under BCAA deprivation.  

**f.** Colocalization of Mitotracker and RexMito fluorescence in Mia Paca-2 cells. Mitotracker (red), RexMito (green), and DAPI (blue). Experiments were repeated independently three times with similar results.  

**g.** Substrate-specific oxygen consumption rate (OCR) in permeabilized pancreatic cancer cells.  

n = 4 biologically independent samples.  

**h.** OCR of Panc-1 cells after BCAT2 and DBT knockdown.  

n = 18 biologically independent samples.  

**i.** Substrate-specific OCR of BCAT2 knockdown pancreatic cancer cells.  

n = 4 biologically independent samples.  

**j.** Substrate-specific of DBT knockdown pancreatic cancer cells.  

n = 4 biologically independent samples.  

*P < 0.0001.  

Data are presented as mean ± s.d.  

One-way ANOVA with Tukey’s post hoc comparison (a, j); two-way ANOVA with Dunnett’s multiple comparison test (i, j).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | CAFs have upregulated collagen uptake under BCAA deprivation. **a**, BCAT activity in CAFs treated with Gabapentin measured by spectrophotometric assay. $n = 6$ biologically independent samples. **b**, Growth rate of Panc-1 cancer cells with Gabapentin, BCKAs, and CAF coculture under BCAA deprivation. **c**, The effect of knockdown of BCAT1 in CAFs on CAF growth rates. $n = 4$ biologically independent samples. **d**, Uptake of DQ-Collagen by CAFs after 24 h measured using confocal imaging. Experiments were repeated independently three times with similar results. **e**, Uptake of DQ-Collagen by PDAC cell lines and CAFs after 24 h measured using confocal imaging. Experiments were repeated independently three times with similar results. **f**, Flow cytometry assay of MRC2 expression in PDAC cell lines. Experiments were repeated independently three times with similar results. **g**, Flow cytometry assay of MRC2 expression in CAFs. Experiments were repeated independently three times with similar results. *$P < 0.0001$. Data are presented as mean ± s.d. One-way ANOVA with Tukey’s post hoc comparison (b).
Extended Data Fig. 6 | CAFs uptake collagen through the proteasome. a. Uptake of DQ-Collagen by CAFs transfected with siControl or siuPARP measured using confocal imaging after 24 h. Experiments were repeated independently three times with similar results. b. CAFs are cultured with 13C-BCAAs for 12 h prior to inducing BCAA deprivation. Spent media and cells are collected after 6, 12, 24, and 48 h under deprivation. Media samples are analyzed for secreted BCKAs using LC-QTOF and intracellular samples are analyzed for BCAAs using GC-MS. c. Intracellular BCAA levels measured after 6, 12, 24 and 48 h under BCAA deprivation. Mole percent enrichment of intracellular BCAAs measured after 6, 12, 24, and 48 h under BCAA deprivation. n = 3 biologically independent samples.
d. Influence of TGF-β and BCAA deprivation on the proteasome activity in CAFs (n = 6). e. Relative growth rates of Mia Paca-2 and Panc-1 cells cocultured with CAFs treated with MG-132 under BCAA deprivation conditions. n = 8 biologically independent samples.
f. Mass isotopomer distribution of BCAAs after acid hydrolysis of decellularized ECM proteins produced by CAFs cultured with 13C-BCAAs. n = 3 biologically independent samples. g. Fractional enrichment of amino acids after acid hydrolysis of decellularized ECM proteins produced by CAFs cultured with 13C-BCAAs. n = 3 biologically independent samples. Data are presented as mean ± s.d.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Stromal BCAT1 is regulated by cancer-cell derived TGF-β. a. BCAT2 expression in NOFs treated with pancreatic cancer cell conditioned media (CM). n = 8 biologically independent samples. b. α-smooth muscle actin, (α-SMA) expression in NOFs cultured with pancreatic cancer cell-CM over 4 weeks. n = 8 biologically independent samples. c. Fibroblast specific protein (FSP1) expression in NOFs cultured with pancreatic cancer cell-CM over 4 weeks. n = 8 biologically independent samples. d. Podoplanin (PDPN) expression in NOFs cultured with pancreatic cancer cell-CM over 4 weeks. n = 8 biologically independent samples. e. BCAT2, α-SMA, FSP-1 and PDPN expression in MSCs treated with pancreatic cancer cell CM. n = 6 biologically independent samples. f. Expression of BCAA related genes in CAFs treated with pancreatic cancer cell-CM. n = 8 biologically independent samples. g. BCAT2 expression in CAFs treated with TGF-β and BCAT1 expression in cancer cells treated with TGF-β. n = 8 biologically independent samples. h. BCAT2 and α-SMA expression in NOFs cultured with pancreatic cancer cell CM in presence of anti-TGFB1 antibodies or isotype antibodies for 3 weeks. n = 8 biologically independent samples. Data are presented as mean ± s.d.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Cancer cells regulate stromal BCAT1 through SMAD5. 

a. Representative images from IF analysis of BCAT1 and α-SMA expression in NOFs cultured with pancreatic cancer cell CM in presence of anti-TGFB1 antibodies or isotype antibodies for 3 weeks. Experiments were repeated independently twice with similar results. 
b. BCAT1 and BCAT2 expression in control and integrin αvβ5 KO CAFs cultured with pancreatic cancer cell CM for 3 weeks. n = 4 biologically independent samples. 
c. ELISA for TGF-β1 secretion levels from CAFs and PDAC cell lines. n = 8 biologically independent samples. 
d. SMAD2 expression in NOFs treated with pancreatic cancer cell CM. n = 8 biologically independent samples. 
e. SMAD3 expression in NOFs treated with pancreatic cancer cell CM. n = 8 biologically independent samples. 
f. SMAD4 expression in NOFs treated with pancreatic cancer cell CM. n = 8 biologically independent samples. 
g. SMAD5 binding motif. 
h. ChIP assays performed with control IgG and anti-SMAD4 antibodies in CAFs treated with PBS control or TGF-β1. n = 6 biologically independent samples. *P < 0.0001. Data are presented as mean ± s.d. Multiple, two-tailed, unpaired, Student’s t-test (b); one-way ANOVA with Tukey’s post hoc comparison (c).
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Validation of stromal BCAT1 and PDAC DBT in patient-derived CTCs. 

a. Representative images of CTCs separated by Labyrinth. Cells are stained with DAPI (blue), cytokeratin (red), CD45 (green) and Vimentin (pink). Experiments were repeated independently three times with similar results.

b. The influence of BCAAs and BCKAs on the growth of CTCs. n = 8 biologically independent samples.

c. Extracellular concentration of BCKAs secreted by CAFs in monoculture and cocultured with CTCs over 6, 12, 24, and 48 h. n = 3 biologically independent samples.

d. Extracellular concentration of BCKAs secreted by CAFs in monoculture or cocultured with CTCs, and CTCs in monoculture for 48 h. n = 4 biologically independent samples.

e. Relative growth rate of PDAC cell lines and CTC lines under BCAA deprivation but supplemented with αKG, malate, acetate, citrate, NEAA mixture, or a combination in BCAA-deprived media. n = 8 biologically independent samples.

f. Schematic of the protocol used to generate CTC derived organoid with CAF secreted ECM.

g. Representative images from a CTC-derived organoid. Cytokeratin is shown in green and the nuclei stained with DAPI are shown in blue. Experiments were repeated independently three times with similar results.

h. Representative FACS data of Pan-Cytokeratin positive tumor cells in CTC derived organoids. Experiments were repeated independently three times with similar results.

i. Representative images of CTC derived organoids. Cells are stained with DAPI (blue), cytokeratin (red), CD45 (green) and Vimentin (pink). Experiments were repeated independently twice with similar results. *P < 0.0001. Data are presented as mean ± s.d. Multiple, two-tailed, unpaired, Student’s t-test (b).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Validation of stromal BCAT1 and PDAC DBT in patient-derived tissue slices. a. Representative Live Dead assay of tissue slice at Day 0 and Day 14. Live cells fluoresce bright green, whereas dead cells fluoresce red. Negative controls were fixed by methanol. Experiments were repeated independently three times with similar results. b. Efficiency of BCAT1 and DBT siRNAs in the human PDAC tissue slices. Expression of BCAT1, BCAT2, DBT, BCKDHA and BCKDHB in the human PDAC tissue slices treated with BCAT1 and DBT siRNAs. n = 6 biologically independent samples. Data are presented as mean ± s.d.
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|     | Give P values as exact values whenever suitable. |
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code.

Data collection
Confocal images were collected on a Nikon A1Si Laser Scanning Confocal Microscope using NIS-Elements Software v4.50. SEM images were obtained using a FEI Nova 200 Nanolab Dualbeam FIB scanning electron microscope. Real time quantitative PCR was performed on the QuantStudio 3. Flow cytometry samples were analyzed on a Bio-Rad ZE5 Cell Analyzer and exported with ZE5 Everest Software v2.3. Oxygen consumption was measured using a Seahorse Bioanalyzer running Wave software v2.6. Plate reader data was collected using a SpectraMax M5 Microplate Reader using SoftMax Pro 5 software. Immunobots were imaged using a Biorad ChemiDoc MP Imaging System running image Lab 6.0. Metabolomics and tracer experiment data was obtained from either an Agilent GC-MS (7890/5977B) or an Agilent LC-QTOF (Infinity LC/6520 QTOF).

Data analysis
Quantification of confocal images and densitometry of immunobots was performed using NIS-Elements Software v4.50. Flow data was analyzed with Flowjo software v10.0. Oxygen consumption was calculated using the Agilent Seahorse XF Mito Stress Test Report Generator. Real-time PCR data was analyzed with QuantStudio V1.5. Metabolomics and tracer experiment data was acquired and analyzed with Agilent MassHunter software v4.2. All statistical analysis was done using GraphPad Prism v 8.4. Single Cell RNAseq analyses were performed in R [v.3.5] following the Seurat v 2.3.4 pipeline. The cluster information based on cell-type markers was used to generate a dotplot (Seurat v 2.3.4) for visualizing the differential gene expression.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical methods were used to predetermine sample size. Sample size was based on experimental feasibility, sample availability, and N necessary to obtain definitive, significant results.

Data exclusions
No data were excluded

Replication
Experimental findings were successfully replicated. The number of replicates for each experiment is indicated in the figure panels/methods as required.

Randomization
To the best of the authors’ knowledge, all allocation into experimental groups were random

Blinding
Blinding was utilized for the GC-MS and LC-MS studies. For other experiments, blinding is not applicable because the investigator who set up the experiment is the same person doing analyses.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|-------------------------------|---------|
| n/a | Involved in the study | r/a | Involved in the study |
| ☑ | Antibodies | ☑ | ChiP-seq |
| ☑ | Eukaryotic cell lines | ☑ | Flow cytometry |
| ☑ | Palaeontology | ☑ | MRI-based neuroimaging |
| ☑ | Animals and other organisms | | |
| ☑ | Human research participants | | |
| ☑ | Clinical data | | |

Antibodies

Antibodies used

- HSP90 (CST, 4877), Vinculin (Santa Cruz Biotechnology, sc-25336), BCAT1 (Novus Biologicals, NBP2-01826), BCAT2 (Cell Signalling Technologies [CST], 9432S), DBT (Abcam, ab151991), SMAD4 antibody (CST, 38454), SMAD5 antibody (CST, 12534), BCAT1 (Sigma, HPA048592, 1:200), SMAD5 (Sigma, HPA058831, 1:200), Pan-Cytokeratin [CK] (Bio-Rad, MCA1907) CD45 (Bio-Rad, MCA87GA), Vimentin [Vim] (CST, 5741), Proteasome 20S alpha + beta (Abcam, ab22673), Puromycin AAF/647 [Sigma, MABE343-AF647], DMSA [Sigma, A5228, 1:500], Ki-67 (Santa Cruz Biotechnology, sc-239001, 500), PCNA (Santa Cruz Biotechnology, sc-5625), anti-Mouse IgG3a, Alexa Fluor 488 (Thermo-A21131), anti-Mouse IgG1, Alexa Fluor 546 (Thermo-A21213) and Anti-rabbit IgG (H+L), Alexa Fluor 647 (Thermo-A21245). All antibodies were diluted to 1:1000 with the exception of BCAT1 and SMAD5 for IHC which were diluted to 1:200.

Validation

All antibodies were validated by the manufacturers and by extensive use in published work. A complete list of citations are available on manufacturers’ websites.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  CAF1-5, B-9, GFP KO and αvβ3-integrin KO CAFs were provided by E. Cukierman (Fox Chase Cancer Center). CAF7 was provided by A. Mastra (MD Anderson Cancer Center). CAF6, 10 were provided by M. Sherman (MD Anderson Cancer Center). AsPC-1, BxPC-3, Mia Paca-2, Panc.1 and Patu 8988t PDAC cell lines and IMR 90, MRC-5 fibroblast cell lines were purchased from ATCC.

Authentication  All cell lines were STR profiled for authenticity.

Mycoplasma contamination  All cell lines were routinely tested negative for mycoplasma.

Commonly misidentified lines

(See [CLAC register](#))

None used.

Human research participants

Policy information about studies involving human research participants

Population characteristics  Pancreatic adenocarcinoma; pre-treatment, age 18 years or greater;

Recruitment  The experimental protocol was approved by the University of Michigan Medicine Institutional Review Board and all patients gave their informed consent to participate in the study. Patients were diagnosed with metastatic pancreatic ductal adenocarcinoma (PDAC) and were treatment naive at the time of the first sample collection. Patients for tissue slice samples were recruited from the Rogel Cancer Center Clinics with no perceived bias since all eligible patients are offered enrollment on the Pancreatic Biospecsatory. For tissue slices, ethical approval was obtained and approved by the University of Michigan Institutional Review Board (approved protocol #HUM00072034). Specimens were obtained via the Tissue Procurement Services of the University of Michigan. Formal consent was obtained from the patient/donor following meeting with a research coordinator.

Ethics oversight  All subjects were enrolled on a HIPAA compliant, University of Michigan IRB approved study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation  Sample preparation listed in Methods

Instrument  Bio-Rad ZE5 Cell Analyzer

Software  FACs data were analyzed with FlowJo software v10.1 (FlowJo, LLC). Data was further analyzed with Prism.

Cell population abundance  For all studies, 100,000 cells were collected per sample.

Gating strategy  First, for an SSC-A (y) vs FSC-A (x) dot plot, a “cells” gate was used to exclude small and large debris that fell in the bottom right corner or off-scale on either axis. Cancer cells then gated base on GFP/PanCK histogram, cells were analyzed for Puromycin AF647 intensity or EdU positive percentage.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.