LETTER

Epithelial–to–mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer

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Diagnosis of pancreatic ductal adenocarcinoma (PDAC) is associated with a dismal prognosis despite current best therapies; therefore new treatment strategies are urgently required. Numerous studies have suggested that epithelial-to-mesenchymal transition (EMT) contributes to early-stage dissemination of cancer cells and is pivotal for invasion and metastasis of PDAC1–4. EMT is associated with phenotypic conversion of epithelial cells into mesenchymal-like cells in cell culture conditions, although such defined mesenchymal conversion (with spindle-shaped morphology) of epithelial cells in vivo is rare, with quasi-mesenchymal phenotypes occasionally observed in the tumour (partial EMT)5,6. Most studies exploring the functional role of EMT in tumours have depended on cell-culture-induced loss-of-function and gain-of-function experiments involving EMT-inducing transcription factors such as Twist, Snail and Zeb1 (refs 2,3,7–10). 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Figure 1  | EMT inhibition does not alter primary tumour progression.

a. Representative haematoxylin and eosin (H&E)-stained primary tumours (scale bar, 100 μm). b. Relative percentages of each primary tumour histological tissue phenotype. n = 31 (KPC), 14 (KPC;Twist<sup>ΔKO</sup>) and 30 (KPC;Snail<sup>ΔKO</sup>) mice; error bars represent s.d. c. Local invasiveness n = 31 (KPC), 14 (KPC;Twist<sup>ΔKO</sup>) and 30 (KPC;Snail<sup>ΔKO</sup>) mice; error bars represent s.d. d. Overall survival n = 29 (KPC), 12 (KPC;Twist<sup>ΔKO</sup>) and 33 (KPC;Snail<sup>ΔKO</sup>) mice. e. Twist1 or Snai1 in situ hybridization (black) with CK8 (red) immunolabelling in primary tumours (n = 3 mice for all groups; scale bar, 50 μm). Relative percentages of Twist1<sup>+</sup>CK8<sup>+</sup> or Snai1<sup>+</sup>CK8<sup>+</sup> double-positive cells are shown below (two-tailed t-test).

while being positive for E-cadherin and Ki-67 (Extended Data Fig. 3g, h). The proliferation rate of cancer cells in the metastases was similar in KPC, KPC;Snai1<sup>ΔKO</sup> and KPC;Twist<sup>ΔKO</sup> mice (Extended Data Fig. 3h). Collectively, the results indicated that the deletion of Twist1 or Snai1 in genetically engineered mouse models of PDAC did not reduce metastatic disease.
To evaluate whether cancer cells from the pancreas with and without EMT program differentially benefit from impaired proliferation to form secondary tumours, we isolated cancer cells from KPC, KPC;Twist\textsuperscript{cKO} and KPC;Snail\textsuperscript{cKO} mice to assay their organ colonization potential. Twist\textsuperscript{1} was significantly reduced and Snail\textsuperscript{1} expression was undetectable in cancer cells isolated from Twist- and Snail-deleted tumours, respectively (Fig. 2f). Short-term potential to form tumour spheres (associated with putative cancer stem phenotype) appeared similar in Twist\textsuperscript{cKO} and Snail\textsuperscript{cKO} KPC cells when compared to control KPC cells (Fig. 2g). Lung colonization frequencies following i.v. injection of KPC cancer cells (Twist- or Snail-deleted) were similar to the control KPC cancer cells (Fig. 2h). These results suggest that a favoured epithelial phenotype of cancer cells (via suppression of EMT) did not impact the capacity to form tumour spheres or their ability for organ colonization\textsuperscript{17}.

Cancer cell EMT is associated with gemcitabine drug resistance in PDAC patients and in the orthotopic mouse models of PDAC\textsuperscript{1,2,8,9,18–23}. Moreover, enhanced frequency of EMT\textsuperscript{+} cancer cells in pancreatic tumours is associated with poor survival\textsuperscript{14,25}. To determine whether EMT suppression enhances PDAC sensitivity to gemcitabine chemotherapy, we tested the gemcitabine sensitivity of cancer cells with suppressed EMT in KPC mice. Equilibrative nucleoside transporter (ENT1) and concentrating nucleoside transporter (Cnt3) were significantly upregulated in cancer cells lacking Snail and Twist, while ENT2 expression was unchanged (Fig. 3a–c). KPC, KPC;Snail\textsuperscript{cKO} and KPC;Twist\textsuperscript{cKO} mice were treated with gemcitabine and tumour burden was monitored by MRI (Extended Data Table 3). Tumour progression was suppressed in KPC;Snail\textsuperscript{cKO} and KPC;Twist\textsuperscript{cKO} mice when compared to treated KPC control mice (Fig. 3d). KPC;Snail\textsuperscript{cKO} and KPC;Twist\textsuperscript{cKO} mice treated with gemcitabine showed improved histopathology and increased survival (Fig. 3e–g).

Cancer cells isolated from the tumours of KPC;Snail\textsuperscript{cKO} and KPC;Twist\textsuperscript{cKO} mice showed epithelial morphology (Extended Data Fig. 4a) and reduced expression of mesenchymal genes compared to KPC cancer cell lines (Extended Data Fig. 4b). However, in tissue culture conditions (2D culture on plastic), equilibrative nucleoside transporters (ENT1/ENT2/ENT3) showed similar expression patterns (Extended Data Fig. 4b) and expression of concentrating nucleoside transporters (Cnt1/Cnt3) was not detected (data not shown). Increased proliferation of KPC;Snail\textsuperscript{cKO} and KPC;Twist\textsuperscript{cKO} cancer cells compared to KPC control cells (Extended Data Fig. 4c) probably accounted for

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**Figure 2** EMT inhibition does not alter invasion and metastasis. a, b, Primary tumour immunolabelling for cleaved caspase-3 (a; n = 6 mice for all groups; scale bar, 50 μm) and Ki67 (b; n = 7 (KPC), 7 (KPC;Twist\textsuperscript{cKO}) and 9 (KPC;Snail\textsuperscript{cKO}) mice; scale bar, 100 μm). c, Percentage of YFP\textsuperscript{+} circulating tumour cells (CTCs) (n = 8 mice for both groups; two-tailed t-test; error bars represent s.d.). d, Kras\textsuperscript{G12D} expression in whole blood cell pellets (n = 5 (KPC), 3 (KPC;Twist\textsuperscript{cKO}) and 5 (KPC;Snail\textsuperscript{cKO}) mice; error bars represent s.d.). e, Haematoxylin and eosin staining and CK19 immunolabelling of metastatic liver nodules. Metastatic tumour nodules (T) outlined by a dotted line (scale bar, 100 μm). A table presenting the number of positive tissues out of total tissues examined is shown below (χ\textsuperscript{2} analysis). f, Expression analysis of Twist\textsuperscript{1} and Snail\textsuperscript{1} expression in cultured primary tumour cell lines (n = 4 (KPC) and 5 (KPC;Twist\textsuperscript{cKO}) individual cell lines (Twist\textsuperscript{1}) or 4 (KPC) and 6 (KPC;Snail\textsuperscript{cKO}) individual cell lines (Snail\textsuperscript{1})); one-tailed t-test of ΔC\textsubscript{T} ( error bars represent s.d.). g, Bright-field or YFP images and quantification of sphere number in cultured tumour cell lines (n = 3 (KPC), 2 (KPC;Twist\textsuperscript{cKO}) and 3 (KPC;Snail\textsuperscript{cKO}) individual cell lines; scale bar, 50 μm). h, Haematoxylin and eosin images (scale bar, 100 μm) of colonized lungs from intravenously injected cultured primary tumour cell lines KPC (n = 5 (cell line 1) and 5 (cell line 2) mice injected) and KPC;Twist\textsuperscript{cKO} (n = 11 (cell line 1) and 4 (cell line 2) mice injected) and KPC;Snail\textsuperscript{cKO} (n = 4 (cell line 1), 5 (cell line 2) and 5 (cell line 3) mice injected). A table presenting the number of colonized tissues out of total tissues examined is shown below (χ\textsuperscript{2} analysis). Unless otherwise indicated error bars represent s.e.m and significance was determined by one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant; ND, not detected.
an enhanced sensitivity of EMT-suppressed cancer cells to gemcitabine. Both ENT2 and Cnt3 were upregulated in EMT-suppressed tumours (Fig. 4g). These data support a possible mechanistic connection between EMT and resistance to chemotherapy in PDAC.

Collectively, our studies provide a comprehensive functional analysis of EMT in PDAC progression and metastasis. Absence of either Twist1 or Snail did not alter cancer progression or the capacity for local invasion or metastasis to lung and liver in genetically engineered mouse models of PDAC. Metastasis occurs despite a significant loss of EMT with either the deletion of Snail or Twist, and in both set-

**: ANOVA comparing mean tumour volumes on day 0 and day 19, error bars represent s.d. e, Survival on gemcitabine treatment to end point (day 21). f, Haematoxylin and eosin-stained primary tumours (scale bar, 100 μm). g, Relative percentages of each histological tissue phenotype of end-point mice (n = 3 (KPC + gem.), 9 (KPC; TwistKO + gem.) and 11 (KPC; SnailKO + gem.) mice; error bars represent s.d.; two-tailed t-test). *P < 0.05, **P < 0.01; NS, not significant.
However, such compensation is not observed with respect to chemoresistance, and previous studies have demonstrated that EMT and cancer cell dissemination are observed even before PDAC lesions are detected in KPC mice. Our study demonstrates that EMT results in suppression of cancer cell proliferation and suppression of drug transporter and con- 
ducting proteins, therefore inadvertently protecting EMT

cells from anti-proliferative drugs such as gemcitabine. The correlation of decreased survival of pancreatic cancer patients with increased EMT is probably due to their impaired capacity to respond to gemcitabine and chemotherapeutics, which is a standard of care for most patients. A compromised response to chemotherapy probably also explains higher metastatic disease in association with decreased survival of patients with enhanced EMT signatures. Collectively, our study offers the opportunity to evaluate the potential of targeting EMT to enhance efficacy of chemotherapies and targeted therapies.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 10 June; accepted 8 October 2015.

Published online 11 November 2015.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We wish to thank D. Lundy, S. Yang, Z. Xiao, R. Deitz-Aguirre, T. Miyake and S. Lovisa for technical support and K. M. Ramirez and J. Jewell in the South Campus Flow Cytometry Core Laboratory of MD Anderson Cancer Center for flow cytometry cell sorting and analyses (partly supported by NCI grant no. P30CA16672). We also wish to thank E. Chang for scanning slides of histopathological specimens. This study was primarily supported by the Cancer Prevention and Research Institute of Texas. The research in the LeBleu laboratory is supported by UT MDACC Khalifa Bin Zayed Al Nahya Foundation.

Author Contributions R.K. conceptually designed the strategy for this study and provided intellectual input. V.S.L. helped design experimental strategy, provided intellectual input, supervised the studies, performed immunohistochemistry and culture experiments, generated the figures and wrote the manuscript. X.Z. performed experiments to generate the genetically engineered mouse models and helped characterize the mouse phenotype, performed culture experiments, collected the tissue for analysis and contributed to the manuscript writing. J.L.C. characterized the mouse phenotype, analyzed the data related to the genetically engineered mouse models, collected data, generated the figures and helped with manuscript writing and editing. H.S. performed experiments with mice and injected cancer cells and helped collect tissue, J.Ki., M.S., J.Ka., and C.-C.W. performed reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper.
METHODS

Mice. Characterization of disease progression and genotyping for the *Pdx1-cre;LSL-KrasG12D;P53R16H+* (herein referred to as KPC) and *Ptf1a* (P48)-cre;*LSL-KrasG12D;Tgby2* (herein referred to as KTC) mice were previously described31–33. These mice were bred to *Snai1*−/− (herein referred to as *Snai1*−/−), *Twist1*−/− (herein referred to as *Twist1*−/−), and *R26*-LSL-EYFP31, *Snai1*−/− mice were kindly provided by S. J. Weiss. *Twist1*−/− mice were kindly provided by R. R. Behringer via the Mutant Mouse Regional Resource Center (MMRRC) repository. The resulting progeny were referred to as KPC, KPC;Snai1−/−, KTC, KTC;Snai1−/− mice and were maintained on a mixed genetic background. Both males and females were used indiscriminately. Mice were given gencatibine (G-4177, LC Laboratories) via intraperitoneal injection (i.p.) every other day at 50 mg kg−1 of body weight. Hypoxyprobe was injected in a subset of mice i.p. at 60 mg kg−1 of body weight 30 min before euthanasia. For *in vivo* colonization assays, one million KPC, KPC;*Twist1*−/− and KPC;*Snai1*−/− tumour cells in 100 μl of PBS were injected intravenously via the retro-orbital venous sinus. Four to eleven mice were injected per cell line. All mice were euthanized at 15 days post injection. All mice were housed under standard housing conditions at MD Anderson Cancer Center (MDACC) animal facilities, and all animal procedures were reviewed and approved by the MDACC Institutional Animal Care and Use Committee. Tumour growth met the standard of a diameter less than or equal to 1.5 cm. Investigators were not blinded to group allocation but were blinded for the assessment of the phenotypic outcome by histological analyses. No statistical methods were used to predetermine sample size and the experiments were not randomized.

Histology and histopathology. Histology, histopathological scoring, Masson's trichrome staining (MTS), and Picrosirius Red have been previously described33,34. Formalin-fixed tissues were embedded in paraffin and sectioned at 5 μm thickness. MTS was performed using Gomori’s Trichrome Stain Kit (38016552, Leica Biosystems). Picrosirius red staining to identify collagen was performed using 0.1% picrosirius red (Direct Red 80; Sigma) and counterstained with Weigert's haematoyxlin. Sections were also stained with haema3toxylin and eosin (H&E). Histopathological measurements were assessed by scoring H&E-stained tumours for relative percentages of each histopathological phenotype: normal (non-neoplastic), PanIN, well-differentiated PDAC, moderately-differentiated PDAC, poorly-differentiated PDAC, sarcomatoid carcinoma, or necrosis. When tumour histology was missing or of poor quality, the mice were excluded from primary tumour histological analysis and this was determined blinded from genotype information. A histological invasion score of the tumour cells into the surrounding stroma was scored on a scale of 0 to 2, with 0 indicating no invasion and 2 indicating high invasion, where invasion is defined as tumour cell dissemination throughout the stroma away from clearly defined epithelial 'nests'. Microscopic metastases were observed in H&E-stained tissue sections of the liver, lung and spleen. Positivity (one or more lesions in a tissue) was confirmed using CK19 and YFP immunohistochemistry. This data has been presented as a contingency table (Fig. 2e) and represented as the number of positive tissues out of the number of tissues scored. The 'Any' metastasis score is the number of mice positive for a secondary lesion found anywhere throughout the body out of the total number of mice scored.

Immunochemistry and Immunofluorescence. Tissues were fixed in 10% formalin overnight, dehydrated, and embedded in paraffin and 5-μm-thick sections were then processed for analyses. Immunohistochemical analysis was performed as described18,35. Heat-mediated antigen retrieval in 1 mM EDTA + 0.05% Tween20 (pH 8.0) for one hour (pressure cooker) was performed for Snail and Twist, 10 mM citrate buffer, pH 6.0, was used for one hour (microwave) for Ki67 or 10 min for all other antibodies. Primary antibodies are as follows: oSMa (M0851, DAKO, 1:400 or ab5694, Abcam, 1:400), cleaved caspase-3 (9661, Cell Signaling, 1:200), CD3 (A0452, DAKO, 1:200), CD31 (Dia310M, DiaNova, 1:10), CK8 (TROMA-1, Developmental Studies Hybridoma Bank, 1:50), CK19 (ab52625, Abcam, 1:100), Cnox (11696, BD Bio, 1:400), SMA, Pimonidazole, Slug (9585, Cell Signaling, 1:200), Snail2 (ab180714, Abcam, 1:100), Zeb1 (ab19930, Abcam, 1:100), Zeb2 (NPBI-05987, Novus, 1:500), and SMA (ab5694, Abcam, 1:400) followed by Alexa Fluor 680 conjugated secondary antibody. Primary antibody staining was observed on a Leica DM1000 light microscope or the Perkin Elmer 3D Histotach Slide Scanner. Fluorescence imagery was obtained on a Zeiss Axio Imager.M2 or the Perkin Elmer Vectra Multispectral imaging platform. The images were quantified for per cent positive area using NIH ImageJ analysis software (oSMa, Pimonidazole, Slug, and CD31), for per cent positive cells using InForm analysis software (Ki67 and CD3), or for scored intensity either positive or negative (oSMa/CK8 dual staining, oSMa, CK19, YFP, Zeb1, Zeb2, Sox2, E-cadherin and cleaved caspase-3) or on a scale of 1–3 (E-cadherin) or 1–4 (ENT1, ENT2 and Cat3).

In situ hybridization. *In situ* hybridization (ISH) was performed on frozen tumour sections as previously described41. In brief, 10-μm-thick sections were hybridized with antisense probes to *Twist1* and *Snail1* overnight at 65 °C. After hybridization, sections were washed and incubated with AP-conjugated sheep anti-DIG antibody (1:2,000; Roche) for 90 min at room temperature. After three washes, sections were incubated in BM Purple (Roche) until positive staining was seen. Digoxigenin-labelled *in situ* riboprobes were generated with an *in vitro* transcription method (Promega and Roche) using a PCR template. The following primers were used to generate the template PCR product. *Twist1*, forward, 5′-CGGCGAAGGTACAGCTGATCT-3′; reverse, 5′-TAATAGGACTCATCAGTATG-3′; *Snail1*, forward, 5′-AACGGGTGTCCGTGAC-3′; reverse, 5′-AAATGACTACTATAGGGACACCTTTAAAAATGACATTTCTCC-3′. Gene expression profiling. Total RNA was isolated from tumours of KPC control, KPC;*Twist1*−/− and KPC;*Snai1*−/− mice (n = 3 in each group) by TRIzol (15596026, Life Technologies) and submitted to the Microarray Core Facility at MD Anderson Cancer Center. Gene expression analysis was performed using MouseWG-6 v2.0 Gene Expression BeadChip (Illumina). The Limma package from R Bioconductor was used for quantile normalization of expression arrays and to analyse differently expressed genes between oKo and control sample groups. Gene expression microarray data have been deposited in GEO (Accession number GSE66981). Genes upregulated in cells acquiring an EMT program were expected to be downregulated in the *Twist1* and *Snai1* tumours compared to control tumours.

CTC assays. Blood (200 μl) was collected from KPC;LSL-YFP and KPC;*Twist1*−/−;LSL-YFP (ROSA-LSL-YFP lineage tracing of cancer cells) mice and incubated with 10 ml of ACK lysis buffer (A1069201, Gibco) at room temperature to lyse red blood cells. Cell pellets were resuspended in 2% FBS containing PBS and analysed for the number of *YFP*+ cells by flow cytometry (BD LSFortessa X-20 Cell Analyzer). The data was expressed as the percentage of *YFP*+ cells from gated cells, with 100,000 cells analysed at the time of acquisition. Whole blood cell pellets were also assayed for the expression of *KrasG12D* transcripts, using quantifying real-time PCR analyses (described below).

Primary pancreatic adenocarcinoma cell culture and analyses. Derivation of primary PDAC cell lines were performed as previously described46. Fresh tumours were minced with sterile razor blades, digested with dispase II (17105041, Gibco, 4 mg ml−1)/collagenase IV (17104019, Gibco, 4 mg ml−1) at 37°C, filtered through a 70 μm cell strainer, resuspended in RPMI20%FBS and then seeded on collagen I-coated plates (087747, Fisher Scientific). Cells were maintained in RPMI medium with 20% FBS and 1% penicillin, streptomycin and amphotericin B (PSA) antibiotic mixture. Cancer cells were further purified by FACS based on YFP or E-cadherin expression (anti-E-cadherin antibody, 50-3249-82, ebioscience, 1:100). The sorted cells, using BD FACSAria™ II sorter (South Campus Flow Cytometry Core Lab of MD Anderson Cancer Center) were subsequently expanded in vitro. All studies were performed on cells cultivated less than 30 passages. As these are primary cell lines, no further authentication methods were applicable and no mycoplasma tests were performed.

MTT and drug sensitivity assays. MTT assay was performed to detect cell proliferation and viability by using Thiazolyl Blue Tetrazolium Bromide (MTT; M2128, Sigma) following the manufacturer's recommendations of two hours at 37°C. For the drug treatment studies, a cell line derived from each of the KPC, KPC;*Snai1*−/− and KPC;*Twist1*−/− mice was treated with 20 μM gemcitabine (G-4177, LC Laboratories) or 100 μM erlotinib (5083S, NEB) for 48 h. The relative cell viability was detected using MTT assay with a cell line derived from each of the KPC, KPC;*Snai1*−/− and KPC;*Twist1*−/− mice. *n* is defined as the number of biological replicates of a single cell line. Control conditions included 1% DMSO vehicle for erlotinib. The relative absorbance was normalized and control (time 0h or vehicle-treated) arbitrarily set to 1 or 100% for absorbance or drug survival, respectively.

Quantitative real-time PCR analyses (qPCR). RNA was extracted from whole blood cell pellets following ACK lysis using the PicoPure Extraction kit as directed (K10204, Arcturus), or from cultured primary pancreatic adenocarcinoma cells using TRIzol (15596026, Life Technologies). cDNA was synthesized using TaqMan Reverse Transcription Reagents (N8080234, Life Technologies)
Primers for KrasG12D recombination are: forward, 5′-ACTTGTGGTGGTTGGACAGC-3′; reverse, 5′-TAGGGTCATACTCATCCACAA-3′. 1/ΔCt values are presented to show KrasG12D expression in indicated experimental groups, statistical analyses were performed on ΔCt. Primer sequences for EMT-related genes are listed in Supplementary Table 1, GAPDH was used as an internal control. The data are presented as the relative fold change and statistical analyses were performed on ΔCt.

Tumour sphere assay. Tumour sphere assays were performed as previously described. Two million cultured primary tumour cells were plated in a low-adherence 100-mm dish (FB0875713, Fisherbrand) with 1% FBS, Dulbecco’s modified Eagle’s medium, and penicillin/streptomycin/amphotericin. Cells were incubated for 7 days and formed spheres were counted at 100× magnification. Three, two and three cell lines were analysed for KPC control, KPC;TwistcKO and KPC;Snail cKO groups, respectively, five field of views per cell line were quantified.

MRI analyses. MRI imaging was performed using a 7T small animal MR system as previously described. To measure tumour volume, suspected regions were drawn blinded on each slice based on normalized intensities. The volume was calculated by the addition of delineated regions of interest in mm² × 1 mm slice distance. None of the mice had a tumour burden that exceeded 1.5 cm in diameter, in accordance with institutional regulations. All mice with measurable tumours were enrolled in the study (see Extended Data Table 3). Mice were imaged twice, once at the beginning of the enrolment (day 0), and a second time 20 days (day 19) afterwards. Surviving animals were euthanized at end point (day 21) for histological characterization.

Statistical analyses. Statistical analyses were performed on the mean values of biological replicates in each group using unpaired two-tailed or one-tailed t-tests (qPCR only), or one-way ANOVA with Tukey’s multiple comparisons test using GraphPad Prism, as stipulated in the figure legends. χ² analyses, using SPSS statistical software, were performed comparing control to cKO groups for metastatic or colonization frequency across multiple histological parameters in all mice and mice ≥120 days of age in Extended Data Table 1. Fisher’s exact P value was used to determine significance. Results are outlined in Extended Data Table 2. Kaplan–Meier plots were drawn for survival analysis and the log rank Mantel-Cox test was used to evaluate statistical differences, using GraphPad Prism. Data met the assumptions of each statistical test, where variance was not equal (determined by an F-test) Welch’s correction for unequal variances was applied. Error bars represent s.e.m. when multiple visual fields were averaged to produce a single value for each animal which was then averaged again to represent the mean bar for the group in each graph.

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Extended Data Figure 1 | EMT inhibition is specific to tumour epithelium. a, Representative images of haematoxylin and eosin-stained small intestine (SmInt), kidney, and heart (scale bar, 100 μm). b, Pancreatic mass of 29 (KPC), 13 (KPC;Twist<sup>KO</sup>) and 28 (KPC;Snail<sup>KO</sup>) mice, error bars represent s.d.; one-way ANOVA. c, Merge of Twist1 or Snail in situ hybridization (black) followed by CK8 (red) immunolabelling in tumours from KPC and KPC;Twist<sup>KO</sup> or KPC;Snail<sup>KO</sup> mice, respectively. White arrows highlight positive cells in the stroma, yellow arrows highlight negative epithelium (scale bar, 20 μm). d, Twist or Snail immunostaining in KPC and KPC;Twist<sup>KO</sup> or KPC;Snail<sup>KO</sup> tumours, respectively. Black arrows highlight positive cells in the stroma, red arrows highlight negative epithelium (scale bar, 20 μm). e, Channel separations of the representative images of αSMA immunolabelling in YFP lineage-traced tumours found in Fig. 1f (scale bar, 50 μm). f, EMT gene expression signature analysis in KPC, KPC;Twist<sup>KO</sup> and KPC;Snail<sup>KO</sup> cohorts (n = 3 mice). Red arrows indicate reduced Twist1 and Snail expression in KPC;Twist<sup>KO</sup> and KPC;Snail<sup>KO</sup> cohorts, respectively.
Extended Data Figure 2 | General suppression of EMT markers does not affect desmoplasia. a, E-cadherin immunolabelling and quantification of primary KPC (n = 5 mice), KPC;Twist\textsuperscript{KO} (n = 5 mice) and KPC;Snail\textsuperscript{KO} (n = 4 mice) (scale bar, 100 μm). b, Zeb2 immunolabelling and quantification of primary KPC (n = 6 mice), KPC;Twist\textsuperscript{KO} (n = 5 mice) and KPC;Snail\textsuperscript{KO} (n = 7 mice) (scale bar, 50 μm; inset scale bar, 20 μm). c, Sox4 immunolabelling and quantification of primary KPC (n = 7 mice), KPC;Twist\textsuperscript{KO} (n = 6 mice) and KPC;Snail\textsuperscript{KO} (n = 8 mice) (scale bar, 50 μm; inset scale bar, 20 μm). d, Slug immunolabelling and quantification of primary KPC (n = 4 mice), KPC;Twist\textsuperscript{KO} (n = 4 mice) and KPC;Snail\textsuperscript{KO} (n = 4 mice) tumours (scale bar, 50 μm; inset scale bar, 20 μm). e, Sirius Red staining and quantification of primary KPC (n = 21 mice), KPC;Twist\textsuperscript{KO} (n = 8 mice) and KPC;Snail\textsuperscript{KO} (n = 11 mice) (scale bar, 100 μm; error bars represent s.d.) f, αSMA immunolabelling and quantification of primary KPC (n = 5 mice), KPC;Twist\textsuperscript{KO} (n = 5 mice) and KPC;Snail\textsuperscript{KO} (n = 5 mice) (scale bar, 100 μm). g, CD31 immunolabelling and quantification of primary KPC (n = 4 mice), KPC;Twist\textsuperscript{KO} (n = 4 mice) and KPC;Snail\textsuperscript{KO} (n = 3 mice) (scale bar, 200 μm, inset scale bar, 100 μm). h, Pimonidazole staining and quantification of primary KPC (n = 4 mice), KPC;Twist\textsuperscript{KO} (n = 4 mice) and KPC;Snail\textsuperscript{KO} (n = 4 mice) (scale bar, 100 μm). i, CD3 immunolabelling and quantification of primary KPC (n = 5 mice), KPC;Twist\textsuperscript{KO} (n = 5 mice) and KPC;Snail\textsuperscript{KO} (n = 5 mice) (scale bar, 100 μm; inset scale bar, 25 μm). Unless otherwise indicated error bars represent s.e.m., and significance determined by one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001; ns, not significant.
Extended Data Figure 3 | EMT suppression does not alter epithelial characteristics of metastases. a, Immunolabelling of primary tumours (n = 3 mice) for αSMA (red), CK8 (green), Ki67 (white) and DAPI (blue); yellow arrows indicate EMT+ cells (scale bar, 20 μm). b, Representative dot plots of circulating YFP+ cells (scale bar, 20 μm). c, Images of serial sections of KPC;LSL-YFP lung and liver metastasis stained for haematoxylin and eosin or immunolabelled for CK19 or YFP. Yellow dashed box represents magnified areas in panel below (scale bar, 200 μm; magnification scale bar, 50 μm). d, KPC metastatic tumours stained for Twist and Snail (n = 3 mice; scale bar, 20 μm; inset scale bar, 10 μm). e, Zeb1 immunolabelling and quantification of metastatic KPC (n = 4 mice), KPC;TwistKO (n = 3 mice) and KPC;SnailKO (n = 4 mice) (scale bar, 50 μm; inset scale bar, 20 μm). f, αSMA immunolabelling and quantification of metastatic KPC (n = 3 mice), KPC;TwistKO (n = 3 mice) and KPC;SnailKO (n = 3 mice) (scale bar, 50 μm; inset scale bar, 20 μm). g, E-cadherin staining on serial sections of αSMA immunolabelling and quantification of metastatic KPC (n = 4 mice), KPC;TwistKO (n = 3 mice) and KPC;SnailKO (n = 4 mice) (scale bar, 50 μm; inset scale bar, 20 μm). h, Ki67 immunolabelling and quantification of metastatic KPC (n = 7 mice), KPC;TwistKO (n = 3 mice) and KPC;SnailKO (n = 3 mice) (scale bar, 50 μm; inset scale bar, 20 μm). Unless otherwise indicated error bars represent s.e.m., percentages indicated represent percent decrease from control, and significance was determined by one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001; ns, not significant.
Extended Data Figure 4 | EMT suppressed primary tumour cells have reduced mesenchymal markers and show resistance to chemotherapy in vitro. a, Bright-field micrograph of cultured primary KPC, KPC;Twist$^{cKO}$ and KPC;Snail$^{cKO}$ cells (scale bar, 50 μm). b, EMT- and gemcitabine-transport-related gene expression shown by qPCR analysis in KPC ($n=3–4$ cell lines), KPC;Twist$^{cKO}$ ($n=5$ cell lines) and KPC;Snail$^{cKO}$ ($n=5–6$ cell lines) (error bars represent s.d., one-tailed t-test, *$P<0.05$, numbers list non-significant $P$ values. nd, not detected, ns, not significant). c, MTT assay showing cell proliferation in KPC, KPC;Twist$^{cKO}$ and KPC;Snail$^{cKO}$ cells ($n=8$, 8 and 8 biological replicates of a cell line for each genotype). d, Relative cell viability (MTT assay) in cultured KPC, KPC;Twist$^{cKO}$ and KPC;Snail$^{cKO}$ cells treated with gemcitabine or erlotinib ($n=8$, 8 and 8 biological replicates of a cell line for each genotype). Unless otherwise indicated error bars represent s.e.m., significance was determined by one-way ANOVA. **$P<0.01$, ***$P<0.001$, ****$P<0.0001$. 
**Extended Data Figure 5** | EMT inhibition in KTC mice mirrors phenotype observed in KPC mice.

**a.** Representative images of haematoxylin and eosin-stained primary tumours (scale bar, 100 μm).

**b.** Relative percentage of each histological tissue phenotype of KTC (n = 8 mice) and KTC;Snail^KO^ (n = 6 mice) primary tumours (error bars represent s.d.).

**c.** Primary tumour invasiveness in KTC (n = 8 mice) and KTC;Snail^KO^ (n = 6 mice) (error bars represent s.d.).

**d.** Pancreatic mass in KTC (n = 5 mice) and KTC;Snail^KO^ (n = 6 mice) (error bars represent s.d.).

**e.** Immunolabelling and quantification of primary KTC (n = 5 mice), KTC;Snail^KO^ (n = 4 mice) for αSMA (red), CK8 (green) and DAPI (blue); white arrows indicate double-positive cells (scale bar, 20 μm); Zeb1 (scale bar, 50 μm; inset scale bar, 20 μm), cleaved caspase-3 (scale bar, 50 μm; n = 4 mice for both groups); K67 (scale bar, 100 μm), ENT2 (scale bar, 100 μm) and CNT3 (scale bar, 100 μm); error bars represent s.e.m. Significance was determined by two-tailed t-test. *P < 0.05, ***P < 0.001; ns, not significant.
Extended Data Figure 6 | Desmoplasia is unaffected in EMT suppressed tumours with or without gemcitabine. a, b, Staining and quantification of KTC (n = 5 or 6 mice), KTC;Snail<sup>cKO</sup> (n = 4 or 5 mice), KTC plus gemcitabine (+ GEM; n = 4 or 5 mice), KTC;Snail<sup>cKO</sup> + GEM (n = 5 mice) for Masson's trichrome stain (MTS) (scale bars, 100 μm), Sirius Red staining (scale bars, 100 μm), and ENT1 (scale bars, 100 μm). Error bars represent s.d. (MTS and Sirius Red) or s.e.m. (ENT1), and significance was determined by two-tailed t-test. ns, not significant.
## Extended Data Table 1 | Pathological spectrum of primary disease and metastasis in KPC, KPC;TwistcKO and KPC;SnailcKO cohorts

| Pathological Spectrum within cohorts | KPC (104) | TwistcKO (111) | SnailcKO (103) |
|-------------------------------------|-----------|----------------|----------------|
|                                     | AGE | PDA | Differentiation | Histology 1 | Histology 2 | Liver | Lung | Spleen | Any | Moribund |
|-------------------------------------|-----|-----|----------------|------------|------------|-------|------|--------|-----|----------|
| ID                                  |     |     |                |            |            |       |      |        |     |          |
| 1                                   | 158 | Y   | W              | S          | G          | Y     | Y    | Y      | N   | Y        |
| 2                                   | 165 | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| 3                                   | 148 | Y   | P              | S          | G          | N     | N    | N      | N   | Y        |
| 4                                   | 135 | M   | S              | G          | Y          | N     | Y    | Y      | N   | Y        |
| 5                                   | 95  | Y   | M              | G          | N          | Y     | N    | N      | N   | N        |
| 6                                   | 42  | Y   | M              | G          | N          | N     | N    | N      | N   | N        |
| 7                                   | 55  | Y   | P              | G          | S          | Y     | N    | N      | N   | Y        |
| 8                                   | 91  | Y   | M              | G          | N          | N     | N    | N      | N   | N        |
| 9                                   | 87  | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| 10                                  | 63  | Y   | P              | G          | Y          | Y     | Y    | Y      | N   | N        |
| 11                                  | 108 | Y   | P              | S          | G          | Y     | N    | N      | N   | Y        |
| 12                                  | 110 | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| 13                                  | 104 | Y   | W              | G          | Y          | N     | N    | N      | N   | Y        |
| 14                                  | 54  | Y   | W              | S          | G          | N     | N    | N      | N   | N        |
| 15                                  | 108 | Y   | P              | S          | G          | N     | N    | N      | N   | Y        |
| 16                                  | 115 | Y   | W              | G          | Y          | N     | N    | N      | N   | N        |
| 17                                  | 68  | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| 18                                  | 107 | Y   | P              | G          | N          | N     | N    | N      | N   | N        |
| 19                                  | 87  | Y   | P              | G          | N          | N     | N    | N      | N   | N        |
| 20                                  | 48  | Y   | P              | G          | N          | N     | N    | N      | N   | N        |
| 21                                  | 109 | Y   | P              | G          | S          | Y     | Y    | Y      | N   | Y        |
| 22                                  | 81  | Y   | P              | G          | N          | Y     | N    | N      | N   | N        |
| 23                                  | 151 | Y   | W              | G          | N          | Y     | N    | N      | N   | N        |
| 24                                  | 47  | Y   | M              | G          | S          | N     | Y    | N      | N   | Y        |
| 25                                  | 143 | Y   | P              | G          | S          | N     | Y    | N      | N   | Y        |
| 26                                  | 122 | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| 27                                  | 115 | Y   | P              | G          | Y          | N     | N    | N      | N   | N        |
| 28                                  | 122 | Y   | W              | G          | N          | Y     | N    | Y      | N   | N        |
| 29                                  | 122 | Y   | M              | S          | G          | Y     | N    | N      | Y   | Y        |
| 30                                  | 97  | Y   | P              | G          | N          | N     | N    | N      | N   | N        |
| 31                                  | 107 | Y   | W              | G          | N          | N     | N    | N      | N   | N        |
| **Totals (Median)**                 |     |     |                | 31/31      |            | 11/31 | 11/31 | 2/30   | 17/31|          |
|                                     | %   |     |                | 100.0%     |            | 35.5% | 35.5% | 6.7%   | 54.8%|          |
| **TwistcKO (111)**                 |     |     |                | 14/14      |            | 6/14  | 4/14  | 2/14   | 8/14 |          |
|                                     | %   |     |                | 100.0%     |            | 42.9% | 28.6% | 14.3%  | 57.1%|          |
| **SnailcKO (103)**                 |     |     |                | 30/30      |            | 13/30 | 9/30  | 5/29   | 18/30|          |
|                                     | %   |     |                | 100.0%     |            | 43.3% | 30.0% | 17.2%  | 60.0%|          |

Y, yes; N, no; W, well; M, moderate; P, poor; G, glandular; S, sarcomatoid; FD, found dead; –, no tissue.
Extended Data Table 2 | Results of $\chi^2$ analysis of KPC cohorts in Extended Data Table 1

| $\chi^2$ Analysis | Parameter                   | Fisher's Exact $P$ value |
|--------------------|-----------------------------|--------------------------|
| **Differentiation**| All Ages                    |                          |
| Control vs. Twist$^{\text{KO}}$ | Early Tumor progression    | 0.458                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.106                    |
| Control vs. Twist$^{\text{KO}}$  | Late Tumor progression      | 0.458                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.106                    |
| Control vs. Twist$^{\text{KO}}$  | Sarcomatoid                 | 0.108                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.446                    |
| **Differentiation**| $\geq$ 120 days             |                          |
| Control vs. Twist$^{\text{KO}}$ | Early Tumor progression    | 0.580                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.569                    |
| Control vs. Twist$^{\text{KO}}$  | Late Tumor progression      | 0.580                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.569                    |
| Control vs. Twist$^{\text{KO}}$  | Sarcomatoid                 | 1.000                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.119                    |
| **Metastasis**     | All Ages                    |                          |
| Control vs. Twist$^{\text{KO}}$ | Liver Metastasis            | 0.744                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.605                    |
| Control vs. Twist$^{\text{KO}}$  | Lung Metastasis             | 0.743                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.786                    |
| Control vs. Twist$^{\text{KO}}$  | Spleen Invasion             | 0.581                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.254                    |
| Control vs. Twist$^{\text{KO}}$  | Any Metastasis              | 1.000                    |
| Control vs. Snail$^{\text{KO}}$  |                             | 0.797                    |
| **Metastasis**     | $\geq$ 120 days             |                          |
| Control vs. Twist$^{\text{KO}}$ | Liver Metastasis            | 0.627                    |
| Control vs. SnailcKO |                             | 1.000                    |
| Control vs. Twist$^{\text{KO}}$  | Lung Metastasis             | 0.592                    |
| Control vs. SnailcKO |                             | 1.000                    |
| Control vs. Twist$^{\text{KO}}$  | Spleen Invasion             | 0.559                    |
| Control vs. SnailcKO |                             | 1.000                    |
| Control vs. Twist$^{\text{KO}}$  | Any Metastasis              | 0.473                    |
| Control vs. SnailcKO |                             | 0.608                    |
## Extended Data Table 3 | Survival and primary tumour burden determined by MRI in KPC, KPC;Twist<sup>−/−</sup> and KPC;Snail<sup>−/−</sup> cohorts treated with gemcitabine

### KPC Gemcitabine cohorts

| ID  | Start Age (Days) | Start Volume (mm<sup>3</sup>) | End Volume (mm<sup>3</sup>) | Survival (Days) |
|-----|------------------|-------------------------------|------------------------------|-----------------|
| KPC + GEM | (89) |                      |                              |                |
| 1   | 148              | 1610.4                        | D                            | 7               |
| 2   | 72               | 29.7                          | D                            | 13              |
| 3   | 72               | 439.8                         | 902.8                        | 21*             |
| 4   | 80               | 44.1                          | D                            | 14              |
| 5   | 100              | 536.3                         | 592.3                        | 21*             |
| 6   | 89               | 167.0                         | D                            | 2               |
| 7   | 94               | 52.7                          | D                            | 7               |
| 8   | 122              | 90.2                          | D                            | 14              |
| 9   | 164              | 217.9                         | D                            | 8               |
| 10  | 143              | 212.8                         | D                            | 18              |
| 11  | 84               | 323.8                         | 897.2                        | 21*             |
| 12  | 58               | 76.7                          | D                            | 4               |
| 13  | 58               | 116.2                         | D                            | 8               |
| Mean | (Median)        | 301.4                         | 797.4                        |                |
| Stdev |                  | 406.9                         | 145.1                        |                |

### Twist<sup>−/−</sup> + GEM (79)

| ID  | Start Age (Days) | Start Volume (mm<sup>3</sup>) | End Volume (mm<sup>3</sup>) | Survival (Days) |
|-----|------------------|-------------------------------|------------------------------|-----------------|
| 1   | 117              | 243.0                         | 644.2                        | 21*             |
| 2   | 75               | 47.2                          | 180.0                        | 21*             |
| 3   | 75               | 45.4                          | 460.9                        | 21*             |
| 4   | 78               | 54.6                          | 47.5                         | 21*             |
| 5   | 46               | 53.7                          | 66.5                         | 21              |
| 6   | 96               | 63.1                          | D                            | 13              |
| 7   | 90               | 23.9                          | D                            | 13              |
| 8   | 79               | 101.0                         | D                            | 14              |
| 9   | 52               | 28.5                          | D                            | 14              |
| 10  | 52               | 49.4                          | 98.706                       | 21*             |
| 11  | 104              | 43.4                          | 127.0                        | 21*             |
| 12  | 104              | 53.5                          | 12.1                         | 21*             |
| 13  | 68               | 56.7                          | D                            | 15              |
| 14  | 122              | 650.1                         | 164.1                        | 21*             |
| 15  | 104              | 181.8                         | 78.6                         | 21*             |
| Mean | (Median)        | 113.0                         | 187.9                        |                |
| Stdev |                  | 154.8                         | 193.0                        |                |

### Snail<sup>−/−</sup> + GEM (96)

| ID  | Start Age (Days) | Start Volume (mm<sup>3</sup>) | End Volume (mm<sup>3</sup>) | Survival (Days) |
|-----|------------------|-------------------------------|------------------------------|-----------------|
| 1   | 188              | 255.2                         | D                            | 12              |
| 2   | 181              | 854.7                         | D                            | 4               |
| 3   | 127              | 32.0                          | 59.6                         | 21*             |
| 4   | 127              | 58.7                          | 107.4                        | 21*             |
| 5   | 142              | 109.8                         | D                            | 14              |
| 6   | 54               | 33.6                          | 57.2                         | 21*             |
| 7   | 89               | 17.0                          | D                            | 13              |
| 8   | 78               | 54.9                          | 39.6                         | 21*             |
| 9   | 78               | 3.1                           | D                            | 15              |
| 10  | 104              | 209.7                         | 134.3                        | 21*             |
| 11  | 96               | 220.0                         | 280.2                        | 21*             |
| 12  | 96               | 24.1                          | 46.2                         | 21*             |
| 13  | 119              | 711.0                         | D                            | 18              |
| 14  | 126              | 655.6                         | 805.4                        | 21*             |
| 15  | 119              | 168.6                         | D                            | 18              |
| 16  | 82               | 453.8                         | 517.4                        | 21*             |
| 17  | 82               | 56.7                          | 74.1                         | 21*             |
| 18  | 90               | 40.0                          | D                            | 16              |
| 19  | 67               | 80.5                          | D                            | 10              |
| 20  | 66               | 49.5                          | 226.2                        | 21*             |
| Mean | (Median)        | 204.4                         | 213.4                        |                |
| Stdev |                  | 250.7                         | 231.7                        |                |

D, died; *euthanized at end point.