Mutant GNAS drives pancreatic tumourigenesis by inducing PKA-mediated SIK suppression and reprogramming lipid metabolism

Krushna C. Patra1,2, Yasutaka Kato1, Yusuke Mizukami1,3,4, Sebastian Widholz1, Myriam Boukhali1, Iulia Revenco1, Elizabeth A. Grossman5, Fei Ji5,6, Ruslan I. Sadreyev7,8, Andrew S. Liss1, Robert A. Screaton9,10, Kei Sakamoto11,15, David P. Ryan1,2, Mari Mino-Kenudson1,7,8, Carlos Fernandez-del Castillo1,12,13, Daniel K. Nomura4, Wilhelm Haas1 and Nabeel Bardeesy1,2*

G protein αs (GNAS) mediates receptor-stimulated cAMP signalling, which integrates diverse environmental cues with intracellular responses. GNAS is mutationally activated in multiple tumour types, although its oncogenic mechanisms remain elusive. We explored this question in pancreatic tumourigenesis where concurrent GNAS and KRAS mutations characterize pancreatic ductal adenocarcinomas (PDAs) arising from intraductal papillary mucinous neoplasms (IPMNs). By developing genetically engineered mouse models, we show that GnasR201C cooperates with KrasG12D to promote initiation of IPMN, which progress to invasive PDA following Tp53 loss. Mutant Gnas remains critical for tumour maintenance in vivo. This is driven by protein-kinase-A-mediated suppression of salt-inducible kinases (Sik1-3), associated with induction of lipid remodelling and fatty acid oxidation. Comparison of Kras-mutant pancreatic cancer cells with and without Gnas mutations reveals striking differences in the functions of this network. Thus, we uncover Gnas-driven oncogenic mechanisms, identify Siks as potent tumour suppressors, and demonstrate unanticipated metabolic heterogeneity among Kras-mutant pancreatic neoplasms.

The GNAS gene encodes the Gαs stimulatory subunit of heterotrimeric G proteins, which mediate G-protein-coupled receptor (GPCR) signalling, a central mechanism by which cells sense and respond to extracellular stimuli. Ligand stimulation of GPCRs promotes GDP for GTP exchange on Gαs, enabling Gαs to engage effectors. The primary target is adenyl cyclase, which generates the second messenger cAMP, an activator of protein kinase A (PKA), effectors. The primary target is adenylyl cyclase, which generates cAMP in ~41–75% of IPMNs9,10,15 and ~2–11% of total PDAs11,12,15,16). GNAS mutations, whereas KRAS mutations characterize pancreatic intraepithelial neoplasias (PanINs) and IPMNs both exhibit frequent mutations and amplifications are particularly common in pancreatic tumorigenesis where their presence distinguishes two major precursors of invasive PDA; pancreatic intraepithelial neoplasias (PanINs) and IPMNs both exhibit frequent KRAS mutations, whereas GNAS mutations are exclusive to IPMN (present in ~41–75% of IPMNs9,10,15,18 and ~2–11% of total PDAs11,12,15,16).

**Results**

Pancras-specific GnasR201C and KrasG12D mutations cooperate to promote IPMNs. To examine the functions of mutually activated Gnas in the murine pancreas and its cooperative interactions with oncogenic Kras, we generated a knock-in strain expressing GnasR201C controlled by a doxycycline (Dox)-inducible promoter (Teto-GnasR201C) and crossed these mice with the Ptf1a-Cre, Rosa26-LSL-rtTA and LSL-KrasG12D strains, to establish GC (GnasR201C; Cre), KC (KrasG12D; Cre) and KGC (KrasG12D; GnasR201C; Cre) cohorts (Fig. 1a). KGC mice rapidly developed cystic pancreatic tumours requiring euthanasia (mean 9.7 weeks) (Fig. 1b–e). At this time point, KC mice had only focal PanINs7,12 and GC mice had no abnormalities (Fig. 1c and Supplementary Fig. 1a). Histologic and immunostaining analyses of KGC tumours suggested equivalence to human gastric- and pancreatobiliary-type IPMN, with positive staining for cytokeratin-19, Muc5AC and Muc1, but not Muc2 or Cdx2 (Fig. 1d and Supplementary Fig. 1b). Long-term monitoring revealed eventual development of invasive PDA in KC mice and low-grade IPMN in GC mice (mean survival of 61 and 106 weeks, respectively) (Fig. 1b, f–g).

Serial analyses of KGC mice following Dox-mediated induction of GnasR201C revealed neoplastic lesions with ductal morphology within one week, which increased in size and number to encompass...
Fig. 1 | Pancreas-specific Gnas<sup>R201C</sup> and Kras<sup>G12D</sup> mutations cooperate to promote IPMNs. **a**, Schematic of mouse strains. **b**, Kaplan-Meier analysis for time until tumour progression necessitated euthanasia (KGC: N = 13; KC: N = 15; GC: N = 12; C: N = 14; N represents mouse numbers). Kaplan-Meier curves were analysed by log-rank test. P < 0.05 was considered statistically significant. **c**, Photographs depicting pancreata from 12-week-old mice of the indicated genotypes. Representative of 10 mice per group. **d**, H&E-stained sections (top panels) and IHC stained sections for the indicated markers (lower panels). Representative of 10 mice per group for H&E and 3 mice per group for IHC. Inset: higher magnification. **e**, H&E staining of KC and KGC pancreata at the indicated time points (representative of 3 mice per group). Right: higher magnification of the boxed regions. **f**, Gross photograph of the pancreas from a representative GC mouse and control mouse at age 61 weeks. **g**, H&E-stained section of the GC pancreas at age 61 weeks. The background in **f** was edited in Photoshop for presentation purposes. Data in **f**, **g** are representative of 4 GC mice. Scale bars: 1 cm (**c**), 200 μm (**d**, main), 40 μm (**d**, inset), 200 μm (**e**, left), 40 μm (**e**, right), 1 cm (**f**), 2 mm (**g**, top) and 40 μm (**g**, bottom). Source data are provided in Supplementary Table 2.
the entire organ after 3–7 weeks (Fig. 1e). The lesions exhibited staged histological progression, gradually acquiring papillary architecture, nuclear stratification and atypical nuclei. Collectively, these studies show that GNAS<sup>R201C</sup> alone is weakly oncogenic in the pancreas, but potently synergizes with Kras<sup>G12D</sup>, shifting the PanIN phenotype to that resembling high-grade IPMNs and accelerating tumorigenesis.

**Tp53 loss facilitates progression of Kras/Gnas-mutant IPMNs to invasive PDA.** Consistent with the clinical behaviour of most human IPMNs, we did not observe invasion in KGC mice despite the presence of high-grade dysplasia (Supplementary Fig. 1c). Tp53 mutations arise as a late event in advanced human IPMNs and coexist with GNAS mutations in a subset of PDA<sup>20,21,23,24</sup> (ref. <sup>18</sup> accessed from www.cbioportal.org), prompting us to examine the impact of this genetic alteration. We generated mice with combinations of conditional heterozygous Kras<sup>G12D</sup>, Gnas<sup>R201C</sup> and Tp53<sup>α/α</sup> alleles. KGC mice exhibit rapid onset of end-stage disease due to extensive IPMN burden; therefore, we used the Ptf1a-Cre<sup>ER<sub>2</sub></sup> system to recombine the mutant alleles more focally in adult pancreatic acinar cells (Fig. 2a). Notably, KGPCER mice (Kras<sup>G12D</sup>; Gnas<sup>R201C</sup>; p53<sup>LoxP/−</sup>; Cre<sup>ER<sub>2</sub></sup>) developed malignant ascites and invasive tumours with significantly shorter latency compared to KPC<sup>ER<sub>2</sub></sup> (Kras<sup>G12D</sup>; Gnas<sup>R201C</sup>; p53<sup>LoxP/−</sup>; Cre<sup>ER<sub>2</sub></sup>) animals (KGPCER, mean 25.8 weeks; KPCER, mean 38.3 weeks); invasive disease was never observed in the other cohorts. Histological analysis of end-stage KGPCER mice revealed PDAs contiguous with high-grade IPMNs and showing liver and peritoneal dissemination, whereas age-matched KGC<sup>ER<sub>2</sub></sup> and KPCER mice had only IPMNs and PanINs, respectively (KGPCER<sup>ER<sub>2</sub></sup> and KGC<sup>ER<sub>2</sub></sup> N<sub>5</sub> mice) (Fig. 2b–d). Loss of wild-type Tp53 was observed in three of four KGPCER<sup>ER<sub>2</sub></sup> PDAs (Fig. 2e). To extend these data, we tested directly whether Tp53 inactivation enables IPMN-to-PDA progression using primary cultures established from KC tumours. Orthotopic injection of parental KC<sup>G12D</sup>R201C<sup>Cmin</sup> cells into SCID mice resulted in non-invasive IPMNs, recapitulating the phenotype to that resembling high-grade IPMNs and showing liver and peritoneal dissemination, whereas age-matched KGC<sup>Cmin</sup> and KPCER<sup>Cmin</sup> mice had only IPMNs and PanINs, respectively (KGC<sup>Cmin</sup> and KGC<sup>Cmin</sup> N<sub>8</sub> mice per group; KPCER<sup>Cmin</sup>, N<sub>5</sub> mice) (Fig. 2f,g). Thus, IPMNs harbouring hallmark Gnas and Tp53 mutations can progress to PDA, and Tp53 is an important barrier to the onset of malignancy.

**Gnas<sup>R201C</sup> is critical for pancreatic tumour maintenance.** The circuits supporting the growth of established IPMNs and resulting PDA have yet to be defined. Our Dox-inducible system enabled us to investigate whether Gnas<sup>R201C</sup> is required for tumour maintenance. To this end, KCG mice were provided with Dox until palpable tumours were present, and then Dox was withdrawn (Fig. 3a, Dox-Off group). These mice showed immediate improvements in body condition, including reductions in abdominal size and cyst fluid (Fig. 3b and Supplementary Fig. 2a). Remarkably, they remained healthy for >20 weeks, whereas the Dox-On group required euthanasia within 1–3 weeks of tumour detection (Fig. 3c). Serial analyses of tumours following Dox withdrawal revealed rapid loss of dysplastic papillary features, reduced mucins and decreased proliferation (Fig. 3d), without evidence of apoptosis (Supplementary Fig. 2b). Furthermore, organoids derived from KGC IPMNs and KGPCER PDAs remained dependent on Gnas<sup>R201C</sup> for tumour formation and maintenance, as assessed by injection into SCID mice±Dox supplementation and by acute Dox withdrawal following tumour establishment, respectively (Fig. 3e–g). Thus, Gnas<sup>R201C</sup> is critical for sustained pancreatic tumour growth, despite the presence of concurrent Kras and Tp53 mutations. This tumour maintenance function and induction of PDA from IPMNs rather than PanINs suggest that mutant Gnas may establish a unique molecular program in PDA distinct from that resulting from Kras/Tp53 mutations alone.

**Gnas<sup>R201C</sup> supports pancreatic tumour growth via cAMP–PKA signalling.** We used tumour-derived organoids from the KGC (IPMN) and KGC<sup>R201C</sup> (PDA) models to define the oncogenic mechanisms of Gnas<sup>R201C</sup>. Dox withdrawal abrogated organoid growth in vitro, indicating that Gnas<sup>R201C</sup>-driven tumour maintenance involves cell-intrinsic mechanisms. Gnas<sup>R201C</sup> extinction led to a decrease in cAMP whereas treatment with the adenylyl cyclase agonist forskolin (FSK) boosted cAMP levels and rescued organoid growth (Fig. 4a–d and Supplementary Fig. 3a–c). These data are consistent with a primary role for adenylyl cyclase/cAMP signalling downstream of Gnas<sup>R201C</sup>, rather than engagement of non-canonical targets<sup>3,17</sup>, cAMP activates multiple effectors (Fig. 4c) including PKA, whose subunits are abundantly expressed in KGC cultures, and EPAC1/2, which are virtually undetectable (Supplementary Fig. 3d). Accordingly, activation of PKA with the cAMP analogue Sp-8-Br-cAMPS (8-bromoadenosine-3′,5′-cyclic monophosphorothioate, Sp-isomer, acetoxyethyl ester) rescued colony formation, whereas the EPAC-specific agonist 8-pCPT-2′-O-Me-cAMPS (8-(4-chlorophenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate) had no effect (Fig. 4d; PKA activation by FSK and Sp-8-Br-cAMPS is shown in Supplementary Fig. 3e). Moreover, Gnas<sup>R201C</sup> extinction decreased PKA signalling in vitro and in vivo as reflected by phosphorylation of VASP and proteins detected with phospho-PKA substrate antibody (Fig. 4f and Supplementary Fig. 3g; Dox-dependent regulation of Gnas expression in vivo is shown in Supplementary Fig. 3f,g). Correspondingly, ectopic expression of a dominant-negative PKA mutant (PKA<sup>DN</sup>) attenuated growth of KG and KGC<sup>R201C</sup> organoids in vitro, and suppressed tumorigenesis following organoid injection in vivo (Fig. 4g–i and Supplementary Fig. 3h,i; inhibition of PKA signalling by PKA<sup>DN</sup> is shown in Fig. 3c). These experiments establish PKA as a principal effector of oncogenic Gnas<sup>R201C</sup>.

**The salt-inducible kinases are critical targets of oncogenic Gnas-PKA signalling in pancreatic tumours.** Creb, the classic PKA substrate, and several other direct or indirect PKA targets implicated in growth control either showed no phosphorylation changes (Creb, Yap and β-catenin) or increased activating phosphorylation (ERK1/2) following Gnas<sup>R201C</sup> silencing (Supplementary Fig. 4a–d). A broader network by which PKA integrates responses to hormonal cues involves phosphorylation of salt-inducible kinases (Sik1, Sik2 and Sik3), which prevents SIKs from phosphorylating key substrates<sup>20,21</sup> (Fig. 5a). Similarly to cAMP/PKA signalling, SIKs have context-dependent tumour-promoting and -suppressing roles<sup>22–25</sup>. Consistent with Gnas<sup>R201C</sup>-mediated control of this pathway in KCG cells, Dox withdrawal reduced Sik2 phosphorylation at each documented PKA site (Fig. 5b) and, accordingly, there was increased phosphorylation of established SIK targets, CrtcRT2C271, Crtc2-S275 and Hdac7-155 (Fig. 5b) and, PKA<sup>DN</sup> expression recapitulated these effects (Fig. 5c). Thus, oncogenic Gnas<sup>R201C</sup>-PKA signalling leads to sustained inhibition of SIKs in pancreatic tumour cells. Sik1, Sik2 and Sik3 have overlapping functions and each is readily detectable in Gnas-mutant organoids (Sik2 and Sik3 are visualized by immunoblot with available antibodies whereas Sik1 is not, but has the highest mRNA levels; Fig. 5f and Supplementary Fig. 4b). This prompted us to study their role as a group using small-molecule pan-SIK inhibitors (HG-9-91-01 and KIN-112)<sup>26,28</sup>. Remarkably, these compounds rescued proliferation of KGC organoids following Gnas<sup>R201C</sup> silencing, with effects proportional to the extent of SIK inhibition gauged by Crtc2 phosphorylation (Fig. 5d,e). Conversely, expression of the Sik2-S4A mutant<sup>27,28</sup>, which is resistant to cAMP/PKA-dependent phosphorylation, strongly suppressed growth of KGC organoids compared to WT Sik2 (Supplementary Fig. 4c). We corroborated these studies with CRISPR-mediated gene editing using two independent sets of sgRNAs to knock out Sik1-3 in KGC cells. qRT-PCR and immunoblot confirmed that Sik1–3<sup>−/−</sup> cells lost Sik1–3 expression and lacked induction of Crtc2 phosphorylation following
Dox withdrawal (Supplementary Figs. 4d and 5f). Importantly, Sik1–3KO rescued both organoid growth in vitro and subcutaneous tumour growth following GnasR201C silencing (Fig. 5g,h). Thus, the cAMP-PKA-SIK axis is critical for GnasR201C-driven tumour growth.

To test this circuit in human PDA, we generated a cell line (950-5-BLK) from a human PDA harbouring KRASG12V, GNASR201H and TP53R273C mutations (Fig. 6a), and compared this model with a series of KRAS-mutant/GNASWT patient-derived and established PDA cell lines. Immunoblot analysis revealed that 950-5-BLK cells had high relative levels of phospho-PKA substrates and phospho-VASP and low phospho:total CRTC2 levels, consistent with constitutive PKA activity and impaired SIK function (Fig. 6b).
Moreover, shRNA-mediated knockdown of GNAS promoted SIK activity specifically in 950-5-BLK cells, as reflected by increased CRTC2 phosphorylation (Fig. 6c). Ectopic expression of PKADN also increased CRTC phosphorylation in 950-5-BLK cells (Fig. 6e). Importantly, PKADN inhibited the growth of 950-5-BLK tumour spheres and subcutaneous xenografts, while having minimal effects in three human GNAS-WT PDA models (Fig. 6d–f). Therefore, oncogenic GNAS-PKA-SIK signalling is a conserved mechanism of murine and human pancreatic tumour maintenance.

Mutant GNAS reprograms lipid metabolism in pancreatic tumour cells. The GNAS-PKA-SIK pathway modulates lipolysis and lipid synthesis in liver and adipose tissue. Notably, global inhibitors rescued growth of PKADN expressing organoids (Fig. 6g).
Fig. 4 | GnasR201C supports pancreatic tumour growth via cAMP-PKA signalling. a, Organoid cultures established from two independent KGC tumours (lines A and B) were tested for in vitro growth ±Dox (200 ng ml\(^{-1}\)) after 7 days. Left, gross photos; graph: quantification. b, Immunoblot showing Gnas regulation in KGC cells. c, Schematic of GNAS signalling and targets of agonists. d, Quantification of KGC organoid growth ±Dox and ± the indicated agonists. e, Immunoblot showing PKA regulation in KGC cells as determined by VASP mobility shift (PKA-phosphorylated VASP), and by anti-phospho-PKA substrate (subst.) antibody. f, IHC staining using anti-phospho-PKA substrate antibody in KGC tumours in mice ±Dox or following Dox withdrawal. Bottom: higher magnification of boxed regions. Data are quantified in the chart (right). g, h, Growth of KGC (g) and KGC\(^{R201C}\) (h) organoids expressing empty vector (V) or PKADN cultured ±Dox. i, Tumour volume at 6 weeks following subcutaneous injection of KGC organoids expressing empty vector (V) or PKADN. Scale bars: 500 μm (a), 200 μm (f, top) and 40 μm (bottom). a, d, N = 3 independent biological replicates; b, e, immunoblots were performed two times with similar results; f, N = 4 mice per group; g, N = 6 independent biological replicates; h, N = 4 independent biological replicates; i, N = 8 tumours per group. Error bars (a, d, f–i) are ±s.e.m. Significance was analysed using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Source data are provided in Supplementary Table 2. Original scans of the immunoblots are shown in Supplementary Fig. 8.

quantitative proteomics of KGC tumour organoids ±Dox and gene set enrichment analysis (GSEA) revealed enrichment of processes relating to lipid metabolism and in components of the peroxisome (microbody), an organelle required for processing of very long-chain fatty acids and generation of ether lipids\(^{33,34}\) (Fig. 7a and Supplementary Fig. 5a). QRT-PCR following Dox removal or treatment with the PKA inhibitor KT5720 indicates that GnasR201C-PKA signalling supports the transcription of multiple lipid metabolism enzymes (Supplementary Fig. 5b). Comparable expression changes were observed following acute GnasR201C silencing in subcutaneous KGC tumours in vivo (Supplementary Fig. 5c). Moreover, PKADN caused similar expression changes in human GNAS-mutant 950-S-12-MD.BLK cells, while not significantly affecting GNAS-WT PDA models (Supplementary Fig. 5d).

Based on these observations and the central role of GNAS-PKA-SIK signalling in regulating lipid metabolism in normal tissues\(^{1,29,31,35}\), we examined the potential functions of oncogenic GnasR201C in reprogramming tumour cell metabolism. Free fatty acids synthesized de novo or imported into the cell are rapidly esterified to glycerolipids/triglycerides (TG)\(^{29,36–38}\). Thus, complementary lipolytic processes are required to provide fatty acid substrates for signalling, structural, and metabolic purposes, and recent studies have shown that various oncogenes coordinate this lipid network in different tumour types\(^{29,38–40}\). Using BODIPY staining and lipidomic profiling of KGC organoids, we found that GnasR201C inactivation caused a decrease in total lipids including TG, as well as specific depletion of several lipid classes (Supplementary Fig. 6a–c).

There was a particularly pronounced reduction in ether lipids, in keeping with the downregulation of the ether lipid biosynthesis enzymes Far1 and Apps (Supplementary Figs. 5bc and 6d,e). However, the ratio of monoglycerides/triglycerides (MG:DG) was decreased following
Fig. 5 | SIKs are critical targets of oncogenic Gnas-PKA signalling in pancreatic tumours. a, Schematic of PKA-SIK signalling. b, Immunoblots of lysates from KGC organoids grown ±Dox, demonstrating Gnas<sub>ΔC</sub>-mediated regulation of phosphorylation of Sik2, Crtc2, and Hdac7. Note: Crtc2 phosphorylation is indicated by phosphospecific antibodies and mobility shift. c, Immunoblot of lysates from the indicated KGC organoids grown ±Dox and expressing empty vector (V) or PKADN, showing that PKADN effectively inhibits PKA signalling and activates SIKs. d–f, KGC organoids were cultured ±Dox and ±the indicated SIK inhibitor (250 nM) for 7 days. g, Immunoblots documenting PKA-SIK signalling changes. CRTC2 protein and defective Crtc2 phosphorylation following Dox withdrawal (g1, g2). Scale bars in a, e.g. 750 μm. Immunoblots in b–d,f were performed two times with similar results; e.g., N=3 and 4 independent biological replicates, respectively; h, GFPg1 ±Dox (N=5), GFPg1 –Dox (N=7), Sik(1-3)g1 +Dox (N=7), Sik(1-3)g1 –Dox (N=5) Sik(1-3)g2 +Dox (N=7), Sik(1-3)g2 –Dox (N=7); N represents number of tumours. Error bars (e.g.h) are ±s.e.m. Significance was analysed using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Source data are provided in Supplementary Table 2. Original scans of the immunoblots are shown in Supplementary Fig. 8.
The GNAS-PKA-SIK axis drives growth of human patient-derived PDA cells harbouring concurrent GNAS, KRAS and TP53 mutations.

a. Sequencing chromatograms of 950-5-BLK cells, derived from a human PDA patient, showing GNAS<sup>R201H</sup>, KRAS<sup>G12V</sup> and TP53<sup>R273C</sup> mutations. Sequencing was performed from two independent DNA isolations and PCR reactions.

b. Immunoblot of a series of PDA cell lines, including those from the ATCC repository and newly established patient-derived lines. The single GNAS mutant line, 950-5-BLK shows highest PKA activity and uniquely exhibits CRTC2 primarily in its unphosphorylated form.

c. Immunoblot of the indicated PDA cell lines expressing two different shRNAs against GNAS or control shGFP. GNAS knockdown blocks PKA signalling and specifically activates SIK-mediated CRTC2 phosphorylation in 950-5-BLK cells.

d–f. The indicated human PDA cell lines were engineered to express PKA<sup>N1</sup> or empty vector and tested for relative growth as matrigel spheroid cultures (d), subjected to immunoblot analysis for activity of PKA and SIK (e) and tested for tumorigenicity following subcutaneous injection in SCID mice (f).

g. Growth as spheroids of 950-5-BLK cells expressing PKA<sup>N1</sup> treated with increasing amounts of the indicated pan-SIK inhibitor (HG-9-91-01). Cells transduced with empty vector are shown as controls. The data are graphed at the right (the inhibitor-treated vector-expressing cells are not represented in photomicrographs). Scale bars in g, 500 μm. Immunoblots in b,c,e were performed two times with similar results; d,g, N = 3 independent biological replicates; f, PANC-1 and YAPC (N = 8 tumours per group) and 1925-2-BHG and 950-5-BLK (N = 10 tumours per group). Error bars (d,f,g) are ±s.e.m.). Significance was analysed using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Source data are provided in Supplementary Table 2. Original scans of the immunoblots are shown in Supplementary Fig. 8.
Fig. 7 | Mutant Gnas reprograms lipid metabolism in pancreatic tumour cells. a, GSEA was performed using quantitative proteomics data from two independent KGC organoid lines grown ±Dox. GnasR201C positively regulates fatty acid metabolism and peroxisomal gene sets as defined in the biological process (BP) and cellular compartment (CC) categories from the Gene Ontology (GO) database. Data are from two independent quantitative proteomics experiments with the following number of replicates per condition: experiment no. 1, two replicates per condition for both organoid lines; experiment no. 2, three replicates for organoid line A and two replicates for organoid line B. P values are calculated based on 1,000 permutations and adjusted for multiple hypothesis testing. b, KGC organoids grown ±Dox were analysed by global lipidomics. The graph shows that the relative MG:TG ratio and MG:DG ratios are decreased following Dox withdrawal. c, Immunoblot showing GnasR201C-dependent phosphorylation of HSL (Ser660) in KGC organoids. d, Relative FAO rate assessed by 14CO2 trap in KGC cultures grown ±Dox (d) or +Dox and ±PKA DN (e). f, Relative acetyl-CoA levels in KGC cultures grown ±Dox, detected by LC-MS. g, Immunoblot of organoids from Gnas mutant (KGC; IPMN) and (KGCshp53; PDA) and Gnas WT PDA (KPC, KIC, and KPIC) models (see Methods). h, Relative growth of Gnas mutant and WT organoids ±FSK (10μM). i, Relative FAO rates. j,k, Impact of Cpt1a knockdown using two different shRNAs on organoid growth (j) and tumorigenesis (k) following subcutaneous injection. b,d,e,h,N = 3; f, N = 5; j, N = 4 independent biological replicates; c, immunoblots were performed two times with similar results; i, n = 4 cell lines for GnasMUT and n = 3 cell lines for GnasWT; k, N = 6 tumours per group. Error bars (b,d,f,h-k) are ±s.e.m. Significance was analysed using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Source data are provided in Supplementary Table 2. Original scans of the immunoblots are shown in Supplementary Fig. 8.

Dox withdrawal (Fig. 7b), suggesting that GnasR201C promotes lipid hydrolysis in addition to lipid synthesis and remodelling.

In Kras-mutant pancreatic cancers lacking Gnas mutations, both lipid accumulation and fatty acid oxidation (FAO) are restricted by Kras signalling. By contrast, our data indicate that these processes are activated by GnasR201C (despite concurrent KrasG12D mutations), prompting us to examine lipid metabolism in further detail. In adipocytes, the Gnas-PKA-SIK axis controls lipolysis through PKA-mediated phosphorylation events as well as SIK-dependent transcriptional changes. Accordingly, GnasR201C extinction in KGC organoids led to loss of activating phosphorylation (Ser660) of hormone-sensitive lipase (HSL) (Fig. 7c) and reduced expression of other lipases (Supplementary Fig. S8f). Reduced phospho-HSL was detected by LC-MS. GnasR201C positively regulates fatty acid metabolism and peroxisomal gene sets as defined in the biological process (BP) and cellular compartment (CC) categories from the Gene Ontology (GO) database. Data are from two independent quantitative proteomics experiments with the following number of replicates per condition: experiment no. 1, two replicates per condition for both organoid lines; experiment no. 2, three replicates for organoid line A and two replicates for organoid line B. P values are calculated based on 1,000 permutations and adjusted for multiple hypothesis testing. b, KGC organoids grown ±Dox were analysed by global lipidomics. The graph shows that the relative MG:TG ratio and MG:DG ratios are decreased following Dox withdrawal. c, Immunoblot showing GnasR201C-dependent phosphorylation of HSL (Ser660) in KGC organoids. d, Relative FAO rate assessed by 14CO2 trap in KGC cultures grown ±Dox (d) or +Dox and ±PKA DN (e). f, Relative acetyl-CoA levels in KGC cultures grown ±Dox, detected by LC-MS. g, Immunoblot of organoids from Gnas mutant (KGC; IPMN) and (KGCshp53; PDA) and Gnas WT PDA (KPC, KIC, and KPIC) models (see Methods). h, Relative growth of Gnas mutant and WT organoids ±FSK (10μM). i, Relative FAO rates. j,k, Impact of Cpt1a knockdown using two different shRNAs on organoid growth (j) and tumorigenesis (k) following subcutaneous injection. b,d,e,h,N = 3; f, N = 5; j, N = 4 independent biological replicates; c, immunoblots were performed two times with similar results; i, n = 4 cell lines for GnasMUT and n = 3 cell lines for GnasWT; k, N = 6 tumours per group. Error bars (b,d,f,h-k) are ±s.e.m. Significance was analysed using two-tailed Student’s t-test. P < 0.05 was considered statistically significant. Source data are provided in Supplementary Table 2. Original scans of the immunoblots are shown in Supplementary Fig. 8.

Correspondingly, assessment of CO2 release from 14C-labelled oleate in KGC cultures ±Dox demonstrated that GnasR201C was required to sustain FAO activity (Fig. 7d). The use of PKADN confirmed that this effect was PKA-dependent (Fig. 7e). PKADN also suppressed FAO in human Gnas-mutant (950-5-BLK) but not GNAS-WT PDA cells (Supplementary Fig. 6g). Importantly, FSK or SIK inhibitor treatment or SIK1-351 rescued FAO following GnasR201C extinction (Supplementary Fig. 6i,j). Thus, GnasR201C activates multiple aspects of lipid metabolism including potentiating FAO through the PKA-SIK axis.

LC-MS/MS-based profiling of 95 polar metabolites spanning central metabolic pathways revealed that GnasR201C silencing in KGC cells led to a decrease in acetyl-CoA (Fig. 7f), which is a main product of FAO. Moreover, shRNA-mediated silencing of carnitine palmitoyltransferase 1a (Cpt1a), the rate-limiting enzyme for fatty acid transport into mitochondria, reduced acetyl-CoA levels in KGC organoids but not in GnasWT PDA organoids from a Kras-mutant/Tp53-deficient genetically engineered mouse model.
Metabolic reprogramming is a hallmark of PDA driven by oncogenic Kras and involves enhanced glycolysis and attenuated FAO\(^{41,45}\). Our studies indicated that Gnas\(^{R201C}\) induces a distinct set of metabolic processes, suggesting that there may be differences in the circuitry of Kras-mutant pancreatic tumours depending on Gnas mutational status. Notably, comparison of four independently-derived lines per group of murine Gnas-mutant and Gnas\(^{WT}\) PDA organoids revealed opposite patterns of PKA and SIK activity (Fig. 7g), consistent with observations in human PDA cells (Fig. 6b). Moreover, FSK treatment compromised the growth of multiple Gnas\(^{WT}\) PDA organoids while promoting that of Gnas-mutant organoids (Fig. 7h). Conversely, Gnas-mutant organoids were highly sensitive to PKA\(^{DN}\) expression (Fig. 4h, top) or treatment with the PKA inhibitor, KT5720, while Gnas\(^{WT}\) PDA organoids were largely unaffected (Supplementary Fig. 7a,b). Thus, the biological effects and activation state of the cAMP-PKA-SIK pathway distinguishes these subsets of Kras-mutant pancreatic tumours.

Notably, Gnas\(^{R201C}\)-expressing cells had a greater FAO rate relative to Gnas\(^{WT}\) cells (Fig. 7i and Supplementary Fig. 7c). Furthermore, Gnas-mutant organoids were specifically responsive to inhibition of lipid degradation, using the lipase/carboxylesterase inhibitor WWL113\(^{46}\) (Supplementary Fig. 7d), and showed preferential sensitivity to FAO inhibition by Cpt1a knockdown or treatment with the FAO inhibitor 4-bromocrotonic acid (BrCA) (Fig. 7j and Supplementary Fig. 7e,f). Cpt1a knockdown also impaired tumourigenicity in vivo (Fig. 7k). By contrast, Gnas-mutant and Gnas\(^{WT}\) organoids responded similarly to glycolysis inhibition using 2-deoxyglucose (2DG) or oxamate, indicating that Gnas-mutant pancreatic cancer cells do not exhibit general increased vulnerability to metabolic stress and reinforcing the specificity of the observed responses to inhibition of lipid metabolism (Supplementary Fig. 7g,h).

**Discussion**

The oncogenic functions of GNAS remain under-investigated despite increasing evidence for its mutational activation in human tumours. Moreover, there is a critical need to understand the diversity of pancreatic cancer subtypes, including IPMN-associated tumours, which comprise ~10% of PDA\(^{54,55}\). By establishing an inducible GEM model that recapitulates the human IPMN-PDA sequence, we demonstrate that Gnas\(^{R201C}\) cooperates with Kras\(^{G12D}\) and Tp53 loss to drive tumour initiation and progression and has a persistent role in maintenance of advanced malignancy. This function is mediated by PKA-dependent inactivation of Sik1-3, establishing SikS as critical tumour suppressors and suggesting that the therapeutic potential of inhibition of GNAS-PKA signalling in GNAS-mutant pancreatic tumours. Comparison of Kras\(^{G12D}\)-driven pancreatic tumour models defined opposing roles for the PKA-SIK pathway and distinct metabolic requirements depending on Gnas mutational status. Thus, Kras-mutant pancreatic tumour cells have heterogeneous circuits for growth and nutrient utilization governed by their combinations of cooperating gene mutations.

Our studies reveal negative regulation of SikS as a primary mechanism by which Gnas\(^{R201C}\)-PKA drives pancreatic tumorigenesis. Whereas inactivating mutations of SikS are infrequent in human cancers, perhaps reflecting redundancy, the mutant-GNAS-PKA pathway inhibits Sik1–3, thereby unmasking their tumour suppressor activity. Genetic alterations in pathways potentially converging on SikK signalling are found across several sporadic tumour types and include other instances of GNAS mutations, mutation of PKA subunits, and loss-of-function of Lkb1/Stk11, an activator of SikS\(^{34–36}\). Moreover, genetic syndromes involving GNAS (McCune-Albright syndrome), PKA (Carney complex) and Lkb1 (Peutz-Jeghers syndrome) have overlapping features including abnormal pigmentation and predisposition to a spectrum of tumours, including IPMN\(^{37,38}\). Thus, our findings suggest the possibility that Sik inactivation may comprise a common node contributing to these different diseases.

The GEM models provide insights into the natural history of IPMNs and their relationship to PanIN and PDA. Gnas\(^{R201C}\) induction promotes IPMN with long latency and shows dramatic cooperation with Kras\(^{G12D}\), shifting Kras\(^{G12D}\)-driven PanIN to more proliferative and dysplastic IPMN lesions (see also previous work\(^{40}\) reporting a non-inducible transgenic model of GNAS-/KRAS-mutant IPMN). Additionally, while previous studies have suggested that the distinct features of IPMN versus PanIN may reflect differing cells of origin (proposed to arise from ducts and acinar cells, respectively)\(^{44}\), our observations using the Ptf1a-Cre\(^{ER}\) system show that acinar cells readily give rise to IPMNs (Fig. 2b), indicating that underlying mutations (rather than cell of origin) direct this neoplastic phenotype. Moreover, in humans, IPMNs often remain benign, and GNAS mutations are associated with relatively indolent tumours across several tissues; therefore, there is uncertainty regarding the relationship between IPMNs and PDA and the potential of mutant-GNAS to drive invasive tumour growth\(^{45}\). Consistent with having constrained malignant potential, GNAS/KRAS-mutant murine IPMNs did not typically progress to PDA. However, Tp53 deficiency in the KGPC\(^{28}\) model and acute Tp53 inactivation in KGK organoids resulted in invasive PDA, revealing Tp53 as a central barrier for IPMN advancement and reinforcing the paradigm of direct IPMN-to-PDA progression (Fig. 2c).

Established murine KGK and KGPC tumours remained dependent on mutant-Gnas/PKA signalling for sustained growth. Our data in 950-5-BLK cultures and xenografts indicate that at least a subset of GNAS-mutant human PDA is likely to remain dependent on this pathway. The KGK tumours exhibit rapid and progressive responses to Gnas\(^{R201C}\) inactivation (Fig. 3e–g). Within days of Dox withdrawal, cell proliferation, mucin expression and dysplastic histologic features were reduced, followed by a gradual loss of papillary architecture (Fig. 3d). Unlike Kras\(^{G12D}\) extinction in the KPC model of PDA\(^{49,50}\), there was no evidence of apoptosis (Supplementary Fig. 2). These observations highlight distinct modes of oncogene addiction whereby different activated oncogenes support the neoplastic state by variously controlling proliferation, survival, differentiation and/or tumour–stroma interactions.

Our data also link oncogenic Gnas signalling to regulation of lipid metabolism. Mutant-Gnas promoted both maintenance of triglyceride levels and supported lipid breakdown via FAO. Coordinated triglyceride synthesis and degradation have been observed in other tumour types\(^{45,56–58}\), including subsets of lung cancers where mutant-Kras induces lipid uptake, triglyceride production, and FAO\(^{59}\). We also present evidence that mutant-Gnas contributes to steady-state levels of acetyl-CoA, the substrate for fatty acid synthesis, TCA cycle activity and protein acetylation. Whereas acetyl-CoA is primarily derived from glucose or acetate in many cancers\(^{60}\), our data suggest that FAO may be an important contributor to acetyl-CoA pools in Gnas-mutant pancreatic tumours cells. Overall, our study highlights the broad rewiring of lipid metabolism by Gnas\(^{R201C}\), although fully deciphering the metabolic processes controlled by mutant Gnas will require metabolic labelling analysis and assessment of how intracellular and extracellular nutrient sources are mobilized.

Finally, our study illustrates the dichotomous oncogenic versus tumour suppressive functions of cAMP/PKA signalling. We show that in the context of common Kras\(^{G12D}\) ± p53 mutations the cAMP-PKA pathway has opposite effects on pancreatic tumour cell proliferation depending on Gnas mutational status. Thus, Kras-mutant pancreatic tumours have heterogeneous circuits for growth and governed by their combinations of cooperating gene mutations.
Since >30% of FDA-approved drugs target GPCRs and largely function as cAMP–PKA modulators, understanding the effects of cAMP on tumour growth may predict new treatment avenues repurposing these compounds.

Methods
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41556-018-0122-3.

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Author contributions

K.C.P., Y.M. and N.B conceived and designed the study. Y.M. generated the TetO-GnasCHF mouse strain. K.P. performed most of the animal and cell-based experiments, with assistance of Y.K., Y.M., S.W., and I.R. Y.K. and M.M-K performed histological analysis. M.B. and W.H. performed proteomics analysis and interpreted the data. F.J. and R.I.S. performed bioinformatics analysis. E.A.G. did LC-MS for polar metabolites, and D.K.N. analysed and interpreted the data. A.S.L., R.A.S., and K.S. provided essential resources. A.S.L., R.A.S., K.S., D.P.R., M.M-K. and C.F.C provided important intellectual input and data interpretation. K.C.P. and N.B. wrote the manuscript with feedback from all authors. N.B. supervised the studies.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Chemicals, drugs, supplements and other materials. Laminin (23017-95, CyQUANT NF (C53006), bovine pituitary extract (13038-14), HBSS, RPMI, DMEM, DMEM:F12, penicillin-streptomycin, phytoxic solution, fetal bovine serum (10093-023, BOH), EGF (35040-018), ROCK inhibitor (R05426), B-27 (no. 42292), B27 supplement (50c), confirm that the coating (17504444) were from Life Technologies; recombinant human FGF-basic (100-188) from Peprotech; doxycycline hyclate (D9891), nicotinamide (N3376), dexamethasone (D4902), 2-deoxyglucose (D6134), sodium ozide (O2751), collagenase (C7657), glycosaminoglycan (T5648), sodium oleate (O7501), bovine pituitary (CML-00030), Alcian Blue (9607), Roche (C8576). EDITA-pro tease Inhibitor Cocktail (1183617001), ROCK inhibitor: Y-27632 (B2298) were from TCI America; IqQ universal Sybr Green Supermix (BIO-RAD), protein block (X0909; DAKO); MACH HRP polymer (HRP250H) and betaZoid DAB chromogen kit (BDBS201H) from Biocare Medical; M1-9-011 and KIN112 were kindly provided by N. Gray.

Lentiviral and retroviral vectors. Lentiviruses and retroviruses were produced in 293T cells by standard protocols with the packaging plasmids (pCMV-dR8.91, pCMV-dR8, pMD2.G, pR8) (Addgene no. 12259). All adenoviral plasmids (Addgene no. 25897), information on the shRNA and gRNA targeting sequences are shown in Supplementary Table 1.

Animal studies. Mice were housed in pathogen-free animal facilities. All experiments were conducted under protocol 2005N000148 approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital and comply with all regulations for the ethical conduct of research. Mice were maintained on a mixed 129SV/129Bl6 background. Data presented include both male and female mice. All mice included in the survival analysis were euthanized when criteria for disease burden were reached (including abdominal distension that impeded movement, loss of >15% of body weight, laboured breathing and/or abnormal postural). Mouse strains were obtained from Jackson Laboratories (Rosa26-LSL-rtTA, Jax stock no. 005572) and kindly provided by colleagues (Ptf1a-Cre; Sik3-KrasG12d; Sik3-TP53; Sik3-MURR1). Mariner Tp53 was knocked down using the mir-30b targeted LMS sh-PS3.1224 retrovector14. All other knockdowns were performed using pLK0-based lentiviral shRNA vectors from The RNAi Consortium (TRC) library (Broad Institute), and were purchased from Molecular Profiling Laboratory, MGH. The CRI/SPR guides against Sk1, Sk2 and Sk3 were cloned into pLentiCRISPRV2 (Addgene no. 52961). Information on the shRNA and gRNA targeting sequences are shown in Supplementary Table 1.

Histology and immunohistochemical analysis. Tissue samples were fixed overnight in 4% buffered formaldehyde, embedded in paraffin, and then sectioned and stained with haematoxylin and eosin by the MGH Pathology Core. Histological examination was performed by a gastrointestinal pathologist (M.M.-K). Immunohistochemistry (IHC) was performed on paraffin-embedded sections (5 mm thickness) after microwave antigen retrieval for 10min with 3% H2O2 at room temperature to block endogenous peroxidase activity. Specimens were brought to the boil in 10mM sodium citrate buffer (pH 6.0, 5min, pressure cooker) for antigen retrieval. Slides were blocked for 1hour in TBS-0.05% Tween 20 (Fishier Scientific), 1 drop per 1ml of Protein Block (Dako X0909) and incubated with primary antibody for 1hour at room temperature. Primary antibodies were diluted with PBS-Protein Block (1drop/ml) as follows; anti-MUC1 1:100, anti-MUC2 1:50, anti-MUC5AC 1:100, anti-CX3CR1 1:200. Specimens were reacted for 30min with an Odyssey Dual-band multimode detection system. For quantification of IPMN epithelial cells and phospho-PAK1 substrate by IHC, % of positively stained IPMN epithelial cell nuclei was counted automatically using Image software (NIH; http://rbi.info.nih.gov/ij). Quantification of mucin-producing IPMN epithelial cells was performed by manually counting % IPMN epithelial cells with Alcian Blue-positive cytoplasm. For quantification of papillary lesions, the % of the total neoplastic ductal area exhibiting papillary structures was measured by manually counting % IPMN producing IPMN epithelial cells. For quantification of papillary lesions, the % of the total neoplastic ductal area exhibiting papillary structures was performed by manually counting % IPMN producing IPMN epithelial cells.

Isolation and culture of primary tumour cells. Murine pancreatic tumour cells were derived from the following GEM models: KGC (KrasG12d; Rosa26-LSL-rtTA, Tet-O-GnasR201C, Ptf1a-Cre); KCPC (Ptf1a-Cre; KrasG12d; Rosa26-LSL-rtTA, Tet-O-GnasR201C, p53Lox/Lox); KPC (Pdx1-Cre; KrasG12d; Rosa26-LSL-rtTA, LSL-KrasG12d, LSL-rtTALox/Lox); KPC (Pdx1-Cre; KrasG12d; mKl); PCK (Pdx1-Cre; KrasG12d; Ink4a/ArfLox/Lox; KPC (Pdx1-Cre; KrasG12d; p53Lox/Lox; p16Lox/Lox). Tumour tissues were collected in ice cold 20ml DME with 1% Antibiotic-Antimycotic. A transection of the tumour tissue was taken for histological verification and the rest was washed twice with 10ml HBSS and then minced aseptically with a razor blade on a 10cm dish. The minced tissue was digested in a 1:50 ratio for 1 hour in differm in 261Liebreth/D1 in 10ml DME containing 1% Antibiotic-Antimycotic at 37 °C. 10ml tissue homogenates were transferred to a 50ml Falcon tube and centrifuged at 1,000rpm, 5min, 4°C. The pellet was then washed with HBSS 2–3 times by centrifugation. A 100μm strainer was used to remove undigested large tissue debris. The flow-through was centrifuged at (RCF 335) x g for 5 minutes, and the pellet was resuspended with full duct media (DMEM:F12, 5mg/ml Glucose, 2.12mg/ml nicotinamide, 5mM 3,3,5-tri-iodo-L-thyronine, 1μM dexamethasone, 5nM T3+T4, 1×Pen/Strep, 20ng/ml mouse EGF, 5% NaSerum IV and 25μg/ml bovine pituitary extract) with Rho-associated protein kinase (ROCK) inhibitor (10μg/ml) and plated on irradiated feeder cells. Media was supplemented with 200ng/ml doxycycline. The frozen cells were thawed on feeder layer with ROCK inhibitor and grown on organoids in matrigel or collagen matrix as described unless otherwise stated. For viral infections primary cells were plated in feeder or laminin-coated multi-well plates and infected with spinoculation. KGC/CGG cells were initially generated by infecting KCG cells with the LMS sh-PS3 retrovirus. PDA cell lines derived from
Human PDA cell lines. 1473-2-AMK, 1108-2C926, 1319-3-NE, 1925 2-BHG and 950-5-BLK cells were isolated from patient derived xenografts (PDXs) from MGH pancreatic tumour bank. All study participants provided IRB-approved informed consent for their medical records and tissue samples to be used in this study. Patient clinical data was entered into a de-identified clinical database allowing for the anonymous analysis of demographic, clinical and pathological variables. PDx were processed as described above and 2D cultures were established in 10% FBS, 1% DMEM, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. RNA isolation from all cell lines was performed as described68. The data were normalized to the total number of cells in each replicate sample, collected from two independent KGC lines (A and B) grown in LC/MS-grade water and 400 μl of HPLC-grade chloroform (without amylenes) were added to the tube. Then 300 μl of LC/MS-grade methanol at 80°C were added to the tube. The organic extract was vortexed until analysis. LC-MS/MS-based lipidomic experiments and data analyses were performed as described69. The data were normalized to the total number of cells in the organoids from replicate wells.

Polar metabolite profiling. For polar metabolite profiling7, 2 million cells cultivated on laminin-coated plates with growth-factor reduced duct media were harvested as 5 replicates and flash frozen. Cell pellets were extracted in 40:40:20 acetonitrile/methanol/water including 10 mM D3-15N-serine as internal standard. Insoluble debris was separated by centrifugation at 15,871 × g (RCF 15,871) for 15 min at 4°C. After phase separation the bottom layer was collected carefully. The extract was dried in a speed vac and stored at −80°C until analysis. LC-MS/MS-based lipidomic experiments and data analyses were performed as described69. The data were normalized to the total number of cells in the organoids from replicate wells.

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Neutral lipid imaging. KGC cells were grown in growth factor-reduced duct media and incubated overnight in growth factor-reduced duct media (duct media lacking EGF and containing 2.5 ml−1 ITS) containing 60 μM oleic acid-BSA and 2 mM carnitine. The next day, the cells were washed and incubated for 4 hours in fresh media supplemented with [1-14C]oleic acid (0.5 μCi) for 4 hours. The media was transferred to a microfuge tube containing Whatman filter paper saturated with phenylethylamine. The reaction was then terminated by adding with 3 M perchloric acid and the tube was closed. The 14CO2 resulting from oxidized fatty acids was captured on the saturated filter paper for 1 hr at room temperature. The radioactivity was measured by scintillation counting and was normalized to total protein content.

Cyclic AMP (cAMP) was measured using the Cyclic AMP Assay Kit (4339; Cell Signaling Technology) in KGC cells were grown on laminin-coated plates as above. Acetyl-CoA in Extended Data Fig. 8k was measured using the PicoProbe Acetyl-CoA Fluorometric Assay Kit (K317-100; Biovision). All assays were done according to the manufacturers’ protocols.

Statistics and reproducibility. In all cases results are expressed as mean ± standard deviation (s.d.) or mean ± standard error of mean (s.e.m). Significance was analysed using two-tailed Student’s t-test where a p value of less than 0.05 was considered statistically significant. Kaplan-Meier curves were analysed by log-rank test. All statistical calculations were performed by Prism® software (GraphPad Software, USA). No samples or animals were excluded from analysis and sample sizes were not predetermined. Animals were randomized in experiments in KGC mice comparing sustained Dox exposure and Dox withdrawal. Also, additional control studies comparing KGC mice provided Dox at 4 weeks versus those that never received Dox were also randomized in a similar manner. Further randomization for both studies involved allocation of females and males into each study condition. All the ImmunobLOTS and IHC were performed two times and generated similar results. For all other experiments, the number of biological replicates is indicated in the figure captions.

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

Data availability. The proteomics data have been deposited in MassIVE proteomics data depository (https://massive.ucsd.edu/) with accession number MSV000082305. The RNA-seq data have been deposited in the NCBI GEO database with accession number GSE114348. The lipidomic and polar metabolite data can be accessed under https://doi.org/10.6084/m9.figshare.6182501 and https://doi.org/10.6084/m9.figshare.6182513 from Figshare repository (https://figshare.com). Source data for Figs. 1 and 3–7 and Supplementary Figs. 3–7 are provided in Supplementary Table 2. Unprocessed immunoblot scans are presented in Supplementary Fig. 8. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Policy information about availability of computer code

- **Data collection**: No computer code was used
- **Data analysis**: ImageJ software (NIH; http://rsb.info.nih.gov/ij). Prism6 software (GraphPad Software, Inc, USA).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The proteomics data have been deposited in MassIVE proteomics data depository (https://massive.ucsd.edu/) with accession number MSV000082305. The RNA-seq data have been deposited in the NCBI GEO database with accession number GSE114348. The lipidomic and polar metabolite data can be accessed under 10.6084/
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- **Life sciences**
- 🌐 Behavioural & social sciences
- 🌐 Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

| Sample size | No statistical method was used to predetermine sample size. |
|-------------|---------------------------------------------------------------|
| Data exclusions | No samples or animals were excluded from analysis. |
| Replication | All attempts at replication generated reliable and similar results. The reproducibility of all the experiments is described in the figure legends. In all cases results are expressed as mean ± standard deviation (s.d.) or mean ± standard error of mean (s.e.m). Significance was analyzed using 2-tailed Student’s t test where a p value of less than 0.05 was considered statistically significant. No samples or animals were excluded from analysis and sample sizes were not predetermined. Kaplan-Meier curves were analyzed by log-rank test. All statistical calculations were performed by Prism6 software (GraphPad Software, Inc, USA). |

Randomization

Mice were randomized in experiments in KGC mice comparing sustained Dox exposure and Dox withdrawal (randomization was accomplished by preselecting young mice at the time of initial Dox administration for enrollment in either group. Also, additional control studies comparing KGC mice provided Dox at 4 weeks versus those that never received Dox were also randomized in a similar manner. Further randomization for both studies involved allocation of females and males into each study condition.

Blinding

Blinding was performed for all histological analyses, including morphological assessments and for quantification of histological and immunostains. For quantification of PCNA and phospho-PKA substrate, % of positive IPMN epithelial cell nuclei were determined by IHC and counted automatically using ImageJ software (NIH; http://rsb.info.nih.gov/ij).

Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
|     | - Unique biological materials |
|     | - Antibodies |
|     | - Eukaryotic cell lines |
|     | - Palaeontology |
|     | - Animals and other organisms |
|     | - Human research participants |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     | - ChIP-seq |
|     | - Flow cytometry |
|     | - MRI-based neuroimaging |

**Unique biological materials**

Policy information about availability of materials

 Obtaining unique materials

Will be available upon Material transfer agreement (MTA) and availability of the materials during request.

**Antibodies**

| Antibodies used |
|-----------------|
| Commercial antibodies were purchased from the following vendors. Anti-VASP (#3132), anti-phospho-VASP (Ser157) (3111), anti-phospho-PKA Substrate (RRXS*/T*) (#9624) for immunoblot, anti-phospho-(Ser/Thr) PKA substrate (#9621) for IHC, anti-SIK2 (#6919), anti-phospho-HDAC4(Ser246)/HDAC5(Ser259)/HDAC7(Ser155) (#3443), anti-phospho-CREB(S133) (#9198), anti-CREB (#9197), anti-phospho-YAP (Ser127) (#13008), anti-YAP (14074), anti-phospho-β-Catenin (Ser33/37/Thr41) (#9561), anti-β-Catenin (#8480), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (#9101), anti-p44/p42 MAPK (Erk1/2) (#9102), anti-phospho-HSL (Ser660) (#4126), anti-HSL (#4107), anti-CDX2 (#12306), anti-PCNA (#13110) and anti-cleaved Caspase-3 (#9661), |
Validation of the commercial antibodies is available in the product page and search of relevant literature. Antibodies generated by Dr. Kei Sakamoto and Dr. Robert Screaton are described in reference 1, 2.

References:
1. Sakamaki, J. et al. Role of the SIK2-p35-PIA2 complex in pancreatic beta-cell functional compensation. Nat Cell Biol 16, 234–244, doi:10.1038/ncb2919 (2014).
2. Patel, K. et al. The LKB1-salt-inducible kinase pathway functions as a key gluconeogenic suppressor in the liver. Nat Commun 5, 4535, doi:10.1038/ncomms5535 (2014).

Eukaryotic cells

Policy information about cell lines

Cell line source(s)

Murine pancreatic cancer cells were derived from the following GEM models (KGC: [KRASG12D/+; Rosa26-LSL-rtTAlox/+; TetO-GNASR201C, Ptf1a-Cre]; KGPC: [Ptf1a-CreER KRASG12D/+; Rosa26-LSL-rtTAlox/+; TetO-GNASR201C; pS3Lox/+]; KPC: [Pdx1-Cre; KRASG12D/+; pS3Lox/+]; KIC: [Pdx1-Cre; KRASG12D/+; CDKN2Alox/+]; KPIC: [Pdx1-Cre; KRASG12D/+; pS3Lox/+/p16+/-].

Primary human pancreatic cancer cell lines 1473-2-AMK, 1108-2C926, 1319-3-NE, 1925 2-BHG and 950-5-BLK cells were isolated from patient derived xenografts (PDXs) from the MGH pancreatic tumor bank. The human pancreatic cancer cell lines, PL45, YAPC, PANC-1 and PaTu-8889T, and the 293T cell line are from the ATCC repository.

Authentication

Established Human Lines: ATCC
Primary Human Lines: Sanger Sequencing of relevant loci

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination

Commonly misidentified lines

(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mice were housed in pathogen-free animal facilities. All experiments were conducted under protocol 2005N000148 approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital and comply with all regulations for the ethical conduct of research. Mice were maintained on a mixed 129Sv/CS7Bl/6 background. Data presented include both male and female mice. All mice included in the survival analysis were euthanized when criteria for disease burden were reached (including abdominal distension that impeded movement, loss of >15% of body weight, labored breathing, or abnormal posture).

Mouse strains were obtained from Jackson Laboratories; Rosa26-LSL-rtTA (#005572) and kindly provided by colleagues include Ptf1a-Cre and Ptf1a-CreER (#019378) mice from C. Wright, LSL-KrasG12D (#008179) mice from D. Tuveson and T. Jacks, P53Lox/Lox (#008462) mice from A. Berns. TetO-GNASR201C mouse strain generation is described in the manuscript. Chimeras were crossed to the Ptf1a-Cre strain, and offspring were successively crossed with Rosa26-LSL-rtTA, and LSL-KRASG12D mice.

Compound mutant animals were provided with doxycycline (200 μg/ml) in the drinking water beginning at 4 weeks of age and replaced weekly. A group of control KC and KGC mice that was not provided Dox was euthanized at 12 weeks. Rosa26-LSL-rtTA allele was present in each cohort. Histological examination of the pancreas of these mice revealed a comparable phenotype of focal low grade PanIN (similar to KC mice receiving Dox supplementation); (e.g. see Fig. 1d, e), confirming that the cooperative phenotypes observed in the KGC mice were due to inducible expression of GNASR201C.

Ptf1a-CreER, LSL-KRASG12D, P53Lox/Lox, and Rosa26-LSL-rtTA and TetO-GNASR201C strains were intercrossed to obtain KGPER, KGCE, KPCEr animals and controls. To activate Cre, 3-week old animals were injected intraperitoneally every other day with 100 mg/kg (body weight) tamoxifen dissolved in corn oil (total 4 doses). Doxycycline (200 μg/ml) was provided in the drinking water beginning at 4 weeks of age. To study the role of GNASR201C in tumor maintenance, KC mice were provided Dox supplemented water continually from age 4 weeks (Dox-On) or switched to Dox-free water after tumor detection by palpation at age 8-10 weeks (Dox-Off). The samples were collected at the indicated time points for histological analysis. For abdominal cyst fluid measurement, mice were euthanized at the indicated time points and abdominal cyst fluid was isolated and measured using a 30 ml syringe and 25 g needle.

For subcutaneous tumor studies, 2x105 KCG cells or KGPER cells (or modified versions of the same) were injected subcutaneously into the lower flank of NOD.CB17-Prkdcscid/j mice (6-10 weeks of age) from Jackson Laboratories, strain #001303. Tumor size was assessed at indicated time points by caliper measurements of length and width and the volume was calculated according to the formula [(length x width2)/2]. For orthotopic transplantation studies 2x105 tumor cells were prepared in 50% matrigel and injected in a total volume of 30 μl into the tail of the mouse pancreas as described. At the indicated time points the pancreas was harvested and processed for histology and IHC as described in the relevant sections. In experiments involving Dox treatment, Dox supplemented drinking water (200 μg/ml) was first provided one day prior to injection of the tumor cells. For human pancreatic cancer xenograft studies, 7.5-10x105 cells (in 50% matrigel) were subcutaneously injected into the lower flanks of the NOD.CB17-Prkdcscid/j mice (6-10 weeks of age). Tumor growth was followed by caliper
| Category                          | Description                                      |
|----------------------------------|--------------------------------------------------|
| Wild animals                     | This study did not involve wild animals.          |
| Field-collected samples          | The study did not involve samples collected from the field. |