Syndecan 1 is a critical mediator of macropinocytosis in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) remains recalcitrant to all forms of cancer treatment and carries a five-year survival rate of only 8%1. Inhibition of oncogenic KRAS (hereafter KRAS*)—the earliest lesion in disease development that is present in more than 90% of PDACs, and its signalling surrogates has yielded encouraging preclinical results with experimental agents2–4. However, KRAS*-independent disease recurrence following genetic extinction of Kras* in mouse models anticipates the need for co-extinction strategies5,6. Multiple oncogenic processes are initiated at the cell surface, where KRAS* physically and functionally interacts to direct signalling that is essential for malignant transformation and tumour maintenance. Insights into the complexity of the functional cell-surface-protein repertoire (surfome) have been technologically limited until recently and—in the case of PDAC—the genetic control of the function and composition of the PDAC surfome in the context of KRAS* signalling remains largely unknown. Here we develop an unbiased, functional target-discovery platform to query KRAS*-dependent changes of the PDAC surfome, which reveals syndecan 1 (SDC1, also known as CD138) as a protein that is upregulated at the cell surface by KRAS*. Localization of SDC1 at the cell surface—where it regulates macropinocytosis, an essential metabolic pathway that fuels PDAC cell growth—is essential for disease maintenance and progression. Thus, our study forges a mechanistic link between KRAS* signalling and a targetable molecule driving nutrient salvage pathways in PDAC and validates oncogene-driven surfome annotation as a strategy to identify cancer-specific vulnerabilities.

To annotate changes in cell-surface proteins driven by KRAS* signalling, we used a doxycycline-inducible Kras* mouse model of PDAC (hereafter, iKras*) to acutely induce and thereafter extinguish expression of the activated oncogene, KrasG12D (ref. 7). Stable isotope labelling by with amino acids in cell culture (SILAC)-based mass spectrometry was used to identify changes in the surfome that are induced by KRAS* extinction in three independent cell culture isolates from iKras* tumours. In brief, cells were labelled with either heavy isotope 13C6-Arg and 13C6-Lys or light 12C6-Arg and 12C6-Lys, and surface protein changes were quantified using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) 24 h after doxycycline withdrawal (Fig. 1a), a time point at which there is significant loss of Ras activity (Extended Data Fig. 1a) without obvious changes in cell morphology or proliferation rate. Comparison of KRAS*-ON with KRAS*-OFF paired samples (Extended Data Fig. 1b–e) identified 221 differentially expressed plasma membrane proteins8,9 (according to http://compartments.jensenlab.org)—196 of which were upregulated and 25 of which were downregulated (Supplementary Tables 1, 2)—suggesting that KRAS* primarily drives surface protein enrichment rather than depletion. The top-20 species most significantly upregulated by KRAS* (Extended Data Fig. 1f, g) represent a broad spectrum of known effectors, several of which are known to be upregulated and have a functional role in PDAC pathogenesis. Our proteomic analysis also identified many cell-surface-associated proteins regulated by KRAS*. Ingenuity pathway analysis (IPA) revealed that many KRAS*-upregulated cell-surface proteins are involved in biological processes that are activated in PDAC, including the axonal guidance signalling pathway10 (Fig. 1b and Supplementary Table 3), which supports the notion that KRAS* is a major driver of molecular reprogramming in PDAC.

To assess the functional relevance of our findings to human disease, we compared the mouse surfome data with human surfome data from 11 PDAC cell lines11. We chose 110 proteins that are potentially regulated by KRAS* that are also enriched in human PDAC cells (Supplementary Tables 1, 4), and 37 surfome genes that are highly expressed in human PDAC cells (Supplementary Table 4) to generate a pooled, lentiviral short-hairpin RNA (shRNA) library. We conducted an in vivo loss-of-function screen using a previously described barcoding methodology12 in orthotopically implanted tumours derived from three iKras* mouse cell lines (Fig. 1a). Next-generation sequencing analysis revealed full representation of library complexity, high correlation among tumour replicates and expected behaviour of positive (PSMA1 and RPL30) and negative (Renilla luciferase (Luc)) controls (Extended Data Fig. 2a–c). The screens uncovered 79 genes that were significantly depleted in at least 1 of the 3 models; 36 of these genes were common to at least two models (Extended Data Fig. 2d and Supplementary Table 4). Among 11 hits that were depleted in all three models, SDC1—a member of the heparin sulfate proteoglycan family—was also among the most significantly enriched in the plasma membrane during KRAS* expression (Fig. 1c and Supplementary Table 4). Further, among the top-ten most-enriched surfome proteins modulated by KRAS*, three belonged to the heparin sulfate proteoglycan family (SDC1, SDC4 and GPC1) (Extended Data Fig. 1g), which prompted us to select SDC1 as a top-priority candidate as a mediator of KRAS*-driven cellular reprogramming in PDAC.

Representative mass spectra indicated that membrane expression of SDC1 was upregulated in KRAS*-ON relative to KRAS*-OFF conditions (Fig. 1d). This was confirmed by immunofluorescence and flow
cytometry analysis, in which KRAS* extinction led to a rapid decrease of SDC1 membrane expression that was reversed upon re-expression of KRAS* (Fig. 1e and extended Data Fig. 3a). Membrane localization of other surface proteins, such as α-catenin (CTNNNA1) and calcium pump pan PMCA ATPase, was not altered following KRAS* inactivation, which suggests that it had a specific effect on SDC1 (Extended Data Fig. 3b). KRAS* inactivation did not affect Sdc1 mRNA abundance or total protein expression (Extended Data Fig. 3c–e). In vivo, KRAS* expression in the iKrasG12Dp53L/− PDAC model (p53 is also known as Trp53) induced membrane enrichment of SDC1 in premalignant lesions and advanced tumours, whereas its extinction in established tumours resulted in depletion of SDC1 surface expression (Fig. 1f). Comparable induction of membrane-localized SDC1 was observed in the LSL–KrasG12D model (also known as the KC model) (Extended Data Fig. 3f).

In a primary human PDAC tissue array, we detected SDC1 in premalignant lesions (early pancreatic intraepithelial neoplasia) and in tumour-adjacent lesions reminiscent of chronic pancreatitis, as well as in advanced premalignant lesions and invasive carcinomas (Extended Data Fig. 3g, h). Several published human microarray datasets have reported significantly increased SDC1 expression in PDAC tissue compared with normal pancreas (Extended Data Fig. 3i), which implicates SDC1 in PDAC pathogenesis. Enrichment of surface SDC1 in very early disease could result from oncogenic signalling or inflammatory responses associated with pancreatitis. To differentiate, we induced chronic pancreatitis in iKras* mice with caerulein, followed by doxycycline treatment to induce KRAS* expression. Whereas metastatic lesions were similarly positive for the ductal marker CK19 before and after doxycycline treatment, SDC1 was induced mostly on oncogene induction, but not by caerulein (Extended Data Fig. 3j)—which establishes a definitive correlation between KRAS* and SDC1 expression in PDAC development.

To determine whether SDC1 is required for disease progression, we depleted SDC1 in independent iKras* cultures. SDC1 depletion with shRNAs markedly impaired colony-forming ability (Fig. 2a and Extended Data Fig. 4a–c), which was rescued with the expression of shRNA-resistant Sdc1 (Extended Data Fig. 4d). SDC1 depletion also significantly inhibited tumour growth of subcutaneous xenografts (Fig. 2b and Extended Data Fig. 4e). Additionally, CRISPR-mediated Sdc1 deletion in iKras* tumour cells suppressed colony formation and tumorigenicity in vivo (Fig. 2c, d and Extended Data Fig. 4f–h). Consistent with mouse PDAC models, shRNA-mediated depletion of SDC1 in two established human PDAC cell lines, ASPC1 and HPF11, as well as in the patient-derived xenograft (PDX) model PACTC69 significantly impaired colony formation and suppressed tumour growth in vivo (Fig. 2e, f and Extended Data Fig. 4i–k).

We investigated the role of SDC1 in KRAS*-driven PDAC development by generating p48-cre–LSL–KrasG12Dp53L/− (also known as KPC) mice homozygous or heterozygous for the Sdc1-null allele. As previously reported13, Sdc1−/− mice show normal postnatal development. In the PDAC model, Sdc1 deficiency prolonged survival relative to wild-type mice (Fig. 2g: 25 versus 16.75 weeks, respectively; P < 0.0001) and—notably—median survival was also comparably extended in Sdc1−/− mice (24.3 weeks), which suggests a haplosufficient phenotype. Flow cytometry analysis confirmed that SDC1 is expressed in Sdc1−/− tumours, albeit at significantly reduced levels relative to Sdc1+/+ tumours (Extended Data Fig. 5a). Morphologically, Sdc1−/− and Sdc1+/− tumours exhibited more well-differentiated ductal features accompanied by cystic lesions compared with Sdc1+/+ tumours. Both Sdc1−/− and Sdc1+/− tumours showed decreased tumour cell proliferation (Fig. 2h and data not shown) and less aggressive phenotypes, with distal metastases to lung or liver observed in 5% and 4.2% of mice, respectively, compared with 31% in Sdc1+/+ mice (Fig. 2i). Of note, Sdc1−/− tumours exhibited significantly decreased infiltration of myeloid-derived suppressor cells versus Sdc1+/+ lesions (Extended Data Fig. 5b, c).

We investigated the requirement of major KRAS* surrogate for the surface expression of SDC1. Treatment of iKras* cells with either of two distinct MEK inhibitors (AZD8330 or trametinib), but not with a pan-P13K inhibitor (BKM120), decreased membrane SDC1 expression in a dose-dependent manner, similar to the effect of KRAS* extinction (Fig. 3a and Extended Data Fig. 6a–f). MEK inhibition also blocked membrane relocalization of SDC1 on KrasG12D reactivation (Fig. 3a and

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**Fig. 1 | Functional surfaceome analysis identifies SDC1 as a KRAS*-dependent surface protein that is important for tumour maintenance.** a, Experimental design for the functional surfaceome analysis. Differentially expressed surface proteins upon KrasG12D inactivation were identified using SILAC-based proteomic analysis. In vivo loss-of-function screening was subsequently conducted with a custom barcoded lentiviral shRNA library targeting the KrasG12D-dependent surfaceome. Depletion was observed relative to the reference population (Ref.). b, Top—ten canonical signalling pathways identified with IPA analysis of differentially expressed surface proteins upon KrasG12D activation; n = 3 biologically independent samples, enrichment score (Enrich.) and P-value (two-sided Fisher test; Fisher) are reported. c, Rank of common top-scoring hits from three independent iKras* p53+/− tumour cell lines. Cut-off based on redundant shRNA activity (RSA) algorithms and log10 is shown in the heat map alongside corresponding quantification rank from SILAC-based proteomic analysis. d, Mass spectrum of a representative SDC1 peptide shows membrane SDC1 in the presence (heavy) or absence (light) of Kras(G12D) in iKras* p53+/− tumour cells. e, SDC1 levels in iKras* p53+/− tumour cells in the presence (ON) or absence (OFF) of doxycycline were measured by fluorescence-activated cell sorting (FACS) (top) using an APC-conjugated SDC1 antibody, with quantification of mean fluorescence intensity (MFI) (bottom). ReON, cells were grown without doxycycline for 48 h, and then with doxycycline for 24 h (n = 3 biological replicates; data are mean ± s.d.; P values were determined by paired two-sided Student’s t-test). f, Three-week-old iKras* p53+/− mice received doxycycline-containing water for one, three or seven weeks to induce premalignant lesions or for nine weeks to induce invasive PDAC (T), then doxycycline was withdrawn for one, two or seven days. SDC1 in pancreatic or tumour tissues was analysed by immunohistochemistry. PT, peritumour region. Arrows indicate areas magnified in the insets. Scale bar, 200 μm. d-f, Representative experiments from three independent experiments.
Extended Data Fig. 6a, b), which indicates that KRAS* drives SDC1 membrane localization through the MAPK pathway.

Membrane SDC1 expression is primarily modulated via shedding14 (proteolytic cleavage of the N-terminal domain into the extracellular space) and endocytosis15 (internalization through the endocytic route, which is balanced by endosomal recycling that returns much of the endocytosed proteins back to the cell surface)16. Using enzyme-linked immunosorbent assay to detect shed SDC1 from iKras* cells upon doxycycline withdrawal, we determined that KRAS* extinction inhibited rather than enhanced SDC1 shedding in a time-dependent manner (Extended Data Fig. 6g). Next, we used monovalent anti-syndecan antibodies, which are internalized in a syndecan-dependent manner and with comparable kinetics to bivalent antibodies16, with flow cytometry to measure changes in SDC1 internalization and recycling rates upon oncogene extinction. Whereas KRAS* extinction did not affect SDC1 internalization (Extended Data Fig. 6h), the rate of SDC1 recycling back to the plasma membrane was significantly inhibited in the absence of KRAS* (Extended Data Fig. 6i, j). To validate this result, we removed the ectodomain of preexisting cell-surface-exposed SDC1 by trypsinization and then measured trafficking of endocytosed SDC1 to the cell surface using an antibody that recognizes the SDC1 ectodomain. Recovery of surface SDC1 expression was significantly delayed upon KRAS* inactivation (Fig. 3b), further supporting that KRAS* promotes SDC1 membrane localization by enhancing SDC1 recycling.

Trafficking of syndecan proteins to the plasma membrane is orchestrated by the small GTPase, ARF6.16 KRAS* inactivation resulted in redistribution of SDC1 from the cell surface to the juxtanuclear ARF6 endocytic recycling compartment (Fig. 3c), a characteristic of reduced ARF6 activity.16 Indeed, as previously reported17, ARF6 activity was suppressed upon KRAS* extinction or treatment with the MEK inhibitor AZD8330 (Extended Data Fig. 7a). Type I phosphatidylinositol 4-phosphate 5-kinase (PIPK) is a downstream effector of ARF615, PIPK activity was also inhibited upon KRAS* inactivation or MEK inhibition (Extended Data Fig. 7b). Moreover, expression of the constitutively active ARF6 (Q67L) mutant in iKras* cells caused membrane ruffling and rescued the decrease in SDC1 membrane localization on KRAS* extinction, whereas expression of the dominant-negative ARF6(T27N) inhibited SDC1 membrane localization in the presence of KRAS* (Extended Data Fig. 7c–e). We therefore conclude that KRAS* signalling stimulates ARF6 activity to promote SDC1 trafficking to the plasma membrane.

ARF6 activity is controlled by specific guanine nucleotide exchange factors and GTPase-activating proteins. We mined the expression profiles of iKras* tumour cells following KRAS* extinction for ARF6-specific guanine nucleotide exchange factors and GTPase-activating proteins, and found that expression of the ARF6-specific guanine nucleotide exchange factor 'pleckstrin and Sec7 domain-containing 4' (PSD4), was significantly decreased upon KRAS* extinction (Extended Data Fig. 7f). This was validated using quantitative PCR
with reverse transcription; both KRAS* extinction and MEK inhibitor treatment markedly inhibited PSD4 expression (Extended Data Fig. 7g). Moreover, PSD4 protein levels decreased in a time-dependent manner in iKras* cells rescued by constitutively active RAC1 (RAC1(Q61L)) in SDC1-depleted iKras* tumour cells, in which SDC1 depletion impaired macropinocytosis (Extended Data Fig. 8i–k), supporting an essential role for RAC1 in maintaining macropinocytosis in PDAC cells.

To identify SDC1 domains required for RAC1 activation and macropinocytosis, we constructed a series of truncation mutants of SDC1 that were expressed on the cell surface, as well as for soluble SDC1 protein, which was detected in the culture medium because it lacks the transmembrane and intracellular domains (Extended Data Fig. 9a, b). As expected, expression of full-length SDC1 fully rescued RAC1 activity in SDC1-depleted cells. By contrast, soluble SDC1 did not reactivate RAC1 (Extended Data Fig. 9c), which indicates that the membrane localization of SDC1 is essential for macropinocytosis. Deletion of the extracellular domain of SDC1 also abolished its activity towards RAC1. However, SDC1(GAG), in which the glycosaminoglycan chain modification sites are mutated, exerted a partial rescue effect (Extended Data Fig. 9c), suggesting that glycosaminoglycan chain may not be essential for this function. Of note, the SDC1(C30) mutant, which lacks its PDZ binding domain, also failed to rescue RAC1 activity in SDC1-depleted cells (Extended Data Fig. 9c). The mutant constructs exhibit similar ability to restore macropinocytosis and rescue the in vivo tumour growth of SDC1-deficient cells (Extended Data Fig. 9d–g). The C terminus of SDC1 binds to the PDZ domain containing protein syntenin, and this complex is involved in assembly of multimers that organize intracellular signal-transduction pathways. Ablation of syntenin markedly inhibited RAC1 activity, suppressed macropinocytosis, and impaired clonogenic activity and tumour growth in KRAS*-driven PDAC cells (Extended Data Fig. 9h–m), suggesting that the SDC1–syntenin complex is required to mediate extracellular–intracellular signalling to promote macropinocytosis and tumour growth in this context.

Finally, we validated the role of SDC1 in human AsPC1 and PDX-derived PATC69 cells, in which SDC1 depletion impaired macropinocytosis; this was rescued by ectopic expression of shRNA-resistant mouse Sdc1 (Extended Data Fig. 10a–c). Evaluation of macropinocytosis across 20 KRAS*-driven human PDAC models demonstrated that EFA6-mediated ARF6 activation can induce formation of plasma membrane protrusions and macropinocytosis, which might regulate macropinocytosis. Consistent with previous findings, iKras* tumour cells exhibited high levels of macropinocytosis, as measured by uptake of tetramethylrhodamine-labelled dextran (TMR–dextran). Macropinocytosis was substantially inhibited upon KRAS* inactivation or treatment with the macropinocytosis inhibitor EIPA (Extended Data Fig. 8a, b), as well as in SDC1-depleted mouse PDAC cells derived from iKras* or KPC models (Fig. 4a, b and Extended Data Fig. 8c, d). As anticipated, overexpression of SDC1 or PSD4 attenuated the decrease in macropinocytosis upon KRAS* extinction (Fig. 4c and Extended Data Fig. 8e–g), consistent with SDC1 membrane localization control by PSD4–ARF6 as an essential mechanism by which KRAS* drives macropinocytosis in PDAC.
SDC1 abundance at the membrane was correlated with macropinocytosis (Extended Data Fig. 10d–f). Thus, our data across a variety of models highlight the requirement for SDC1 to mediate macropinocytosis in KRAS*-driven PDAC (Fig. 4f).

Although activation of macropinocytosis by KRAS* in transformed cells has long been established, the molecular mechanisms that control this process have remained unknown. Here we provide evidence that SDC1 serves as a KRAS* effector that induces macropinocytosis in PDAC. To our knowledge, pharmacological inhibition of macropinocytosis has not been accomplished; the critical role for SDC1 to regulate macropinocytosis in KRAS*-driven PDAC suggests SDC1 as a target for therapeutic intervention. Monoclonal antibodies directed against SDC1 (CD138) are being tested for activity in multiple myeloma as antibody–drug conjugates, proving the feasibility of developing targeted therapeutics against this molecule.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-1062-1.

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Author contributions

W.Y. and G.F.D. designed the studies, interpreted the data and wrote the manuscript. Y.A.W. and R.A.D. provided valuable suggestions and intellectual input, W.Y., A.K., A.T. and S.H. performed the proteomic study and analysis. W.Y. and J.L.R. carried out the loss-of-function screen. W.Y., W.W., H.J., J.L., L.Y., A.K., P.H., Z.C., Z.T., L.N., Q.W., V.R. and G.J.M. conducted other experiments. S.S., J.L., J.Y. and Z.T. were responsible for bioinformatics analysis. Z.X., S.J., P. Deng, P. Den, B.H., P.F.P., A.C., S.X.Z., I.L.H., N.F. and T.P.H. provided technical assistance. H.W. and A.M. provided assistance with pathology. J.B.F. provided patient-derived xenograft cells. Y.A.W., A.V., K.A.K. and R.A.D. provided valuable intellectual input and edited the manuscript.

Competing interests

R.A.D. is a co-founder, advisor and director of Tvardi Therapeutics, G.F.D. reports personal fees from and stock ownership in Karyopharm Therapeutics, Forma Therapeutics, Metabomed, BiovelocITA, Nurix and Orionis Biosciences; and personal fees from Blueprint Medicines, Taiho Pharmaceutical, Symphogen and Helsinn Ventures.

Additional information

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filtration through a 0.22-µm filter. Cells and debris were removed by centrifugation at 5,000g and filtration through a 0.22-µm filter.

Mass spectrometry analysis. Samples were next fractionated at the protein level by reverse-phase chromatography followed by desalting for 5 min with 95% mobile-phase A (0.1% trifluoroacetic acid (TFA) in 95% H2O) at a flow rate of 3 ml/min. Proteins were eluted from the RPGS-reversed-phase column (4.6 mm internal diameter × 150 mm, 15 µm, 1,000 A; Column Technology) and collected into 24 fractions, at a flow rate of 2.1 ml/min with a gradient elution that included an increase from 5% to 70% mobile phase B (0.1% TFA in 95% acetonitrile) over 25 min, 70% to 95% mobile phase B for 3 min, a wash step to hold at 95% mobile phase B for 2 min, and a re-equilibration step at 95% mobile phase A for 5 min. The collected fractions from RP-HPLC were dried by lyophilization and subjected to in-solution digestion with trypsin dissolved in 100 mM ammonium bicarbonate containing 2% acetonitrile buffer.

The samples were reconstituted with acetonitrile:water:TFE (3:0.7:1, v/v/v) and individually analysed by LC–MS/MS in a QXactive mass spectrometer coupled to an Easy nanoLC 1000 system (Thermo Scientific) using a 15-cm column (75-µm internal diameter, C18 3 µm, Column Technology) as a separation column, and Symmetry C18 180-µm internal diameter × 20 mm trap column (Waters) over a 120-min gradient. Mass spectrometer parameters were: spray voltage 3.0 kV, capillary temperature 275°C, full scan MS of scan range 350–1,800 m/z, resolution 70,000, AGC target 3 × 106, maximum IT 50 ms and data-dependent MS2 scan of resolution 17,500, AGC target 1 × 105, maximum IT 100 ms and repeat count 10.

Acquired mass spectrometry data were processed by Proteome Discoverer 1.4 (Thermo Scientific). The tandem mass spectra were searched against UniprotX (disease), Swiss-Prot (Disease), SWISS-PROT (Disease), RefSeq (Disease), and UniProtKB (Disease) databases using the SEQUEST HT. A fixed modified peptide (oxidation (+16.94915 Da) was added to Cys and a variable modification of oxidation (+15.994195 Da) was added to Met. SILAC heavy stable isotopes 13C6 (+6.020129) was added to Arg and Lys. The precursor mass tolerance was 10 ppm and the fragment mass tolerance was 0.02 Da. The searched data was further processed with the ‘target decoy PSM validator’ function to filter with FDR 0.05.

Ingenuity pathway analysis. The list of 221 KRAS-regulated genes from the SILAC screen was subject to IPA to find pathways enriched in changed genes. The enrichment of the changes in certain pathways using mouse plasma membrane genes only as a background to ensure the enrichment is not from plasma membrane genes per se. All mouse plasma membrane genes (about 3,000 genes) were subjected to IPA. For a certain pathway, the result from SILAC top genes was compared to that from all mouse plasma membrane genes by the Fisher exact test. An enrichment fold is calculated as percent of pathway-related genes in the SILAC compared to that from all mouse plasma membrane genes by the Fisher exact test.

Immunofluorescence and confocal microscope. Cells were grown on eight-well chamber slides (LabTek), fixed in PFA with or without permeabilization in 0.5% saponin. Cells were further incubated with the primary antibody for 2 h at room temperature. Autofluorescence was quenched and nonspecific protein–protein interactions were blocked with 1% BSA/10% normal goat serum/0.3 M glycine. The secondary antibody was used at a 1/1,500 dilution for 40 min at room temperature. Imaging was performed using a Leica TCS SP5 II confocal microscope (Leica). Cells were incubated with 1 µg/ml of 4,6-diamidino-2-phenylindole (DAPI). Images were captured using an Olympus FV1000 confocal microscope system.

**Antibodies.** Antibodies used were KRAS (Santa Cruz, sc-30), phospho-ERK (Cell Signaling Technologies, 4376), vinculin (Cell Signaling Technologies, 11021), β-actin (Sigma Aldrich, A1325), cyto-keratin 19 (Proteintech, 14965-1-AP); SDC1 (281-2) (Biolegend, 142500); KI67 (Vector Laboratories, VP-RM04); ARF6 (Thermo Scientific, PA1-093X); CD8 (Cell Signaling Technologies, 389,941), CD45R (eBioscience, 14-0452-82), F4/80 (Cell Signaling Technologies, 70076), FOXP3 (eBioscience, 14-4771-80), CD4 (Abcam, 183685), Ly6G (Biogend, 127,602), CD11b (Abcam, ab133537), phospho-AKT473 (Cell Signaling Technologies, 3877); PSD4 (Thermo Scientific, PA5-31837; EPH4A2 (Cell Signaling Technologies, 3699); CD19 (Fisher Scientific, NC0743599); SDC1 (B-A38)(Abcam, 34164); phospho-MEK (Cell Signaling Technologies, 9154); APC-conjugated mouse anti-mouse antibody (Biorad, 142506); APC-conjugated human SDC1 antibody (Biogend, 352308); purified rat IgG2a (Biogend, RTK278).

**Glutamine-deprivation assay.** Glutamine-deprivation was performed following previously published protocols. Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight (LabTek). Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight (LabTek). Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight (LabTek). Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight (LabTek). Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight (LabTek). Twenty-four to forty-eight hours after cell seeding, cells were serum-starved for 12–18 h. Macropinosomes were marked using a high molecular weight.
Data availability
All data are available from the corresponding author upon reasonable request.

Code availability
Code for screen hit analysis are available upon request from the authors.

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Extended Data Fig. 1 | Interrogation of surfaceome changes upon KRAS* signalling by SILAC-based proteomic analysis. a, Western blot for p-ERK and KRAS in iKras* p53L/+ cells in the presence (ON) or absence (OFF) of doxycycline for 24 h. The experiment was repeated twice with similar results. b, c, Venn diagram showing the total number of proteins identified (b) and quantified (c) by SILAC-based proteomic analysis upon Kras* inactivation in three independent iKras* p53L/+ cell cultures. d, e, Venn diagram showing the number of decreased (KRAS* ON/OFF ratio > 1.2) (d) or increased (KRAS* ON/OFF ratio < 0.83) (e) proteins quantified with normalized MS2 counts >2 from the SILAC-based proteomic analysis on Kras* inactivation in three independent iKras* p53L/+ cell cultures. f, Immunohistochemistry for EPHA2 and CD9 in orthotopic xenograft tumours from iKras* p53L/+ model in the presence (ON) or absence (OFF) of doxycycline for 24 h (scale bar, 100 µm). The experiment was repeated twice with similar results. g, Top-20 surfaceome genes preferentially regulated by KRAS*.
Extended Data Fig. 2 | Functional surfacome analysis identified SDC1 as a KRAS*-dependent surface candidate. a, Normalized counts showing the distribution of reference and tumour barcodes to establish library coverage in vivo in three independent iKras* p53L/+ tumour cell cultures, showing similar results across experiments. In vivo screens were conducted in 3–5 mice for each cell culture. b, Correlation matrix among replicates of orthotopic xenograft-derived AK192, AK196 and AK10965 tumours screened with the surfacome-targeting shRNA library (barcode-level fold-change comparison, Pearson correlation coefficient). c, Positive (Psma and Rpl30) and negative (Luc) controls were plotted applying the RSP/logP score. d, Venn diagram showing the number of hits identified from three independent iKras* p53L/+ tumour cell cultures.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | SDC1 membrane expression is elevated in KRAS*-driven PDAC. a, iKras* p53<sup>1/2</sup> tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h and subjected to immunofluorescence for SDC1 (red) and DAPI (blue) (scale bar, 20 µm). For ReON samples, cells were grown in the absence of doxycycline for 48 h followed by doxycycline treatment for 24 h. Representative images are shown; experiments were repeated three times with similar results. b, iKras* p53<sup>1/2</sup> tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline for indicated time periods or treated with MEK inhibitor (AZD8330, 100 nM) for 16–18 h and cell-surface levels of CTNNA1 and PMCA were measured by FACS analysis. Quantification of fluorescence intensity is shown. For ReON samples, cells were grown in the absence of doxycycline for 48 h followed by doxycycline treatment for 24 h. Representative images are shown; experiments were repeated three times with similar results. c, iKras* p53<sup>1/2</sup> tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline for 24 h and Sdc1 mRNA level was measured by quantitative PCR with reverse transcription (RT–qPCR) (n = 3, Data are mean ± s.d.). Experiments were repeated twice with similar results. d, iKras* p53<sup>1/2</sup> tumour cells stably overexpressing SDC1 (Sdc1-OE) or empty vector (Vec) and a stable single clone with double nickase-mediated SDC1 deletion (SC1) derived from iKras* p53<sup>1/2</sup> tumour cells were blotted to validate SDC1 expression. Some samples were also treated with heparinase (Hepa) and chondroitinase (Cho) before western blot analysis. Experiments were repeated twice with similar results. e, iKras* p53<sup>1/2</sup> tumour cells grown in the presence (ON) or absence (OFF) of doxycycline for indicated time periods were processed for western blot analysis to detect SDC1, vinculin, p-ERK and KRAS. Experiments were repeated twice with similar results. f, Representative immunohistochemistry for SDC1 from the LSL-KRAS PDAC mouse model showing membrane SDC1 level in normal pancreas, acinar-to-ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN). Experiments were repeated twice with similar results. Scale bar, 200 µm. g, Left, representative immunohistochemistry for SDC1 in normal pancreas, tumour adjacent pancreatitis (TAP), PanINs and invasive tumours (PDA) from the human PDAC TMA. Right, quantification of the TMA scores. Scale bar, 50 µm. h, Representative H&E staining and SDC1 immunohistochemistry from the TMA analysis. Representative images of SDC1 immunohistochemistry showing staining classified as low (score 1), intermediate (score 2) and high (score 3). Scale bar, 200 µm. i, mRNA expression of SDC1 in public microarray datasets. Data are mean ± s.d.; P values were determined by unpaired two-sided Student's t-test. j, Top, outline of experimental design for sequential cerulein and doxycycline treatment in iKras* mice (top). Chronic pancreatitis was induced in iKras* mice by cerulein injection for six weeks. The mice were then treated with doxycycline for indicated times. Bottom, pancreatic or tumour tissues were subjected to H&E staining or immunohistochemistry for CK19 and SDC1. Experiments were repeated twice with similar results. Scale bar, 100 µm.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | SDC1 is required for tumorigenic activity of KRAS<sup>*</sup>-driven PDAC. a, Representative images of clonogenic assay for two independent iKras<sup>*</sup> p53<sup>Li+/lo</sup> PDAC cells infected with scrambled shRNA or shRNA against Sdc1. Experiments were repeated three times with similar results. b, c, Validation of Sdc1 knockdown by RT–qPCR (b) and FACS analysis with anti-SDC1 antibody (c) in iKras<sup>*</sup> p53<sup>Li+/lo</sup> PDAC cells infected with scrambled shRNA or shRNA against Sdc1. In b, n = 3; data are mean ± s.d.; P values were determined by unpaired two-sided Student’s t-test. In c, experiments were repeated three times with similar results. d, Two independent iKras<sup>*</sup> p53<sup>Li+/lo</sup> tumour cells stably expressing SDC1 or empty vector were infected with scrambled shRNA or shRNA against Sdc1. Quantification of clonogenic assay is shown (n = 4 biological replicates; data are mean ± s.d.). P values were determined by unpaired two-sided Student’s t-test. e, Digital photographs of dissected tumours from iKras<sup>*</sup> p53<sup>Li+/lo</sup> tumour cells containing scrambled shRNA or shRNA against Sdc1. f, Validation of double nickase-mediated Sdc1 deletion in iKras<sup>*</sup> p53<sup>Li+/lo</sup> PDAC cells using 281-2 anti-SDC1 antibody and FACS analysis. Experiments were repeated twice with similar results. g, Representative figure of clonogenic assay for iKras<sup>*</sup> p53<sup>Li+/lo</sup> PDAC cells with wild-type SDC1 or double nickase-mediated Sdc1 deletion. Experiments were repeated twice with similar results. h, Digital photographs of dissected tumours from mice implanted with iKras<sup>*</sup> p53<sup>Li+/lo</sup> tumour cells with wild-type SDC1 or double nickase-mediated Sdc1 deletion. i, Representative figure of clonogenic assay for human PDAC cells infected with scrambled shRNA or shRNA against SDC1. Experiments were repeated twice with similar results. j, Validation of SDC1 knockdown by shRNA in human PDAC cell lines from (i) using DL-101 anti-SDC1 antibody and FACS analysis. Experiments were repeated twice with similar results. k, Digital photographs of dissected tumours from subcutaneous xenografts of mice implanted with PATC69 cells infected with scrambled shRNA or shRNA against SDC1.
Extended Data Fig. 5 | SDC1 loss leads to changes in tumour microenvironment. a, Validation of SDC1 level by FACS analysis in primary cultures derived from the PDAC mouse model with indicated Sdc1 genotypes. Experiments were repeated twice with similar results. b, c, Quantification (a) and representative images (b) from immuno-profiling of SDC1 wild-type and knockout (KO) tumours from the GEMM model by immunohistochemistry or immunofluorescence staining (n = 20 random images from four biological replicates). P values were determined by unpaired two-sided Student’s t-test.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6  |  KRAS* induces SDC1 membrane expression via the MAPK pathway.  

a, b, iKras* p53L/+ PDAC cells were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h. ReON cells were grown in the absence of doxycycline for 48 h and then treated with doxycycline for 24 h. Treatment of ON or ReON samples with trametinib (50 nM) or BKM120 (100 nM) for 16–18 h is indicated. a, Cells were stained with anti-SDC1 antibody and surface SDC1 was measured via FACS. Quantification of fluorescence intensity from biological duplicates is shown as mean ± s.d.  
b, Cell lysates were blotted for phospho-ERK, phospho-AKT and KRAS, with vinculin as loading control. Experiments were repeated twice with similar results.  
c–f, iKras* p53L/+ PDAC cells were treated with different concentrations of the MEK inhibitor tramatinib (c, d) or AZD8330 (e, f) overnight (16–18h), and the live cells were prepared for FACS analysis for SDC1 (c, e; data are mean ± s.d.); cell lysates were blotted for phospho-ERK, phospho-MEK and KRAS, with vinculin as loading control (d, f). Experiments were repeated twice with similar results.  
g, Outline of experimental design to measure SDC1 shedding (left). iKras* p53L/+ PDAC cells were grown in the presence (ON) or absence (OFF) of doxycycline for 24 or 48 h. Medium was collected and shed SDC1 was measured by anti-SDC1 enzyme-linked immunosorbent assay (ELISA) (right) (n = 4 biological replicates; data are mean ± s.d.).  
h, Left, outline of experimental design to measure surface SDC1 internalization. Right, iKras* p53L/+ PDAC cells were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h. Cells were then incubated at 37 °C for indicated times and surface SDC1 was labelled with anti-SDC1 antibody at 4 °C after internalization for indicated times. FACS was performed to detect remaining SDC1 on the cell membrane (n = 2 biological replicates; data are mean ± s.d.).  
i–j, Outline of experimental design to measure surface SDC1 recycling (i, left). iKras* p53L/+ PDAC cells were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h. Surface SDC1 was labelled with anti-SDC1 at 4 °C. Cells were then incubated at 37 °C for 30 min to allow SDC1 internalization. Cells were then incubated on ice to stop internalization; subsequently, cells were returned to 37 °C for indicated times to allow SDC1 recycling. Recycled SDC1 was measured by FACS (i, right).  
j, Histograms of FACS analysis, showing recycled cell-surface SDC1 at the indicated time points. Experiments were repeated twice with similar results.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | The MAPK–PSD4–ARF6 axis mediates KRAS*-dependent SDC1 membrane localization. a, Cells were grown in presence (ON) or absence (OFF) of doxycycline or treated with AZD8330 (100 nM) for 16–18 h. Top, ARF6 activity was measured with GGA3–PBD pull-down assay; Bar graph, ARF6 activity was calculated as ratio of captured ARF6/input ARF6/vinculin. b, iKras* p53+/+ tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline, or treated with AZD8330 (100 nM) for 16–18 h. Cell lysates were used for measurement of PIPK activity (n = 3 biological replicates; data are mean ± s.d.). *P values were determined by unpaired two-sided Student’s t-test. c, Representative images of morphology change in iKras* p53+/+ tumour cells with dominant negative ARF6 (ARF6(T27N)) or constitutively active ARF6 (ARF6(Q67L)). Experiments were repeated three times with similar results. d, Top and middle, iKras* p53+/+ tumour cells stably expressing ARF6(Q67L) or empty vector were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h and surface SDC1 was measured by FACS using anti-SDC1 antibody. Bottom, fluorescence intensity of surface SDC1 (n = 3 biological replicates; data are mean ± s.d.). *P values were determined by paired two-sided Student's t-test. e, iKras* p53+/+ tumour cells stably expressing ARF6(T27N) or empty vector were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h and surface SDC1 was measured by FACS using anti-SDC1 antibody. Representative histograms (top and middle) and bar chart (bottom) of fluorescence intensity are shown (n = 4 biological replicates; data are mean ± s.d.). *P values were determined by paired two-sided Student's t-test. f, mRNA expression of ARF6 GTPase-activating proteins and guanine nucleotide exchange factors in a iKras* p53+/+ tumour cell microarray dataset on KRAS(G12D) inactivation (n = 4 biological replicates). *P values were determined by unpaired two-sided Student’s t-test. g, iKras* p53+/+ tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline or treated with AZD8330 (50 nM) for 16–18 h, and Psd4 mRNA level was measured by RT–qPCR (n = 3). *P values were determined by unpaired two-sided Student’s t-test. h, MiaPaCa2 cells containing doxycycline-inducible shRNA targeting human KRAS were grown in the absence (OFF) or presence (ON) of doxycycline, or treated with trametinib (50 nM), AZD8330 (50 nM) or BKM120 (100 nM) for 18 h. Cell lysates were blotted for PSD4, phospho-ERK and KRAS. Arrow, PSD4 band. Experiments were repeated twice with similar results. i, iKras* p53+/+ tumour cells stably expressing Psd4 or empty vector were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h. ARF6 activity was measured by GGA3–PBD pull-down assay. Input lysates were immunoblotted to validate expression of ARF6, p-ERK, p-MEK, PSD4 and KRAS. Experiments were repeated twice with similar results. j, iKras* p53+/+ tumour cells stably expressing Psd4 or empty vector were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h and surface SDC1 was measured by FACS using anti-SDC1 antibody. Representative histograms of FACS analysis (top) and bar graph of fluorescence intensity (bottom) of surface SDC1 are shown (n = 3 biological replicates; data are mean ± s.d.). *P values were determined by paired two-sided Student’s t-test.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | SDC1 mediates macropinocytosis in KRAS*-driven mouse PDAC cells. a, b, iKras* p53L/+ tumour cells were grown in the presence (ON) or absence (OFF) of doxycycline for 24 h. For a positive control, cells grown in the presence of doxycycline were treated with EIPA (50 µM) for 16 h. Macropinocytosis was visualized with TMR-dextran (scale bar, 20 µm) (a) and quantified (b) (n = 8 random areas for ON/OFF groups, n = 5 for EIPA group; data are mean ± s.d.). Data are representative of three independent experiments with similar results. c, Validation of Sdc1 knockdown by qPCR in iKras* p53L/+ tumour cells (n = 3; data are mean ± s.d.). d, Macropinocytosis index in KPC tumour cells containing scrambled shRNA or shRNA directed against Sdc1 (n = 6 random areas; data are mean ± s.d.). e, f, iKras* p53L/+ tumour cells stably expressing Psd4 or empty vector were grown in the presence (ON) or absence (OFF) of doxycycline for 48 h. Macropinocytosis was visualized with TMR-dextran (f; scale bar, 20 µm) and quantified (e; n = 6 random areas for Vec-ON, PSD4-ON and PSD4-OFF groups, n = 8 for Vec-OFF group; data are mean ± s.d.). Data are representative of two independent experiments with similar results. g, iKras* p53L/+ tumour cells stably expressing Sdc1 or empty vector were grown in the presence (KRAS*-ON) or absence (KRAS*-OFF) of doxycycline for 48 h. Macropinocytosis was visualized with TMR-dextran (scale bar, 20 µm). Experiments were repeated twice with similar results. h, RHOA activity in iKras* p53L/+ tumour cells containing scrambled shRNA or shRNA against Sdc1 was measured by G-LISA activation assay (n = 2 biological replicates; data are mean ± s.d.). i, RAC1 activity in iKras* p53L/+ tumour cells containing RAC1(Q61L) or empty vector was measured by G-LISA activation assay (n = 2 biological replicates; data are mean ± s.d.). j, k, iKras* p53L/+ tumour cells stably expressing RAC1(Q61L) or empty vector were infected with scrambled shRNA or shRNA against Sdc1. Macropinocytosis was visualized with TMR-dextran (scale bar, 20 µm) (j) and quantified (k) (n = 8 random areas; data are mean ± s.d.). Data are representative of two independent experiments with similar results. l, iKras* p53L/+ tumour cells were cultured in medium with serial concentrations of glutamine. Growth rate was measured and the timelapse graph was generated using the Incucyte live-cell analysis system (n = 3 technical replicates; data are mean ± s.d.). Data are representative of two independent experiments with similar results.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | SDCBP is required for SDC1-mediated macropinocytosis. a, iKras* p53L/+ tumour cells containing wild-type or indicated mutant constructs of SDC1 or empty vector were infected with scrambled shRNA or shRNA against Sdc1 and surface expression of SDC1 was measured by FACS; representative histograms are shown. Experiments were repeated twice with similar results. b, Shed SDC1 from iKras* p53L/+ tumour cells stably expressing wild-type or soluble mutant SDC1 (Sol), or empty vector was measured by anti-SDC1 ELISA (n = 5 biological replicates; data are mean ± s.d.). c–e, iKras* p53L/+ tumour cells containing wild type or indicated mutant constructs of SDC1 or empty vector were infected with scrambled shRNA or shRNA against Sdc1 to measure RAC1 activity using G-LISA activation assay (c) (n = 4 biological replicates) or macropinocytosis index by visualizing with TMR–dextran (scale bar, 20 µm) and subsequent quantification (d, e). Data are mean ± s.d. Experiments were repeated twice with similar results. f, g, iKras* p53L/+ tumour cells containing wild-type or different mutant constructs of SDC1 or empty vector were infected with scrambled shRNA or shRNA against Sdc1 and subcutaneously injected into nude mice. Photographs of dissected tumours (f) and tumour volume at indicated time points (g) are shown (n = 5 for Vec Scr, Sol-ShSdc1, Ect-ShSdc1, Gag-ShSdc1 and WT-ShSdc1 groups; n = 4 for Vec-ShSdc1 and C30-ShSdc1 groups; data are mean ± s.d.). h–m, iKras* p53L/+ tumour cells were infected with scrambled shRNA or shRNA against Sdcbp. h, Sdcbp knockdown was validated by western blot. Image of ShSdcbp-2 was cropped from the same membrane as Scr and ShSdcbp-1. Experiments were repeated twice with similar results. i, RAC1 activity was measured by G-LISA activation assay (n = 4 biological replicates; data are mean ± s.d.). j, Macropinocytosis was visualized with TMR–dextran (scale bar, 20 µm) and quantified (n = 5 random areas for Scr and ShSdcbp-2; n = 6 random areas for ShSdcbp-1 group; data are mean ± s.d.). Data are representative of two independent experiments with similar results. k, Representative images of clonogenic assay are shown from two independent experiments with similar results. l, Cells were subcutaneously injected into nude mice (n = 4 for Scr and ShSdcbp-1 groups, n = 5 for ShSdcbp-2 group) and tumour size was measured from photographs of dissected tumours shown in m. P values were determined by unpaired two-sided Student’s t-test.
Extended Data Fig. 10 | SDC1 is critical for macropinocytosis in KRAS*-dependent human PDAC. a–c, AsPC1 or PATC69 PDAC cells expressing mouse SDC1 or empty vector were infected with scrambled shRNA or shRNA against SDC1. Macropinocytosis was visualized with TMR–dextran (scale bar, 20 μm) (a) and quantified (b) (data are mean ± s.d.). Data are representative of two independent experiments with similar results. P values were determined by unpaired two-sided Student’s t-test. c, Surface SDC1 evaluated by FACS using human anti-SDC1 antibody DL101 or mouse anti-SDC1 antibody 281-2. Histograms show representative images from two independent experiments with similar results. d, Macropinocytic index of 20 human PDAC cell lines with high (n = 13) or low (n = 7) SDC1 membrane expression, as determined by FACS analysis. P value was determined by Mann–Whitney test. e, Macropinocytic index was quantified in 20 different PDAC cell lines (data are mean ± s.d.), f. Representative images of TMR–dextran (red) staining (scale bar, 20 μm). Data are representative of two independent experiments with similar results.
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- Clearly defined error bars
  *State explicitly what error bars represent (e.g. SD, SE, CI)*

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Software and code

Policy information about availability of computer code

**Data collection**

- Mass spectrometry data was processed by Proteome Discover 1.4 (Thermo Scientific). Confocal immunofluorescence images were collected with FV10-ASW 4.0 (Olympus). FACS data were collected with BD FACSDiva (BD Biosciences). Illumina sequencing for the in vivo shRNA screen were processed with CASAVA (v.1.8.2).

**Data analysis**

- Data analysis and statistical tests were done using Prism (GraphPad Prism 6) or Excell (Microsoft). Confocal immunofluorescence images were analyzed with ImageJ (Imagej). IPA (Qiagen) were used for pathway analysis. FACS data was analyzed with Flowjo (v10, Flowjo). RSA algorithms (Pubmed), Ggplots (CRAN), Bowtie2 (Pubmed), SAM tools (Pubmed), Flowr(BioRxiv), and R package (CRAN) were used for in vivo shRNA screen analysis. Proteome Discover 1.4 (Thermo Scientific) and Sequest HT were used for mass spectrometry data analysis.

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Life sciences

Study design

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

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

Data exclusions
No data was excluded

Replication
All Experiments were repeated at least twice to ensure reproducibility. Replication numbers are indicated in figure panels/Methods as required.

Randomization
No formal method of randomization was used; Animals were equally divided among the groups being compared.

Blinding
Experiments execution, data collection and data analysis were usually carried out by the same person, therefore no blinding was used.

Materials & experimental systems

Policy information about availability of materials

n/a
Involved in the study

- [x] Unique materials
  - Antibodies
- [ ] Eukaryotic cell lines
- [ ] Research animals
- [x] Human research participants

Antibodies

Antibodies used

- Anti-Kras (Santa Cruz, Cat# sc-30 1:500 for WB); Anti-phospho erk (Cell Signaling Technologies, Cat#4376, 1:1000 for WB); Anti-vinculin (Cell Signaling Technologies, Cat#E19V, 1:3000 for WB); Anti-beta-actin (Sigma-Aldrich, Cat#A5441, 1:10000 for WB); Anti-syntenin (Abcam, Cat#ab19903, 1:50 for IF, 1:1000 for WB); Anti-cytokeratin 19 (Proteintech, Cat#14965-1-AP, 1:100 for IHC); Anti-syndecan1 (281-2)(Biolegend, Cat#142502, 1:200 for IF, 1:500 for IHC, 1:1000 for WB ); Anti-ki67 (Vector Laboratories, Cat#VP-RM04, 1:1500 for IHC); Anti-arf6 (Thermo Scientific, Cat#PA1-093X, 1:50 for IF, 1:500 for WB); Anti-phospho akt473 (Cell Signaling Technologies, Cat#3787, 1:1000 for WB); Anti-sydncan1 (281-2)(Biolegend, Cat#142502, 1:200 for IF, 1:500 for IHC, 1:1000 for WB ); Anti-ki67 (Vector Laboratories, Cat#VP-RM04, 1:1500 for IHC); Anti-arf6 (Thermo Scientific, Cat#PA1-093X, 1:50 for IF, 1:500 for WB); Anti-phospho akt473 (Cell Signaling Technologies, Cat#3787, 1:1000 for WB); Anti-psd4 (Thermo Scientific, Cat#PA5-31837, 1:1000 for WB); Anti-epha2 (Cell Signaling Technologies, Cat#6997, 1:100 for IHC); Anti-cd19 (Fisher Scientific, Cat#NC0743599, 1:100 for IHC); Anti-syndecan1 (B-A38)(Abcam, Cat#34164, 1:100 for IF); Anti-phospho mek (Cell Signaling Technologies, Cat#9154, 1:1000 for WB); APC-anti-mouse syndecan1 antibody (Biolegend, Cat#142506, 1:50 for FACS); APC-anti-human syndecan1 antibody (Biolegend, Cat#352308, 1:50 for FACS and IHC); Purified Rat IgG2a (Biolegend, Cat# RTK2758, 1:50 for FACS); Anti-CTNNA1 (Isbio, Cat# LS-C334181, 1:50 for FACS); Anti-Calcium Pump pan PMCA ATPase (Abcam, Cat# ab2825, 1:50 for FACS); Anti-cdc8 (Cell Signaling Technologies, Cat#98941, 1:400 for IHC); Anti-cdc45R (eBioscience, Cat#14-0452-82, 1:50 for IHC); Anti-F4/80 (Cell Signaling Technologies, Cat#70076, 1:250 for IHC); Anti-foxp3 (eBioscience, Cat#14-4771-80, 1:400 for IF); Anti-cd4 (Abcam, Cat#183685, 1:100 for IF); Anti-ly6g (Biolegend, Cat#127602, 1:50 for IF); Anti-cd11b (Abcam, Cat#ab133757, 1:200 for IF).

Validation
All antibodies were validated by the manufacturers for indicated application. The use of anti-syndecan1 (281-2) for Western blot was validated with Sdc1 deleted cells as shown in Extended Data Figure 3d.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) 293T, ASPC1, MIAPACA2, HPAFII, SW1990, HPAC, PANC1, CAPAN1, BXPC3, PANC0813 and PANC0327 were obtained from ATCC. DAN-G, HUPT3, HUPT4 and PATU8902 were obtained from DSMZ. SNU324 was obtained from AcceGen Biotech. KP4 was obtained from JCRB. Primary PDX lines were established from xenograft tumors maintained in nude mice. Primary mouse PDAC lines were established using primary tumors from the corresponding genetically engineered mouse models.

Authentication

All STR profiles of human PDAC cell lines have been verified at “Characterized Cell Line Core Facility” at the UT MD Anderson Cancer Center. The PDX lines will also be submitted to the “Characterized Cell Line Core Facility” and all unique STR profiles are then added to the database for future MD Anderson reference.

Mycoplasma contamination

All cells have been tested negative for mycoplasma contamination.

Commonly misidentified lines

No such cell lines were used.

Research animals

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

Animals/animal-derived materials

This is reported in Methods. For genetically engineered mouse models, the following strains are used: TetO_Lox-Stop-Lox-Kras-G12D (tetO_LKrasG12D), p48-Cre, Rosa26-Lsl-rtTA-Ires-Gfp (Rosa_rtTA), Trp53Flox, Kras-LSL-G12D, Sdc1-KO. Mice were interbred and maintained on FVB/C57Bl6 hybrid background. Both male and female mice were used. Animals between 10-42 weeks were used for the endpoint analysis. Animals between 3-13 weeks were used for the serial analysis. For xenograft studies, NSG or NCR Nude mice were purchased from Taconic. Both male and female mice of 6-8 week of age were used.

Method-specific reporting

n/a Involved in the study

☑ ChIP-seq

☑ Flow cytometry

☐ Magnetic resonance imaging

Flow Cytometry

Plots

Confirm that:

☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☑ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).

☑ All plots are contour plots with outliers or pseudocolor plots.

☑ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation human or mouse PDAC cells were suspended using Non-enzymatic Cell Dissociation Buffer, pelleted and resuspended in cold PBS with 1% (w/v) BSA (BSA/PBS). (See Methods).

Instrument BD FACSCantoII flow cytometer or BD LSRSortessa analyzer.

Software BD FACSDiva was used for data collection and FlowJo (v10) was used for data analysis.

Cell population abundance at least 10,000 cells were quantified.

Gating strategy unstained cells were used to set up the FSC/SSC gates. Samples stained with isotype control antibody were used to set up gate for positive/negative boundary.

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