Altered exocrine function can drive adipose wasting in early pancreatic cancer

Laura V. Danai, Ana Babic, Michael H. Rosenthal, Emily A. Dennstedt, Alexander Muir, Evan C. Lien, Jared R. Mayers, Karen Tai, Allison N. Lau, Paul Jones-Sali, Carla M. Prado, Gloria M. Petersen, Naoki Takahashi, Motokazu Sugimoto, Jen Jen Yeh, Nicole Lopez, Nabeel Bardeesy, Carlos Fernandez-del Castillo, Andrew S. Liss, Albert C. Koong, Justin Bu, Chen Yuan, Marisa W. Welch, Lauren K. Brais, Matthew H. Kulke, Courtney Dennis, Clary B. Clish, Brian M. Wolpin & Matthew G. Vander Heiden

Malignancy is accompanied by changes in the metabolism of both cells and the organism. Pancreatic ductal adenocarcinoma (PDAC) is associated with wasting of peripheral tissues, a metabolic syndrome that lowers quality of life and has been proposed to decrease survival of patients with cancer. Tissue wasting is a multifactorial disease and targeting specific circulating factors to reverse this syndrome has been mostly ineffective in the clinic. Here we show that loss of both adipose and muscle tissue occurs early in the development of pancreatic cancer. Using mouse models of PDAC, we show that tumour growth in the pancreas but not in other sites leads to adipose tissue wasting, suggesting that tumour growth within the pancreatic environment contributes to this wasting phenotype. We find that decreased exocrine pancreatic function is a driver of adipose tissue loss and that replacement of pancreatic enzymes attenuates PDAC-associated wasting of peripheral tissues. Paradoxically, reversal of adipose tissue loss impairs survival in mice with PDAC. When analysing patients with PDAC, we find that depletion of adipose and skeletal muscle tissues at the time of diagnosis is common, but is not associated with worse survival. Taken together, these results provide an explanation for wasting of adipose tissue in early PDAC and suggest that early loss of peripheral tissue associated with pancreatic cancer may not impair survival.

Models of autochthonous mice with PDAC recapitulate many features of human disease, including cachexia. We confirmed the occurrence of severe adipose tissue and skeletal muscle wasting in advanced disease using two autochthonous mouse models with Kras (G12D) activation and loss of Trp53 function — either via Trp53 deletion (KP−/−/C) or mutant Trp53trp53R172H expression (KPC) (Fig. 1a–f). Protein breakdown in tissues occurs early in PDAC, we assessed the kinetics of tissue wasting in the KP−/−/C model and found a decrease in the mass of the adipose tissue by six weeks of age, whereas the weight of the pancreatic tissue was unchanged and plasma branched-chain amino acids (BCAAs), a measurement of peripheral tissue loss is initiated early in PDAC.

Altered exocrine function can drive adipose wasting in early pancreatic cancer

600 | NATURE | VOL 558 | 28 JUNE 2018

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
a known systemic circulating PDAC-derived factor, promotes early wasting of the adipose tissue.

To investigate how tumour growth in the pancreas promotes adipose tissue wasting, we assessed systemic O$_2$ consumption, CO$_2$ production and calculated the respiratory exchange ratio. Both control and early KP$^{-/-}$ C mice displayed similar respiratory exchange ratios (Fig. 2g), arguing against a shift in whole-body fuel source utilization. However, O$_2$ consumption and CO$_2$ production were lower in early KP$^{-/-}$ C mice (Fig. 2h, i), suggesting decreased nutrient oxidation. Because food intake was similar between groups (Fig. 2i), early KP$^{-/-}$ C mice may metabolize less food and altered pancreatic function could explain these findings as well as the loss of adipose tissue.

To assess endocrine pancreatic function, we measured plasma glucagon and insulin. Whereas we found no significant differences in glucagon levels (Fig. 3a), early KP$^{-/-}$ C mice had lower levels of insulin in the fed state (Fig. 3b). To test for an insulin secretion defect, we measured plasma insulin after a bolus glucose injection and found no significant differences (Fig. 3c). These results indicate that the function of the endocrine pancreas was not altered in early PDAC; rather, impaired dietary absorption or starch breakdown may have caused the lower fed insulin levels. Consistent with this idea, we observed lower fed blood glucose levels in early KP$^{-/-}$ C mice (Fig. 3d). Furthermore, reduced insulin levels may promote increased lipolysis and adipose tissue loss.

To assess exocrine pancreatic function, we measured faecal lipid content and found higher faecal lipids in early KP$^{-/-}$ C mice (Fig. 3e). We also found decreased faecal protease activity and increased faecal protein content in these mice (Fig. 3f, g). Orthotopic implantation of PDAC cells into the pancreas also led to increased faecal protein content during disease progression (Fig. 3h). Decreased exocrine function of the pancreas could explain why early KP$^{-/-}$ C mice had decreased levels of O$_2$ consumption and CO$_2$ production despite normal food intake, leading to a starvation-like response with mobilization of energy stores from peripheral tissues. Indeed, mice bearing subcutaneous PDAC tumours that were fed a calorically restricted diet showed increased adipose tissue loss compared to skeletal muscle loss and reduced tumour size (Extended Data Fig. 3a–d).

To test whether decreased exocrine pancreatic function contributes to tissue wasting in PDAC, we supplemented a diet with pancreatic enzymes (Fig. 3i). Providing pancreatic enzymes attenuated adipose wasting in mice with PDAC (Fig. 3j). Furthermore, whereas mice with early PDAC displayed decreased fed glucose levels (Fig. 3d), mice with PDAC fed a diet supplemented with pancreatic enzymes displayed similar glucose levels to control littermates (Extended Data Fig. 3e). To control for potential food intake differences associated with adding pancreatic enzymes to a diet, we pair-fed mice to assure similar food consumption, and again observed attenuated adipose tissue loss in PDAC when providing pancreatic enzymes (Extended Data Fig. 3f–i). These results confirm that decreased pancreatic exocrine function mediates adipose tissue loss and contributes to peripheral tissue wasting in mice with early PDAC.

Cachexia has been proposed to worsen patient survival in various cancers including PDAC. To determine whether adipose tissue wasting limits survival in PDAC, we assessed whether supplementation with pancreatic enzymes improved disease outcome. Despite reduced adipose tissue wasting, supplementation with pancreatic enzymes decreased survival of mice with PDAC (Fig. 3k), suggesting that peripheral tissue wasting may not always limit survival.
To investigate the association between peripheral tissue wasting and patient survival, we identified 782 patients at five US cancer centres with previously untreated PDAC, available clinical and outcome data, and banked blood samples (Extended Data Table 1). We quantified lumbar visceral and subcutaneous adipose tissue areas using pretreatment computed tomography scans\(^{17,18}\) (Extended Data Fig. 4a). Although adipose tissue area was associated with multiple clinical factors (Extended Data Table 2), no association was found between adipose tissue wasting and patient survival in the full population (Table 1) or by disease stage (Extended Data Table 3a).

Studies of muscle wasting and survival of patients with PDAC have led to conflicting results\(^{17,19–22}\), potentially because of differences in study design. We next used computed tomography imaging to measure the lumbar skeletal muscle index, a marker of muscle mass\(^{17,19,20,22}\). Using previously established cut points for sarcopenia\(^{14}\), we found that 65% of patients displayed sarcopenia at diagnosis, and these patients did not have worse survival (Table 1). Notably, the prevalence of sarcopenia was not different between disease stages (localized, 64%; locally advanced, 70%; metastatic disease, 63%; \(P = 0.40\)). Because an interaction between sarcopenia and body mass index has been proposed\(^{20,22}\), we evaluated whether sarcopenic obesity was associated with worse patient survival and observed no differences in survival between different patient groups (Table 1). Because optimal sarcopenia cut points are not well-defined\(^{14}\), we investigated an agnostic skeletal muscle index classification with gender-specific quintiles and found no association with patient survival (Extended Data Table 3b). We also examined skeletal muscle area and attenuation and did not identify an association of these markers with patient survival (Extended Data Table 3b). Finally, because plasma BCAAs elevations reflect tissue wasting in early PDAC\(^9\), we evaluated whether plasma BCAAs at diagnosis were associated with reduced survival and did not identify worse outcomes with elevated BCAAs (Extended Data Tables 4–6 and Extended Data Fig. 4b). Thus, in this multi-institutional patient population with newly diagnosed, previously untreated PDAC, we found no evidence that early skeletal muscle or adipose tissue wasting was associated with worse survival.

Independent of effects on survival, assessing peripheral tissue loss before overt disease onset may help to identify PDAC at earlier stages. In mouse models, we found that decreased exocrine function contributes to adipose tissue wasting. Many patients with PDAC experience loss of exocrine pancreatic function\(^{21}\); however, whether decreased exocrine function contributes to wasting in patients requires further study. Furthermore, correction of pancreatic exocrine function in mouse models reduces adipose tissue wasting but does not significantly affect muscle mass, suggesting that supplementation with pancreatic enzymes is either insufficient to fully restore the nutritional state or that additional factors contribute to muscle wasting. Other factors may include stroma-derived inflammatory signals that were not tested in this study. Nevertheless, the findings that a starvation-like state in mouse models of PDAC contributes to adipose tissue loss and increases...
Letter reSeArCH

Let us begin our discussion with the key points from Fig. 3: Decreased exocrine pancreatic function in early PDAC disease promotes tissue wasting. (a–d) Endocrine function measurements in male control and early KP−/−C mice. (a) Fasting circulating glucagon levels. n = 15 control and 12 early KP−/−C mice. *P = 0.28. (b) Insulin levels in overnight fasted and fed mice. n = 4 per group. c Insulin levels in mice before and after a glucose injection. n = 5 control and 6 early KP−/−C mice. *P = 0.13 (time = 0) and P = 0.16 (time = 30). (c) Glucose levels in overnight fasted and fed mice. n = 11 control and 12 early KP−/−C mice. *P = 0.0008. (e–g) Faecal analysis of male control and early KP−/−C mice. (e) Total faecal lipid. n = 9 control and 10 early KP−/−C mice. *P = 0.004. (f) Total faecal protease activity. n = 4 per group. *P = 0.0009. (g) Total faecal protein level. n = 6 control and 9 early KP−/−C mice. *P = 0.004. (h) Total faecal protein levels over time in male C57BL/6J mice following orthotopic implantation of PDAC cells into the pancreas. n = 5. P = 0.008 (day 14 versus day 17) and *P = 0.005 (day 14 versus day 21). (i) Schematic of experimental design. (j) Relative weights of KP−/−C male mice fed a control diet or a diet supplemented with pancreatic enzymes. n = 6 control diet and 7 supplemented diet. *P = 0.033. AT, adipose tissue; SM, skeletal muscle. (k) Survival of male KP−/−C mice fed the indicated diet. n = 12 per group. Mantel–Cox test, P = 0.02. Unless otherwise indicated, statistical analysis was performed using unpaired two-sided t-tests, data are mean ± s.e.m. and n represents the number of mice that were analysed.

Table 1 | Hazard ratios for death among cases with pancreatic cancer based on body composition

| Visceral adipose tissue area | Quintiles of body composition areas | 1 | 2 | 3 | 4 | 5 | P trend |
|----------------------------|-------------------------------------|---|---|---|---|---|---------|
| Number of cases            |                                     | 136 | 138 | 138 | 137 | 137 |         |
| Median (cm²)               |                                     | 34.7 | 108.2 | 171.0 | 228.9 | 312.0 |         |
| Median overall survival (months) |                                 | 12.3 | 10.2 | 11.5 | 11.3 | 11.1 |         |
| Hazard ratio (95% confidence interval) |                               | 1.0 | 1.25 (0.96–1.62) | 1.06 (0.82–1.38) | 1.08 (0.83–1.41) | 1.04 (0.79–1.36) | 0.73 |
| Subcutaneous adipose tissue area |                                 | 1.0 | 1.33 (1.01–1.74) | 1.10 (0.82–1.47) | 1.14 (0.84–1.56) | 1.16 (0.82–1.63) | 0.31 |
| Number of cases            |                                     | 136 | 138 | 137 | 138 | 137 |         |
| Median (cm²)               |                                     | 82.7 | 136.9 | 177.5 | 234.7 | 351.0 |         |
| Median overall survival (months) |                                 | 11.9 | 10.6 | 12.0 | 11.6 | 10.2 |         |
| Hazard ratio (95% confidence interval) |                               | 1.0 | 1.18 (0.91–1.53) | 0.97 (0.75–1.26) | 0.92 (0.71–1.19) | 1.06 (0.81–1.38) | 0.80 |
| Sarcopenia¹                 | No                                  | 248 | 462 | P value | 11.6 | 11.3 |         |
| Hazard ratio (95% confidence interval) |                               | 1.0 | 1.03 (0.86–1.24) | 0.74 | 1.04 (0.85–1.27) | 0.72 |         |
| Sarcopenia and obesity²    | Neither                              | 54 | 186 | 191 | 262 | 11.1 | 11.9 |
| Hazard ratio (95% confidence interval) |                               | 1.0 | 0.98 (0.69–1.38) | 1.11 (0.79–1.57) | 0.95 (0.67–1.33) | 1.12 (0.80–1.58) | 0.91 |

¹The two-sided P trend is calculated by entering the quintile-specific median value for adipose tissue area as a continuous variable in the Cox proportional hazards model.
²Cox proportional hazards model adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), institution (Dana-Farber/Brigham and Women’s Cancer Center, Massachusetts General Hospital, Mayo Clinic, Stanford University or University of North Carolina) and cancer stage (local, locally advanced, metastatic or unknown).
³Cox proportional hazards model additionally adjusted for body mass index (continuous), diabetes history (none, ≤4 years, >4 years or unknown) and smoking status (never, past, current or unknown).
⁴Sarcopenia is defined as a skeletal muscle index (the ratio of the skeletal muscle area (in cm²) to the height squared (in m²)) of less than 55.4 cm² per m² for men and less than 38.9 cm² per m² for women. This was measured at baseline computed tomography imaging. Obesity is defined as a body mass index of more than 25 kg per m².
⁵For sarcopenia, 8 patients were excluded owing to missing information on height. For sarcopenia and obesity, a further 17 patients were excluded owing to missing information on weight.

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
survival are consistent with data that suggest that caloric restriction improves survival of mice with PDAC, perhaps via similar mechanisms. Although not examined here, changes in insulin levels may also contribute to survival differences upon reversal of a starvation-like state. The finding that pancreatic enzyme supplementation led to worse survival in mice also suggests that peripheral tissue wasting in early PDAC may be distinct from cachexia associated with late-stage disease. Nutritional intervention and pancreatic enzyme replacement are sometimes used in patients with PDAC and a better understanding of the mechanisms that cause tissue wasting across cancer types and stages of disease is needed to design interventions that reduce functional disability and improve survival of patients with cancer.

**Online content**

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0235-7.

Received: 28 February 2017; Accepted: 21 May 2018; Published online 20 June 2018.

1. Koppenol, W. H., Bounds, P. L. & Dang, C. V. Otto Warburg’s contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer* **11**, 325–337 (2011).

2. Petitruzzelli, M. & Wagner, E. F. Mechanisms of metabolic dysfunction in cancer-associated cachexia. *Genes Dev.* **30**, 489–501 (2016).

3. Dewys, W. D. et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am. J. Med.* **69**, 491–497 (1980).

4. Mueller, T. C., Bachmann, J., Prokopchuk, O., Friess, H. & Martignoni, M. E. Molecular pathways leading to loss of skeletal muscle mass in cancer cachexia—can findings from animal models be translated to humans? *BMC Cancer* **16**, 75 (2016).

5. Fearon, K., Arends, J. & Baracos, V. Understanding the mechanisms and treatment options in cancer cachexia. *Nat. Rev. Clin. Oncol.* **10**, 90–99 (2013).

6. Penna, F. et al. Anti-cytokine strategies for the treatment of cancer-related anorexia and cachexia. *Expert. Opin. Biol. Ther.* **10**, 1241–1250 (2010).

7. Flint, T. R. et al. Tumor-induced IL-6 reprograms host metabolism to suppress anti-tumor immunity. *Cell Metab.* **24**, 672–684 (2016).

8. Hingorani, S. R. et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* **4**, 437–450 (2003).

9. Mayers, J. et al. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat. Med.* **20**, 1193–1198 (2014).

10. Agustsson, T. et al. Mechanism of increased lipolysis in cancer cachexia. *Cancer Res.* **67**, 5531–5537 (2007).

11. Michalak, K. A. et al. Establishment and characterization of a novel murine model of pancreatic cancer cachexia. *J. Cachexia Sarcompenia Muscle* **8**, 824–838 (2017).

12. Rydén, M. et al. Lipolysis—not inflammation, cell death, or lipogenesis—is involved in adipose tissue loss in cancer cachexia. *Cancer* **113**, 1695–1704 (2008).

13. Shaw, J. H. & Wolfe, R. Fatty acid and glycerol kinetics in septic patients and in patients with gastrointestinal cancer. The response to glucose infusion and parenteral feeding. *Ann. Surg.* **205**, 368–376 (1987).

14. Fearon, K. et al. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol.* **12**, 489–491 (2011).

15. Hwang, R. F. et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res.* **68**, 918–926 (2008).

16. Herrington, M. K., Arnelo, U. & Permutt, J. On the role of islet amyloid polypeptide in glucotoxic intolerance and anorexia of pancreatic cancer. *Pancreatology* **1**, 267–274 (2001).

17. Martin, L. et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J. Clin. Oncol.* **31**, 1539–1547 (2013).

18. Mourtzakis, M. et al. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Appl. Physiol. Nutr. Metab.* **33**, 997–1006 (2008).

19. Choi, Y. et al. Skeletal muscle depletion predicts the prognosis of patients with advanced pancreatic cancer undergoing palliative chemotherapy, independent of body mass index. *PLoS ONE* **10**, e0139749 (2015).

20. Prado, C. M. et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol.* **9**, 629–635 (2008).

21. Rolins, K. E. et al. The impact of sarcopenia and myosteatosis on outcomes of unresectable pancreatic cancer or distal cholangiocarcinoma. *Clin. Nutr.* **35**, 1102–1109 (2016).

22. Tan, B. H., Birdsell, L. A., Martin, L., Baracos, V. E. & Fearon, K. C. H. Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. *Cancer Res.* **15**, 6973–6979 (2009).

23. Vujasinovic, M., Valente, R., Del Chiara, M., Perment, J. & Löhr, J. M. Pancreatic exocrine insufficiency in pancreatic cancer. *Nutrients* **9**, 183 (2017).

24. Li, M., Zhu, X., Wang, H., Wang, F. & Guan, W. Roles of caloric restriction, ketogenic diet and intermittent fasting during initiation, progression and metastasis of cancer in animal models: a systematic review and meta-analysis. *PLoS ONE* **9**, e115147 (2014).

25. Lequente, B. et al. Supportive care in pancreatic ductal adenocarcinoma. *Clin. Transl. Oncol.* **19**, 1293–1302 (2017).

**Acknowledgements**

We thank members of the Vander Heiden and Wolpin laboratories for discussions on the Koch Institute Swanson Biotechnology Center, particularly the Animal Imaging and Preclinical Testing Facility, for technical assistance. Major funding for this work was provided by the Lustgarten Foundation to B.M.W. and M.G.V.H. L.V.D. was supported by an NIH Ruth Kirstein Fellowship (F32CA210421). A.B. was supported by P50CA127003 and the Robert T. and Judith B. Hale Fund for Pancreatic Cancer Research. A.M. was supported by F32CA213810. E.C.L. was supported by the Damon Runyon Cancer Research Foundation (DRG-2299-17). A.N.L. is a Robert Black Fellow of the Damon Runyon Cancer Research Foundation (DRG-2241-15). B.M.W. was supported by Robert T. and Judith B. Hale Fund for Pancreatic Cancer Research, NIH/NCI (U01CA210171), Department of Defense (CA130288), Pancreatic Cancer Action Network, Stand Up To Cancer, Noble Effort Fund, Peter R. Leavitt Family Fund, Wexler Family Fund, and Promises for Purple. M.G.V.H. was supported in part by a Faculty Scholar grant from the Howard Hughes Medical Institute, and acknowledges additional funding from Stand Up To Cancer, The Ludwig Center at MIT, the Koch Institute Frontier Awards, the MIT Center for Precision Cancer Medicine, and the NIH (R01CA168653, 530CA14051).

**Reviewer information**

Nature thanks M. Löhr and the other anonymous reviewer(s) for their contribution to the peer review of this work.

**Author contributions**

L.V.D. designed, performed and analysed the animal experiments with input from M.G.V.H.; L.V.D., A.B., B.M.W. and M.G.V.H. wrote the manuscript with assistance from all other authors. E.A.D. and P.J.-S. assisted with animal experimentation. A.M. immortalized and A.N.L. isolated PSCs. E.C.L. performed caloric restriction experiments. J.R.M. performed muscle volume measurements and blood measurements. K.T. performed non-esterified fatty acid and glycerol assays. A.B., M.H.R., C.B.C. and B.M.W. designed the human study. C.M.P., G.M.P., N.T., M.S., J.J.Y., N.L., N.B., C.F.-d.C., A.S.L., A.C.K., J.B., and M.G.V.H. performed caloric restriction experiments. J.R.M. performed muscle volume measurements and blood measurements. K.T. performed non-esterified fatty acid and glycerol assays. A.B., M.H.R., C.B.C. and B.M.W. designed the human study. C.M.P., G.M.P., N.T., M.S., J.J.Y., N.L., N.B., C.F.-d.C., A.S.L., A.C.K., J.B., C.Y., M.W.W., L.K.B., M.H.K. and B.M.W. were involved in patient recruitment and patient data collection. C.D. and C.B.C. were involved in metabolite measurements in patients. A.B. and M.H.R. analysed human data. B.M.W. supervised the human study.

**Competing interests**

The authors declare no competing financial interests; however, M.G.V.H. discloses receiving on the S.A.B. of Agios Pharmaceuticals and Aeglea Biotherapeutics.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0235-7.

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41586-018-0235-7.

**Reprints and permissions information** is available at http://www.nature.com/reprints.

**Correspondence and requests for materials** should be addressed to B.M.W. or M.G.V.H.

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Cell culture. All cells were cultured in DMEM supplemented with 10% FBS (Corning) and 1% penicillin and streptomycin (Corning).

**Western blot analysis.** Antibodies recognizing phospho-P38 (ser323) (4139) and total HSL (4107) were purchased from Cell Signaling Technologies.

**Animal studies.** All experiments performed in this study were approved by the MIT Committee on Animal Care (IACUC). Furthermore, for subcutaneous tumour growth, a maximum tumour burden of 2 cm³ was permitted by our IACUC protocol and these limits were not exceeded. All mice in this study were fully backcrossed to the C57BL/6j background, and housed under a 12-h light and 12-h dark cycle, and cohoused with littersmates with ad libitum access to water and food, unless otherwise stated. Furthermore, all experimental groups were age-matched and assigned based on genotype (or treatment). All animals were numbered and experiments were conducted in a blinded manner. After data collection, genotypes were revealed and animals assigned to groups for analysis. All measurements were collected from distinct animals, n represents biologically independent samples, and mice were analysed as tumours developed. No statistical methods were performed to predetermine sample size. 

For studies using KrasG12DTrp53fl/flPdx1cre (KP−/−) mice, controls included littersmates that lacked the Cre-recombinase allele, LSL-KrasG12D allele or both. For studies using KrasG12DTrp53R172H/Pdx1Cre (KP) mice, control mice included littersmates that lacked the LSL-KrasG12D allele or LSL-P53R172H allele. For studies using KrasG12DTrp53+/−Pdx1Cre (KC) mice, control mice included littersmates expressing the Cre allele.

For subcutaneous or pancreatic orthotopic tumours, C57BL/6j mice (000664) were injected with 10⁶ mouse PDAC cells isolated from C57BL/6j KP−/− mice as previously described39. Phosphate-buffered saline (PBS) or PDAC cells were injected into the right flank (in 100 μl) or the pancreas (50 μl) of 8-week-old C57BL/6j mice. For protein forensic collection studies in mice bearing orthotopic pancreatic tumours, mice were injected with 10⁵ mouse PDAC cells (50 μl) and allowed to recover from surgery for two weeks. Tumour mass was obtained after this two-week recovery time every 2–3 days for approximately 2–3 weeks and placed in −20 °C until the day of analysis. For studies using PSCs, we isolated PSCs as previously described38. After a few passages, PSCs were immortalized and injected at 1:1 ratio of PSCs to cancer cells as previously described39.

For experiments using metabolic cages, 5–6-week-old mice were placed in metabolic cages and food intake, respiratory exchange ratio (RER), volumetric rate of O₂ consumption and volumetric of CO₂ production were measured over a three-day period (TSE Systems). RER was calculated using the following formula: RER = VO₂/VO₂.

For pairing experiments, the amount of diet supplemented with pancreatic enzymes that was consumed per mouse per day was calculated for an average of five days. Animals with early PDAC were weighed, individually housed and randomized to diet groups and the vials were treated separately to ensure both groups of mice had similar starting body weights.

For caloric-restriction studies, mice were injected subcutaneously (into both flanks) with PDAC-derived cells as described above. Animals were randomly placed on AIN-93 G (TD:94045) control diet or on the same diet at 40% restriction after tumours were palpable. Mice were individually housed and fed daily either 3.2 g per day (control mice) or 1.9 g per day (calorie-restricted mice) for a total of three weeks.

**Animal diets.** For diets supplemented with pancreatic enzymes, AIN-93G powdered diet (TD:94045) was purchased from Envigo and mixed with a commercial preparation of pancreatic enzymes as previously described37.

**Assessment of glucose metabolism.** Glucose levels were measured using a Breeze-2 glucose meter (Bayer). For insulin measurements, we used an Ultrasensitive Mouse Insulin ELISA (90080) following the manufacturer's protocol. In vivo insulin secretion, mice were fasted for 16 h and intraperitoneally injected with glucose (1g/kg), blood samples were collected and analysed at the indicated time points.

**Ex vivo lipolysis.** Ex vivo lipolysis assays of adipose explants were performed as previously described24. In brief, epididymal adipose tissue were collected and incubated at 37 °C, non-esterified fatty acids were measured using Wako diagnostics and glycerol release was measured using the Free Glycerol Determination kit (FG0100; Sigma–Aldrich) according to the manufacturer's instructions.

**Hormone and metabolite measurements.** Blood samples were collected in EDTA-containing tubes and centrifuged 3,000 rpm for 15 min (4 °C). Circulating levels of IL-6 (M6008B, R&D Systems), corticosterone (80556, Crystal Chem), amylin (ELI40682), insulin (ELI51001), PYY (ELI55050), GLP-1 (ELI53985), glucagon (ELI53675), leptin (ELI53981), ghrelin (ELI53700), adiponectin (ELI53690), resistin (ELI56098), and adipin (ELI56097) were measured using the manufacturer's instructions. TNF, IFN-γ, IL-10, IL-1β, IL-17 and IL-4 were measured using a Discovery Assay (Eve Technologies). Circulating BCAAs levels were measured as previously described40.

**Micro-computed tomography imaging in mice.** All micro-computed tomography (CT) measurements in mice were performed using GE explore CT120. The scans were conducted at 70kVp, 50 mA and 32 s. There were 720 views, 0.5 degrees apart over a full 360-degree rotation. To assess muscle volume, a 3D Gaussian filter was used in an area extending from the right ankle to the proximal end of the fibula. This filtered dataset was used to determine areas corresponding to leg muscle while excluding other tissues. This was done in MATLAB using the connected components (bwconncomp) function. Having created the mask (segmented the leg muscle from other tissues), a histogram of the muscle region was calculated. Voxels falling within the density range of 160–200 Hounsfield units (HU) were considered muscle. This was done to correct for any overlap of the muscle mask with adjacent bone or adipose (which have higher and lower HU values, respectively).

**Faecal assays.** To assess total faecal protein, 10 mg of faeces was resuspended in lysis buffer (2% SDS, 150 mM NaCl, 0.5 M EDTA), sonicated and the protein concentration was assessed using a BCA assay according to the manufacturer’s instructions. Total faecal protease activity was measured as previously described38. In brief, 10–30 mg of faecal matter was resuspended in 1 ml of buffer A (0.1% Triton X-100, 0.5 M NaCl, 100 mM CaCl₂), sonicated and centrifuged. The supernatant was then incubated with 3% Azo-Casein (Sigma-Aldrich, A2765) at 37 °C for 60 min. The reaction was stopped using 8% trichloroacetic acid and centrifuged. The absorbance of the supernatant (measured at 366 nm) was measured using a spectrophotometer. Total faecal lipids were measured as previously described38. In brief, 1,000 mg of faeces was collected and lipids were extracted using a 2:1 chloroform:methanol solution. The lipid fraction was dried using a stream of gaseous nitrogen and the vials were weighed.

**Histological analysis.** Tissues were fixed overnight with neutral-buffered 10% formalin, paraffin-embedded, sectioned and stained with haematoxylin and eosin or stained with Masson's trichrome using standard protocols.

**Statistics for animal data.** All graphs were generated using Prism (GraphPad) software and data are mean ± s.e.m. Unless otherwise indicated, P values were determined using unpaired two-sided t-tests. Statistical outliers were measured using a Grubb’s outlier test (Prism) and excluded from the final analysis.

**Human population study.** Our study population included patients with pancreatic cancer from five US cancer centres: Dana-Farber/Brigham and Women’s Cancer Center (DF/BWCC), Massachusetts General Hospital (MGH), Mayo Clinic, Stanford University and University of North Carolina–Chapel Hill (UNC). We included 782 patients with pancreatic adenocarcinoma who were diagnosed between 2000 and 2015, and had a stored plasma sample collected before receiving any treatment for their malignancy, including surgery, radiation or chemotherapy. A total of 778 patients at the five institutions met these criteria and had a plasma sample collected within 30 days before their pathological diagnosis and 60 days after this diagnosis. Of these patients, 686 had a CT scan performed during this pathological diagnosis. The overall study was approved by the Dana-Farber/Harvard Cancer Center IRB, and data abstraction and blood sample collection was approved by each individual institutional IRB. All participants provided informed consent.

**Human plasma samples and metabolite profiling.** Blood was collected in sterile EDTA tubes, and processed within 3h (Dana-Farber Cancer Institute, Mayo Clinic, Stanford University and UNC) or 24 h (MGH) for separation into plasma and other components. Plasma was aliquoted into cryovials and stored at −80 °C. Plasma samples were thawed once on wet ice to aliquot into smaller sample volumes for analysis, de-identified and sent for analysis in a single shipment. Liquid chromatography tandem mass spectrometry (LC–MS) analyses were conducted using a Shimadzu Nexera X2 U-HPLC (Shimadzu) coupled to a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific). Polar metabolites were extracted from plasma (10 μl) of 74 samples using a 90 μl v/v v/v acetonitrile:methanol:formic acid containing stable isotope-labelled internal standards (valine-d₅ (Sigma–Aldrich) and phenylalanine-d₅ (Cambridge Isotope Laboratories)). The samples were centrifuged (10 min, 9,000g, 4 °C) and the supernatants were injected directly onto a 150 × 2 mm², 3 μm Atlantis HILIC column (Waters). The column was eluted isocratically at a flow rate of 250 μl min⁻¹ with 5% mobile phase A (10 mM ammonium formate and 0.1% formic acid in water) for 0.5 min followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 min. MS analyses were carried out using electrospray ionization in the positive ion mode using full scan analysis over 70–800 m/z resolution and 3-6 Hz data acquisition rate. Other MS settings were: sheath gas 40, sweep gas 2, spray voltage 3.5kV, capillary temperature 350 °C, 5-lens RF 40, heater temperature 300 °C, microscans 1, automatic gain control target 1 × 10⁶, and maximum ion time 250 ms. Raw data were processed using TraceFinder software (Thermo Finnigan, Thermo Scientific). Metabolite identities were confirmed using authentic reference standards.

To evaluate reproducibility of BCAA measurements, we included 77 blinded quality control plasma samples within the larger sample set, from eight plasma
quality control pools. The mean coefficients of variance were 7.6% for isoleucine, 8.0% for leucine and 7.3% for valine. Because blood samples from MGH were processed after >3 h, we evaluated the reproducibility of BCAA measurements between samples processed at different time points after collection. For 10 patients, we processed blood immediately and at 24 h and measured plasma BCAAs. Spearman correlation coefficients for the BCAA levels at the two time points were 0.95 for isoleucine, 0.95 for leucine and 0.92 for valine. All blood samples were collected into EDTA plasma tubes, except for 28 blood samples collected as serum from MGH patients. To evaluate BCAAs in plasma versus serum tubes, we measured BCAA levels for 10 patients who had simultaneous collection of plasma and serum. Spearman correlation coefficients for plasma BCAs by plasma versus serum samples were 0.84 for isoleucine, 0.76 for leucine and 0.94 for valine. Given the high correlation coefficients for plasma BCAs for both time of blood processing and plasma versus serum collection, we included all MGH patients in our study population.

Computed tomography imaging quantification of muscle and adipose tissue in patients. Muscle, visceral adipose tissue and subcutaneous adipose tissue areas were measured through the third lumbar vertebra landmark on CT imaging as previously published. Scans from the DF/BWCC, MGH, Stanford University and UNC study sites (n = 363) were manually segmented using Slice-O-Matic software (v.4.3; Tomovision) by trained image analysts with final verification from a board-certified radiologist. All paraspinal and abdominal wall muscles were included in the muscle area. The Hounsfield CT attenuation scale was used to constrain adipose tissue and muscle selection. The Hounsfield scale is a linear transformation of the linear attenuation coefficient, with fixed points calibrated for air at −1,000 HU and water at 0 HU. All medical CT scanners are routinely calibrated to this scale. Pixel attenuation constraints from −29 to 150 HU were used for muscle and from −180 to −30 HU for adipose tissue as previously published. The visceral adipose tissue compartment was defined by the peritoneum; all extra-peritoneal adipose tissue was included in the subcutaneous compartment. Pixel dimensions were extracted from scanner parameters embedded within the scan data; the total area was measured as the product of segmented pixel count and pixel area. We calculated skeletal muscle index (SMI) as the ratio of skeletal muscle area (cm²) to height squared (m²). Muscle attenuation was defined as the average Hounsfield attenuation of all pixels in the muscle area. Analyst performance was tested, and a test–retest coefficient of variation <1.1% was observed for all analysts and parameters. Scans from the Mayo Clinic site (n = 324) were analysed using in-house developed software written in MATLAB (MATLAB 2015b, MathWorks) with manual correction by a trained image analyst. The software automatically fits three concentric closed contours at the air–skin boundary, the subcutaneous adipose–muscle boundary and the abdominal wall–peritoneal adipose boundary using hierarchical morphological classification constrained by a prior probability shape model. These boundaries defined the same zones as were described for the Slice-O-Matic method. The software also automatically created masks for bone and colonic content and these masks were used to exclude bone and colonic content from being included as muscle or adipose tissue. The final boundaries were verified by a board-certified radiologist. Adipose and muscle areas were then calculated for each compartment using the same attenuation constraints as described for the Slice-O-Matic method.

To ensure consistency across sites, we analysed 20 cases using both methods and found a coefficient of variation of 2.4%, 1.7% and 3.8% between the methods for the skeletal muscle, subcutaneous adipose tissue and visceral adipose tissue areas, respectively. The maximum observed differences were 4.1%, 3.6% and 15.2% for these compartments.

Covariate data and statistical analysis of patient data. Using patient questionnaires and medical records, we extracted information on patient and clinical characteristics, including: age at diagnosis, gender, race/ethnicity, height, weight at blood collection, diabetes history (no diabetes, diabetes of duration ≤4 years, diabetes of duration >4 years), tobacco use (never, past, current), primary tumour location (head or uncinate, body, tail, other), cancer stage (local, locally advanced, metastatic), year of diagnosis and survival time.

Body composition measurements and plasma BCAs by patient characteristics were compared using Wilcoxon rank-sum test or Kruskal–Wallis test. A Pearson correlation test was used to evaluate the linear relationship between body composition measurements, plasma BCAs and other patient characteristics. To evaluate the associations between patient survival, body composition measurements, and plasma BCAs, we used multivariable-adjusted Cox proportional hazards regression and calculated hazard ratios and 95% confidence intervals. Survival time was calculated as the time from diagnosis to death or date of last follow-up if the patient was still alive. In an initial multivariate model, we adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), institution (DF/BWCC, MGH, Mayo Clinic, Stanford University or UNC), and cancer stage (localized, locally advanced, metastatic or unknown). In a second multivariate model, we additionally adjusted for body mass index (BMI) (continuous), history of diabetes (none, ≤4 years or unknown), and smoking status (never, past, current or unknown), which have previously been shown to associate with patient survival.

Because cut-offs to define clinically relevant body composition categories are not well-defined, we also divided patients into quintiles by adipose tissue and muscle tissue measurements. Because the distribution of body composition measurements differed by gender (Extended Data Table 2), we created gender-specific quintiles for all body composition measurements. P-values for linear trends were calculated by entering the quintile-specific median value for body composition measurements in the Cox proportional hazards models. To evaluate sarcopenia, we used the SMI criteria established by a recent international consensus definition of cancer cachexia. The SMI cut-offs were 54.4 cm² per m² for men and 38.9 cm² per m² for women. To evaluate sarcopenic obesity, we generated a combined variable, for which overweight or obesity status is defined as BMI ≥ 25 kg per m², similar to a previous study in patients with PDAC. The resulting four categories were overweight/obese and sarcopenic, overweight/obese and non-sarcopenic, non-overweight/obese and sarcopenic, non-overweight/obese and non-sarcopenic. For plasma BCAs, patients were divided into quartiles, with quartiles 2–4 compared to quartile 1 as the reference. P-values for trends were calculated by entering quartile-specific median values for total plasma BCAs as a continuous variable in the Cox proportional hazards model.

We performed stratified analyses by disease stage and statistical interactions were assessed using the Wald test of the cross-product term between stage (localized, locally advanced, metastatic or unknown) and tissue measurements (continuous). We also calculated the hazard ratio for each institution and measured heterogeneity across centres using the Cochran’s Q statistic. This statistic is a weighted sum of squared deviations of the estimate of an individual study from the overall estimate obtained by meta-analysis. All analyses were performed with SAS 9.2 statistical analysis software, and all P-values are from two-sided tests.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. Source Data for the graphical representations found in Figs. 1–3 and Extended Data Figs. 1–3 can be found in the online version of the paper. All other data that support the findings of this study are available upon request from the corresponding author.

26. Apté, M. Isolation of quiescent pancreatic stellate cells from rat and human pancreas. Pancreas 36, 15175–15184 (2015).
27. Lofthus, S. K. et al. Acanin cell apoptosis in Serpin1-deficient mice models pancreatic insufficiency. Proc. Natl Acad. Sci. USA 105, 15052–15057 (2008).
28. DiStefano, M. T. et al. The lipid droplet protein hyposin-inducible gene 2 promotes hepatic triglyceride deposition by inhibiting lipolysis. J. Biol. Chem. 290, 15175–15184 (2015).
29. Gabbi, C. et al. Pancreatic exocrine insufficiency in LXRrs–/– mice is associated with a reduction in aquaporin-1 expression. Proc. Natl Acad. Sci. USA 105, 15052–15057 (2008).
30. Kraus, D., Yang, Q. & Kahn, B. B. Lipid extraction from mouse feces. Bio Protoc. 5, e1375 (2015).
31. Yuan, C. et al. Survival among patients with pancreatic cancer and long-standing or recent-onset diabetes mellitus. J. Clin. Oncol. 33, 29–35 (2015).
32. Yuan, C. et al. Prediagnostic body mass index and pancreatic cancer survival. J. Clin. Oncol. 31, 4229–4234 (2013).
33. DerSimonian, R. & Laird, N. Meta-analysis in clinical trials. Control Clin. Trials 7, 177–188 (1986).

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | PDAC is associated with adipose and skeletal muscle wasting. a, Circulating BCAAs (valine, leucine, and isoleucine) in male control (n = 12) and early KP−/− C (n = 10) mice. 
b, Representative histology of H&E-stained gastrocnemius skeletal muscle of control and early KP−/− C mice. n = 4 per group. c, Relative myofibre area in male control and early KP−/− C mice. n = 3 per group. *P < .0001. d, Representative 3D μCT imaging reconstruction of soleus and gastrocnemius skeletal muscle (highlighted in red). e, Relative soleus and gastrocnemius skeletal muscle as assessed by micro-CT scan of control and early KP−/− C male mice. n = 10 per group. P = 0.04. 
f, Skeletal muscle tissue mass of the indicated muscle groups in male control (n = 8) and early KP−/− C mice (n = 9). *P = 0.006 (quadriceps), *P = 0.02 (tibialis anterior), *P = 0.004 (soleus). g, Relative mRNA expression of the indicated genes assessed by RT–qPCR. n = 4 per group. P = 0.05 (Mstn), *P = 0.01 (Trim63), *P = 0.07 (Fbxo32), *P = 0.0004 (Map1lc3a), *P = .006 (Gabarapl1). h, Representative histology of H&E-stained epididymal adipose tissue from control and early KP−/− C male mice. n = 4 per group. i, Relative adipocyte area in male control and early KP−/− C mice. n = 3 per group. P < 0.0001. j, Glycerol release in ex vivo adipose tissue explants from control and early KP−/− C male mice. n = 7 per group. P = 0.01. k, Non-esterified fatty acid (NEFA) release in ex vivo adipose tissue explants from control (n = 8) and KP−/− C male (n = 7) mice. P = 0.0002. l, Representative western blot analysis of phosphorylated (p)HSL and HSL expression in adipose tissue of control and early KP−/− C male mice. n = 3 per group. m, Representative H&E histology images of the pancreas of 15-week-old KC male mice. n = 5 per group. Unless otherwise indicated, statistical analysis was performed using unpaired two-sided t-tests, data are mean ± s.e.m. and n represents the number of mice that were analysed.
Extended Data Fig. 2 | Systemic circulating factors are not altered in early PDAC. a–j, Circulating levels of the indicated factors in control and early KP−/− C mice. a, IL-6. n = 16 control and 14 early KP−/− C mice. ns, not significant; P = 0.45. b, TNF-α. n = 12 control and 10 early KP−/− C mice. P = 0.45. c, PTHrP. n = 7 control and 8 early KP−/− C mice. P = 0.94. d, Corticosterone. n = 19 control and 16 early KP−/− C mice. P = 0.40. e, Amylin. n = 7 control and 6 early KP−/− C mice. P = 0.91. f, IL-1β. n = 11 control and 10 early KP−/− C mice. P = 0.39. g, IL-4. n = 12 control and 10 early KP−/− C mice. P = 0.56. h, IFNγ. n = 12 control and 9 early KP−/− C mice. P = 0.08. i, IL-10. n = 12 control and 10 early KP−/− C mice. P = 0.42. j, IL-17. n = 12 and 10 early KP−/− C mice. P = 0.29. Unless otherwise indicated, statistical analysis was performed using unpaired two-sided t-tests, data are mean ± s.e.m. and n represents the number of mice that were analysed.
Extended Data Fig. 3 | Decreased exocrine pancreatic function in early PDAC disease promotes adipose tissue loss. a–d, C57BL/6J mice bearing PDAC-derived subcutaneous tumours fed a control diet or the same diet at 40% caloric restriction (CR) for 3 weeks. n = 8 per group. a, Body weight. *P < 0.0001. b, Epididymal adipose tissue (eWAT) and inguinal adipose tissue (iWAT) mass normalized to body weight. *P < 0.0001 for epididymal adipose tissue and *P = 0.0034 for inguinal adipose tissue. c, Skeletal muscle mass of the indicated muscle groups normalized to body weight. d, Tumour volume normalized to body weight. *P = 0.002. n = 15 tumours from 8 control mice (2 tumours per mouse; for one mouse one of the tumours did not grow) and n = 16 tumours from 8 calorie-restricted mice. e, Fed plasma glucose levels of 7-week-old male control and KP−/− C mice that were fed the indicated diets. n = 4 per group. P = 0.18. f, Tissue weights normalized to body weight in KP−/− C mice pair-fed indicated diets. n = 4 per group. *P = 0.01. ns, P = 0.53. g, h, Tumour weight normalized to body weight of KP−/− C mice that were fed the indicated diets for 1 week. g, KP−/− C mice were fed the indicated diets. n = 6 control and 7 enzyme-supplemented. *P = 0.9. h, KP−/− C mice were pair-fed the indicated diets. n = 4 per group. P = 0.26. i, Representative histology of H&E-stained autochthonous pancreatic tumours of KP−/− C mice that were fed the indicated diets. n = 4 per group. Unless otherwise indicated, statistical analysis was performed using unpaired two-sided t-tests, data are mean ± s.e.m. and n represents the number of mice that were analysed.
Extended Data Fig. 4 | Use of CT imaging to assess patient body composition and relationship between plasma BCAA levels and patient survival by study site. 

**a**, Representative CT image used to analyse body composition. Skeletal muscle is shown in red, intramuscular adipose tissue is shown in green, visceral adipose tissue is shown in yellow and subcutaneous adipose tissue is shown in blue. 

**b**, Hazard ratios (HRs) and 95% confidence intervals (CI) for the association between plasma BCAAs and patient survival, comparing the top and bottom quartile, calculated using Cox proportional hazards model adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), cancer stage (local, locally advanced, metastatic or unknown), BMI (continuous), diabetes history (none, ≤4 years, >4 years or unknown) and smoking status (never, past, current or unknown). The pooled hazard ratios were calculated using the DerSimonian and Laird random-effects model. The solid squares and horizontal lines correspond to the study site-specific multivariate hazard ratio and 95% confidence interval, respectively. The area of the solid square reflects the study site-specific weight (inverse of the variance). The filled diamond represents the pooled hazard ratio and 95% confidence interval. The solid vertical line indicates a hazard ratio of 1.0. n = 778.
Extended Data Table 1 | Characteristics for patients with pancreatic cancer

### a.

| Characteristics for patients with pancreatic cancer | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|-----------------------------------------------------|---------|---------|-----|---------|-----|------|
| No. of patients                                     | 782     | 281     | 58  | 36      | 57  | 350  |
| Diagnosis period, years                             |         |         |     |         |     |      |
| 2000-2005                                           | 110 (14)| 25 (9)  | 0   | 14 (39) | 0   | 71 (20) |
| 2006-2010                                           | 292 (37)| 90 (32)| 18 (31)| 15 (42) | 24 (42) | 201 (41) |
| 2011-2015                                           | 380 (49)| 166 (59)| 40 (69)| 7 (19) | 33 (58) | 134 (38) |
| Age at diagnosis, years                             | 66.0 (10.7)| 64.3 (10.0)| 69.4 (10.2)| 67.9 (13.1)| 68.2 (11.1)| 66.2 (10.7) |
| Female sex                                          | 347 (44)| 133 (48)| 28 (48)| 11 (32) | 31 (54) | 144 (41) |
| White race                                          | 698 (89)| 252 (90)| 39 (67)| 24 (67) | 39 (68) | 344 (98) |
| Body mass index, kg/m²                               | 27.4 (5.2)| 26.7 (5.2)| 27.0 (5.7)| 24.7 (4.7)| 27.4 (4.7)| 28.2 (5.0) |

**Diabetes history**

| Diabetes status | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|-----------------|---------|---------|-----|---------|-----|------|
| No diabetes     | 565 (72)| 194 (69)| 46 (79)| 28 (78) | 32 (56) | 265 (76) |
| Diabetes ≤4 years| 100 (13)| 38 (14) | 5 (9) | 4 (11) | 8 (14) | 45 (13) |
| Diabetes >4 years| 54 (7) | 20 (7) | 4 (7) | 2 (6) | 7 (12) | 21 (6) |
| Unknown          | 63 (8) | 29 (10) | 3 (5) | 2 (6) | 10 (18) | 19 (5) |

**Tobacco use**

| Tobacco status | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|----------------|---------|---------|-----|---------|-----|------|
| Never          | 335 (43)| 127 (45)| 14 (24)| 18 (50) | 20 (35) | 156 (45) |
| Past           | 330 (42)| 121 (43)| 36 (62)| 13 (36)| 25 (44) | 135 (39) |
| Current        | 68 (9) | 30 (11)| 8 (14) | 4 (11) | 12 (21) | 14 (4) |
| Missing        | 49 (6) | 3 (1)  | 0 (0) | 1 (3) | 0 (0) | 45 (13) |

**Primary tumor location**

| Location       | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|----------------|---------|---------|-----|---------|-----|------|
| Head / Uncinate| 481 (62)| 161 (57)| 41 (71)| 24 (87) | 49 (86) | 206 (59) |
| Body           | 142 (18)| 64 (23)| 6 (10)| 8 (22) | 3 (5) | 61 (17) |
| Tail           | 97 (12) | 47 (17)| 11 (19)| 3 (8) | 5 (9) | 31 (9) |
| Body and tail  | 31 (4) | 5 (2) | 0 (0) | 1 (3) | 0 (0) | 25 (7) |
| Head and body  | 22 (3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 22 (6) |
| Other          | 9 (1) | 4 (1) | 0 (0) | 0 (0) | 0 (0) | 5 (1) |

**Cancer stage**

| Stage            | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|------------------|---------|---------|-----|---------|-----|------|
| Local            | 258 (33)| 52 (19)| 51 (88)| 5 (14)| 54 (95) | 96 (27) |
| Locally advanced | 157 (20)| 47 (17)| 0 (0)| 21 (58)| 1 (2) | 88 (25) |
| Metastatic       | 308 (39)| 175 (62)| 4 (7)| 9 (25)| 2 (4) | 118 (34) |
| Unknown          | 59 (8) | 7 (2)  | 3 (5)| 1 (3) | 0 (0) | 48 (14) |

**Median survival, months**

| Survival Type    | Overall | DF/BWCC | MGH | Stanford | UNC | Mayo |
|------------------|---------|---------|-----|---------|-----|------|
| Local            | 20.6    | 20.5    | 25.6| 6.3     | 17.4| 22.4 |
| Locally advanced | 11.0    | 13.0    | n/a | 7.9     | 1.4 | 11.1 |
| Metastatic       | 7.0     | 6.9     | 15.5| 4.9     | 3.9 | 8.2 |

### b.

| Study Site        | Full Study Population | Blood Samples | CT Images |
|-------------------|-----------------------|----------------|-----------|
|                   | (Pt No.)               | (Pt No.)       | (Pt No.)  |
| DF/BWCC           | 281                   | 260            | 244       |
| MGH                | 58                    | 58             | 38        |
| Stanford           | 36                    | 34             | 33        |
| UNC                | 57                    | 56             | 48        |
| Mayo Clinic        | 350                   | 350            | 324       |
| Total              | 782                   | 778            | 687       |

**Notes:**

- a. Characteristics for each study site.
- b. Number of patients for which blood samples and CT images were available. Continuous variables are reported as mean ± s.d.; categorical variables are reported as number (percentage) at time of diagnosis unless otherwise noted.
### Extended Data Table 2 | Body composition characteristics for patients with pancreatic cancer

|                            | Pt No. | SMI (cm$^2$/m$^2$) | Muscle area (cm$^2$) | Muscle attenuation (HU) | Subcutaneous fat area (cm$^2$) | Visceral fat area (cm$^2$) |
|-----------------------------|--------|---------------------|----------------------|-------------------------|--------------------------------|---------------------------|
| **Age**                     |        |                     |                      |                         |                                |                           |
| < 60 yrs                    | 194    | 47.9 (9.9)          | 144.4 (37.4)         | 39.1 (9.4)              | 206.9 (116.6)                 | 155.0 (107.9)             |
| 60 - 670 yrs                | 238    | 46.6 (11.1)         | 134.2 (34.9)         | 36.9 (9.6)              | 214.7 (115.1)                | 163.2 (103.5)             |
| ≥ 70 yrs                    | 255    | 41.7 (7.7)          | 118.4 (28.9)         | 32.4 (9.4)              | 185.3 (94.2)                 | 163.7 (111.0)             |
| *P*-value$^a$               | 9.8×10^{-15} | 3.3×10^{-14} | 3.0×10^{-12} | 0.03 | 0.53 |
| **Gender**                  |        |                     |                      |                         |                                |                           |
| Male                        | 384    | 49.5 (8.8)          | 153.8 (27.6)         | 37.0 (9.4)              | 185.8 (102.2)                | 203.0 (111.9)             |
| Female                      | 303    | 39.5 (8.5)          | 102.6 (17.8)         | 34.5 (10.3)             | 221.5 (113.8)                | 107.8 (72.7)              |
| *P*-value$^a$               | 3.7×10^{-31} | 6.5×10^{-28} | 2.3×10^{-6} | 2.1×10^{-21} |                                |                           |
| **Race**                    |        |                     |                      |                         |                                |                           |
| White                       | 621    | 45.0 (10.0)         | 131.4 (34.4)         | 35.6 (9.8)              | 201.2 (105.5)                | 163.9 (105.5)             |
| Non-White                   | 42     | 46.8 (11.1)         | 129.7 (39.8)         | 37.6 (10.7)             | 216.4 (137.3)                | 117.6 (118.3)             |
| *P*-value$^a$               | 0.24   | 0.60                | 0.15                 | 0.80                    | 0.001                         |                           |
| **Diabetes**                |        |                     |                      |                         |                                |                           |
| No diabetes                 | 498    | 44.4 (9.4)          | 129.4 (35.1)         | 37.0 (9.7)              | 191.4 (99.6)                 | 148.8 (105.6)             |
| Diabetes ≤4 yrs             | 92     | 45.8 (8.5)          | 133.5 (33.0)         | 33.3 (10.1)             | 224.4 (112.3)                | 189.2 (99.0)              |
| Diabetes >4 yrs             | 48     | 48.0 (10.6)         | 144.1 (35.9)         | 35.1 (8.7)              | 216.5 (130.4)                | 207.2 (116.1)             |
| *P*-value$^a$               | 0.01   | 0.01                | 0.004                | 0.03                    | 3.2×10^{-6}                  |                           |
| **BMI, kg/m²**              |        |                     |                      |                         |                                |                           |
| <18.5                       | 14     | 35.1 (4.6)          | 92.4 (15.0)          | 44.9 (12.2)             | 61.3 (51.9)                  | 24.3 (20.7)               |
| 18.5-24.9                   | 220    | 41.0 (8.0)          | 117.8 (31.2)         | 39.0 (8.2)              | 136.5 (64.8)                 | 89.9 (67.2)               |
| >24.9-29.9                  | 260    | 45.6 (8.1)          | 134.7 (32.1)         | 35.4 (8.7)              | 193.6 (73.3)                 | 176.0 (91.1)              |
| >29.9-34.9                  | 117    | 49.4 (10.2)         | 146.4 (36.3)         | 32.9 (8.7)              | 264.3 (83.0)                 | 228.1 (100.2)             |
| >35                         | 54     | 50.7 (10.0)         | 147.8 (35.6)         | 29.2 (10.9)             | 398.4 (121.2)                | 269.1 (128.0)             |
| *P*-value$^a$               | 3.6×10^{-32} | 7.1×10^{-29} | 2.3×10^{-29} | 1.0×10^{-29} | 1.1×10^{-29}                  |                           |
| **Current smoking**         |        |                     |                      |                         |                                |                           |
| No                          | 586    | 45.2 (10.1)         | 131.3 (35.1)         | 35.8 (9.8)              | 201.6 (105.6)                | 163.0 (109.4)             |
| Yes                         | 55     | 42.9 (8.6)          | 121.0 (29.9)         | 35.7 (11.1)             | 183.5 (112.8)                | 123.8 (88.4)              |
| *P*-value$^a$               | 0.11   | 0.05                | 0.81                 | 0.07                    | 0.02                         |                           |
| **Cancer stage**            |        |                     |                      |                         |                                |                           |
| Local                       | 213    | 45.3 (10.6)         | 130.7 (32.9)         | 34.6 (9.5)              | 217.5 (113.3)                | 170.7 (110.9)             |
| Locally advanced            | 147    | 45.1 (9.9)          | 132.6 (35.6)         | 37.8 (10.3)             | 186.5 (103.3)                | 152.5 (99.6)              |
| Metastatic                  | 279    | 45.3 (9.7)          | 131.6 (36.0)         | 35.9 (9.5)              | 190.5 (98.6)                 | 155.6 (106.3)             |
| Unknown                     | 48     | 43.5 (9.3)          | 126.9 (34.5)         | 35.2 (11.3)             | 241.0 (142.0)                | 176.0 (120.3)             |
| *P*-value$^a$               | 1.00   | 0.87                | 0.01                 | 0.02                    | 0.28                         |                           |
| **Primary tumor location**  |        |                     |                      |                         |                                |                           |
| Head/Uncinate               | 423    | 44.6 (10.2)         | 129.0 (33.8)         | 35.5 (10.1)             | 205.0 (110.7)                | 159.4 (105.6)             |
| Body                        | 131    | 45.0 (9.0)          | 130.8 (32.5)         | 36.9 (9.2)              | 185.8 (94.4)                 | 155.9 (104.3)             |
| Tail                        | 78     | 46.5 (9.8)          | 137.0 (38.0)         | 34.5 (9.9)              | 213.9 (112.2)                | 177.9 (122.7)             |
| *P*-value$^a$               | 0.22   | 0.26                | 0.10                 | 0.17                    | 0.51                         |                           |

Body composition measurements reported as mean ± s.d.

*Two-sided *P* values were calculated using Wilcoxon rank-sum tests.
Extended Data Table 3 | Hazard ratios (with 95% confidence intervals) for death among cases of pancreatic cancer by body composition measurements using computed tomography

### a.

| Pt. No. | Extreme Quintilesa,b | Per S.D.a | P-interactionc |
|---------|-----------------------|-----------|----------------|
| **Visceral fat area (cm²)** | | | |
| Localized | 2.02 (0.97-4.21) | 1.15 (0.88-1.50) | 0.59 |
| Locally advanced | 0.53 (0.22-1.32) | 0.81 (0.64-1.04) | |
| Metastatic | 0.98 (0.54-1.76) | 0.97 (0.80-1.17) | |
| **Subcutaneous fat area (cm²)** | | | 0.72 |
| Localized | 1.29 (0.58-2.87) | 1.44 (1.05-1.98) | |
| Locally advanced | 0.64 (0.26-1.60) | 0.83 (0.68-1.00) | |
| Metastatic | 0.99 (0.54-1.80) | 0.91 (0.73-1.12) | |
| **SMI (cm²/m²)** | | | 0.90 |
| Localized | 0.66 (0.30-1.46) | 0.84 (0.64-1.12) | |
| Locally advanced | 0.59 (0.27-1.29) | 0.87 (0.70-1.08) | |
| Metastatic | 1.54 (0.85-2.79) | 1.05 (0.84-1.30) | |
| **Muscle area (cm²)** | | | 0.47 |
| Localized | 0.82 (0.34-1.98) | 0.86 (0.62-1.19) | |
| Locally advanced | 0.40 (0.16-1.00) | 0.81 (0.62-1.06) | |
| Metastatic | 1.56 (0.77-3.18) | 1.10 (0.82-1.45) | |
| **Muscle attenuation (HU)** | | | 0.49 |
| Localized | 0.32 (0.16-0.67) | 0.74 (0.60-0.90) | |
| Locally advanced | 0.99 (0.50-1.96) | 0.94 (0.75-1.19) | |
| Metastatic | 1.01 (0.60-1.69) | 0.96 (0.80-1.16) | |

### Quintiles of Body Composition Measurements

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| **SMI (cm²/m²)** | | | | |
| Median OS (mo.) | 11.1 | 11.0 | 10.8 | 11.6 | 11.7 |
| Hazard ratio (95% CI)d | 1.0 | 0.98 (0.78-1.27) | 1.04 (0.80-1.36) | 0.92 (0.70-1.19) | 0.91 (0.68-1.21) |
| Hazard ratio (95% CI)e | 1.0 | 1.01 (0.78-1.31) | 1.04 (0.79-1.37) | 0.94 (0.71-1.24) | 0.93 (0.68-1.27) |
| **Muscle area (cm²)** | | | | |
| Median OS (mo.) | 10.2 | 12.1 | 10.8 | 11.6 | 11.3 |
| Hazard ratio (95% CI)d | 1.0 | 0.75 (0.58-0.98) | 0.90 (0.69-1.17) | 0.81 (0.62-1.05) | 0.84 (0.63-1.12) |
| Hazard ratio (95% CI)e | 1.0 | 0.78 (0.60-1.02) | 0.91 (0.69-1.20) | 0.82 (0.62-1.10) | 0.87 (0.64-1.20) |
| **Muscle attenuation (HU)** | | | | |
| Median OS (mo.) | 10.2 | 11.2 | 10.8 | 12.7 | 11.7 |
| Hazard ratio (95% CI)d | 1.0 | 0.89 (0.68-1.15) | 0.92 (0.70-1.20) | 0.75 (0.57-0.98) | 0.87 (0.65-1.15) |
| Hazard ratio (95% CI)e | 1.0 | 0.86 (0.65-1.13) | 0.89 (0.67-1.18) | 0.71 (0.53-0.96) | 0.80 (0.57-1.11) |

a. Patients were stratified by cancer stage at diagnosis. b. All patients. n = 687 patients.

dCox proportional hazards model adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), institution (DF/BWCC, MGH, Mayo Clinic, Stanford University or UNC), BMI (continuous), diabetes history (none, ≤4 years, >4 years or unknown) and smoking status (never, past, current or unknown)

eComparing the highest and lowest quintile.

fTwo-sided P interaction calculated by Wald test of the cross-product between body component measurements (continuous) and stage (local, locally advanced, metastatic, unknown).

gCox proportional hazards model adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), institution (DF/BWCC, MGH, Mayo Clinic, Stanford University or UNC) and cancer stage (local, locally advanced, metastatic or unknown).

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
Extended Data Table 4 | Plasma BCAA levels and clinical characteristics of pancreatic cancer cases

|                      | Pt No. | Total BCAAs (µM) |
|----------------------|--------|------------------|
| **Age, years**       |        |                  |
| < 60                 | 220    | 351.6 (174.9)    |
| 60 - <70             | 271    | 327.7 (104.3)    |
| ≥ 70                 | 287    | 297.7 (84.8)     |
| p-value*             |        | 1.3x10⁻⁶         |
| **Gender**           |        |                  |
| Male                 | 432    | 346.1 (101.2)    |
| Female               | 346    | 290.0 (144.1)    |
| p-value*             |        | 3.4x10⁻¹⁸        |
| **Race**             |        |                  |
| White                | 696    | 322.2 (98.3)     |
| Non-White            | 45     | 290.6 (122.2)    |
| p-value*             |        | 0.10             |
| **Diabetes**         |        |                  |
| No diabetes          | 563    | 322.3 (129.0)    |
| Diabetes ≤4 years    | 99     | 330.9 (113.6)    |
| Diabetes >4 years    | 54     | 341.8 (122.7)    |
| p-value*             |        | 0.24             |
| **Body-mass index, kg/m²** |   |                  |
| <18.5                | 14     | 286.3 (120.4)    |
| 18.5-24.9            | 245    | 316.7 (188.0)    |
| >24.9-29.9           | 295    | 322.6 (91.5)     |
| >29.9-34.9           | 131    | 337.0 (111.7)    |
| >35                  | 63     | 338.3 (99.0)     |
| p-value*             |        | 0.01             |
| **Current smoking**  |        |                  |
| No                   | 661    | 323.0 (101.2)    |
| Yes                  | 68     | 328.8 (291.0)    |
| p-value*             |        | 0.04             |
| **Cancer stage**     |        |                  |
| Local                | 256    | 306.6 (89.0)     |
| Locally advanced     | 155    | 321.1 (96.1)     |
| Metastatic           | 308    | 332.9 (110.0)    |
| Unknown              | 59     | 348.1 (283.8)    |
| p-value*             |        | 0.05             |
| **Primary tumor location** |     |                  |
| Head/Uncinate        | 479    | 311.7 (137.8)    |
| Body                 | 141    | 336.4 (107.1)    |
| Tail                 | 96     | 345.5 (70.1)     |
| p-value*             |        | 6.6x10⁻⁷         |

Plasma BCAA measurements reported as mean ± s.d.

*Two-sided P values calculated using Wilcoxon rank-sum test.
Extended Data Table 5 | Pearson correlation coefficients for BCAAs, body composition measurements and patient characteristics

| Variable               | BMI       | Age at diagnosis | Valine   | Leucine | Isoleucine | Total BCAA | SMI     | Muscle area | Muscle attenuation | Subcutaneous fat area | Visceral fat area |
|------------------------|-----------|------------------|----------|---------|------------|------------|---------|-------------|---------------------|-----------------------|------------------|
| BMI                    | 1.00      | -0.14*           | 0.108    | 0.06    | 0.01       | 0.06       | 0.43*   | 0.38*       | -0.30*              | 0.74*                 | 0.58*            |
| Age at diagnosis       | 1.00      | -0.19*           | -0.11*   | -0.14*  | -0.18*     | -0.29*     | 0.87*   | 0.22*       | -0.02*              | 0.09*                 | 0.03             |
| Valine                 | 1.00      | 0.82*            | 0.79*    | 0.86*   | 0.89*      | 0.24*      | 0.29*   | 0.16*       | 0.03                | 0.16*                 | 0.03             |
| Leucine                | 1.00      | 0.91*            | 0.99*    | 0.17*   | 0.24*      | 0.17*      | 0.01    | 0.12*       | 0.07                | 0.01                  | 0.07             |
| Isoleucine             | 1.00      | 0.93*            | 0.10*    | 0.14*   | 0.17*      | 0.13*      | 0.01    | 0.13*       | 0.01                | 0.15                  | 0.37*            |
| Total BCAA             | 1.00      | 0.17*            | 0.24*    | 0.17*   | 0.04*      | 0.20*      | 0.15*   | 0.37*       | 0.44*               | 0.44*                 | 0.44*            |
| SMI                    | 1.00      | 0.84*            | 0.23*    | 0.08*   | 0.40*      | -0.34*     | 0.40*   | 0.43*       | 1.00                | 1.00                  | 1.00             |

n = 687 patients.

*Two-sided P < 0.05.

Two-sided P < 0.0001.
Extended Data Table 6 | Hazard ratios (with 95% confidence intervals) for death among pancreatic cancer cases by plasma BCAA levels at diagnosis

| Quartiles of Plasma Branched Chain Amino Acids | 1     | 2     | 3     | 4     | P-trenda |
|----------------------------------------------|-------|-------|-------|-------|----------|
| **Total BCAAs**                              |       |       |       |       |          |
| Median (µM)                                  | 217.7 | 284.3 | 343.3 | 425.0 |          |
| Range (µM)                                   | 72.9-255.7 | 255.8-312.6 | 312.9-374.3 | 374.5-2327.2 |          |
| Median OS (mo.)                              | 10.6  | 10.8  | 12.4  | 12.1  |          |
| Hazard ratio (95% CI)b                        | 1.00  | 0.95 (0.76-1.19) | 0.79 (0.62-0.99) | 0.81 (0.64-1.02) | 0.04     |
| Hazard ratio (95% CI)c                        | 1.00  | 0.96 (0.76-1.20) | 0.78 (0.62-0.99) | 0.82 (0.64-1.04) | 0.06     |
| **Isoleucine**                               |       |       |       |       |          |
| Median (µM)                                  | 43.0  | 54.9  | 66.1  | 86.0  |          |
| Range (µM)                                   | 12.7-49.8 | 49.9-60.4 | 60.5-73.6 | 73.6-824.7 |          |
| Median OS (mo.)                              | 10.8  | 11.7  | 11.6  | 11.6  |          |
| Hazard ratio (95% CI)b                        | 1.0   | 0.89 (0.71-1.12) | 0.93 (0.74-1.16) | 0.89 (0.71-1.12) | 0.42     |
| Hazard ratio (95% CI)c                        | 1.0   | 0.89 (0.70-1.12) | 0.90 (0.72-1.14) | 0.90 (0.71-1.14) | 0.49     |
| **Leucine**                                  |       |       |       |       |          |
| Median (µM)                                  | 73.6  | 99.9  | 121.8 | 152.5 |          |
| Range (µM)                                   | 23.6-89.0 | 89.3-108.0 | 108.3-132.6 | 132.7-844.6 |          |
| Median OS (mo.)                              | 10.6  | 11.1  | 12.1  | 11.9  |          |
| Hazard ratio (95% CI)b                        | 1.0   | 0.91 (0.73-1.14) | 0.81 (0.64-1.02) | 0.82 (0.65-1.04) | 0.08     |
| Hazard ratio (95% CI)c                        | 1.0   | 0.93 (0.74-1.16) | 0.82 (0.65-1.03) | 0.84 (0.66-1.07) | 0.12     |
| **Valine**                                   |       |       |       |       |          |
| Median (µM)                                  | 98.6  | 129.0 | 155.5 | 191.3 |          |
| Range (µM)                                   | 35.4-114.0 | 114.2-141.9 | 142.0-171.3 | 171.4-656.0 |          |
| Median OS (mo.)                              | 9.9   | 11.5  | 12.4  | 12.1  |          |
| Hazard ratio (95% CI)b                        | 1.0   | 0.78 (0.62-0.99) | 0.71 (0.56-0.90) | 0.71 (0.56-0.91) | 0.01     |
| Hazard ratio (95% CI)c                        | 1.0   | 0.79 (0.62-1.00) | 0.71 (0.56-0.90) | 0.74 (0.57-0.94) | 0.02     |

n = 778 patients.

aTwo-sided P-trend calculated by entering the quartile-specific median value for individual plasma BCAAs as a continuous variable in a Cox proportional hazards model.

bCox proportional hazards model adjusted for age at diagnosis (continuous), gender (male or female), race (white, non-white or unknown), year of diagnosis (2000–2005, 2006–2010 or 2011–2015), institution (DF/BWCC, MGH, Mayo, Stanford University or UNC) and cancer stage (local, locally advanced, metastatic or unknown).

Cox proportional hazards model additionally adjusted for BMI (continuous), diabetes history (none, ≤4 years, >4 years or unknown) and smoking status (never, past, current or unknown).
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

### Experimental design

1. **Sample size**
   
   **Describe how sample size was determined.**

   No sample size calculation was performed for animal studies. The number of animals assigned per condition was selected to provide sufficient statistical power to discern significant differences. This was based on prior experience with the model and based on several published manuscripts using these models. In human studies, sample size was determined to detect a statistically significant difference in survival between patients in highest and lowest quantiles of plasma branched chain amino acids and body composition measurements by CT imaging.

2. **Data exclusions**
   
   **Describe any data exclusions.**

   Statistical outliers were calculated using Grubb’s outlier test (Prism) and excluded from final analysis. No patient data was excluded from the analysis.

3. **Replication**
   
   **Describe whether the experimental findings were reliably reproduced.**

   All attempts at replication were successful.

4. **Randomization**
   
   **Describe how samples/organisms/participants were allocated into experimental groups.**

   For diet studies in PDAC mice (Figure 3j-k, Figure S3e-i), PDAC mice were weighted and randomly assigned either a control diet or a diet supplemented with pancreatic enzymes. Animals were weighted to ensure similar starting body weights in both groups. For caloric restriction studies (Figure S3a-d), C57Bl/6J mice were injected with PDAC-derived cells to develop subcutaneous tumors. After tumors were palpable, tumor volume and mouse body weight were measured to ensure both groups of mice (control and calorically restricted) had similar starting tumor volume and body weight. The study involving pancreatic cancer patients was observational, and therefore no randomization was performed. Experimental groups were defined by quantiles of plasma BCAAs and body composition measurements by CT imaging.

5. **Blinding**
   
   **Describe whether the investigators were blinded to group allocation during data collection and/or analysis.**

   Animals were numbered and experiments conducted in a blinded manner. After data collection, genotypes were revealed and animals assigned to groups for analysis. For subcutaneous and orthotopic allograft tumor implantation, blinding of experiments was not feasible. For the human studies, all collected data including exposure variables (plasma BCAAs and body composition measurements by CT imaging) were obtained with blinding to outcome data (patient survival).

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒         |

- The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

---

**Software**

**Policy information about availability of computer code**

**7. Software**

Describe the software used to analyze the data in this study.

GraphPad Prism, Excel, ImageJ, SAS

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

---

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used are available from the authors.

---

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies recognizing pHSL (Ser563) (#4139) and total HSL (#4107) were purchased from Cell Signaling Technologies. pHSL had been validated in preliminary experiments where adipose tissue explants were treated with isoproterenol to induce lipolysis.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Murine PDAC cell lines were isolated from tumor-bearing C57Bl/6J KP-/-C (Kras G12D; P53 fl/fl; Pdx1-cre) mice. Pancreatic stellate cells were isolated from wild-type C57Bl/6j mice. All cell lines utilized were mycoplasma free.

b. Describe the method of cell line authentication used.

Cell lines used were not authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines were tested and tested negative for Mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used in these studies.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

All animals (Mus Musculus) used in this study were fully back-crossed to the C57Bl/6J background. Unless otherwise stated (Figure 1A-C), all animals were male. All experimental groups included age-matched litter-mate controls and animals were co-housed (unless otherwise stated, Figure S3a-d,f,h). For end-stage tissue weights in KP/-/- C studies, animals were approximately 10-12 weeks of age (Figure 1A-C). For "early KP/-/- C" studies, animals were 6 weeks of age (Fig 1a-c). For KC studies, animals were 15 weeks of age (Figure 1k-l, Figure S1m). For orthotopic and subcutaneous implantation studies (Figure 2a-f, Figure S3a-d), 10-12 week old male C57Bl/6J (Jackson mice, 000664) were utilized.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study included 782 pancreatic cancer patients who received care at five U.S. cancer centers: Dana-Farber/Brigham and Women’s Cancer Center, Massachusetts General Hospital, Mayo Clinic, Stanford University, and University of North Carolina-Chapel Hill. Detailed demographic and clinical characteristics of those patients are shown in Supplementary Table 1.