Supplementary information

Contents

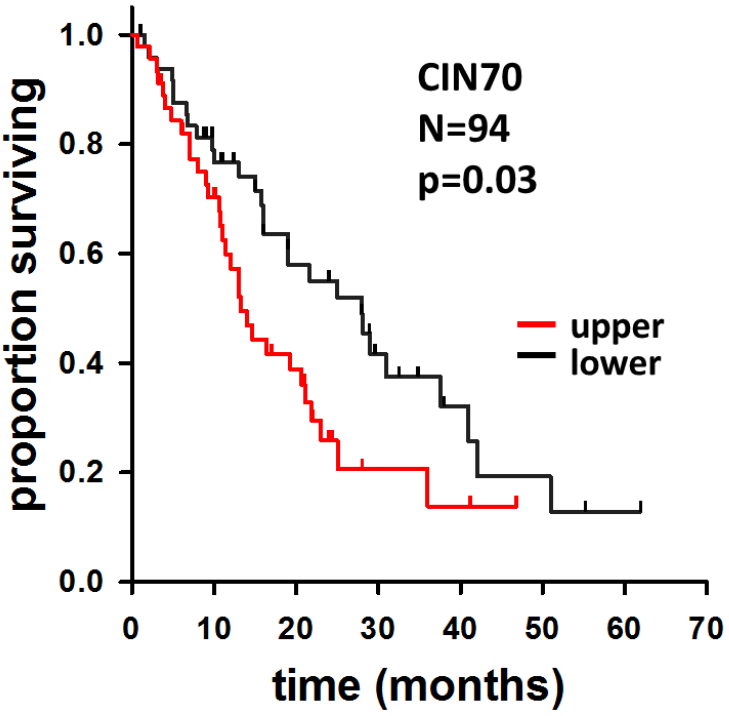

Figure S1 The CIN70 signature predicts survival in PDAC patients. Upper and lower refer to patients in upper and lower halves of the CIN70 score distribution (see methods for details). Upper: median survival was 28 months. Lower: median survival was 13.3 months. P value refers to log-rank test comparing survival curves.

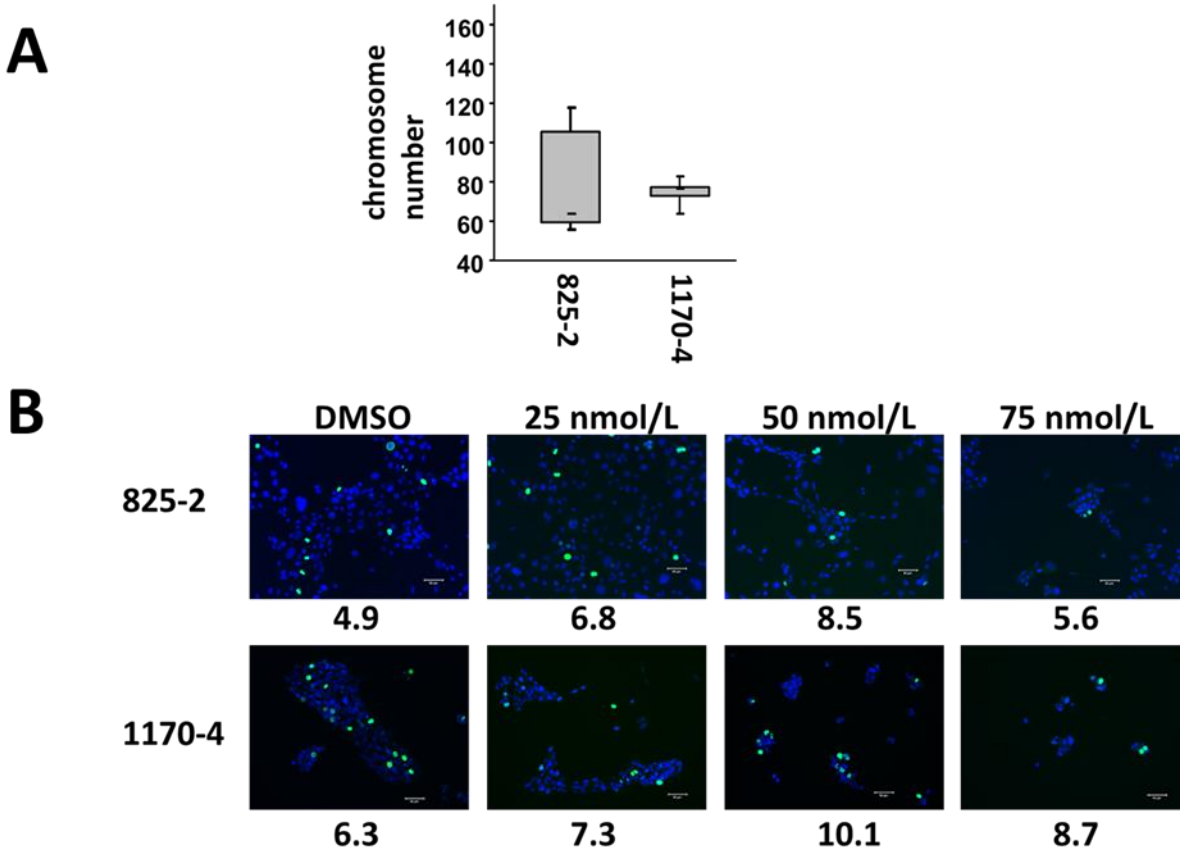

Figure S2 Murine PDAC KRC cells exhibit chromosome instability and evidence of a weakened SAC. A. Box whisker plots of chromosome number in ≥10 metaphase chromosome spreads prepared from untreated 825-2 and 1170-4 cells. Central band and lower and upper box limits denote median, first and third quartiles, respectively. Whiskers denote data within 1.5 interquartiles of upper and lower box limits. **B.** 825 and 1170-4 KRC cells were incubated with DMSO control or nocodazole at the indicated concentrations for ~18h after which the cells were fixed and incubated with a pS10H3 antibody (green). Nuclei are stained with DAPI (blue). Representative images from each treatment are shown. The percentage of cells (n≥200/sample) with positive pS10H3 signals is indicated below each panel and did not differ significantly from the control ($p>0.05$; χ^2 test). Cells decreased in number with increasing nocodazole concentration and no cells survived at >100 nM nocodazole (not shown).

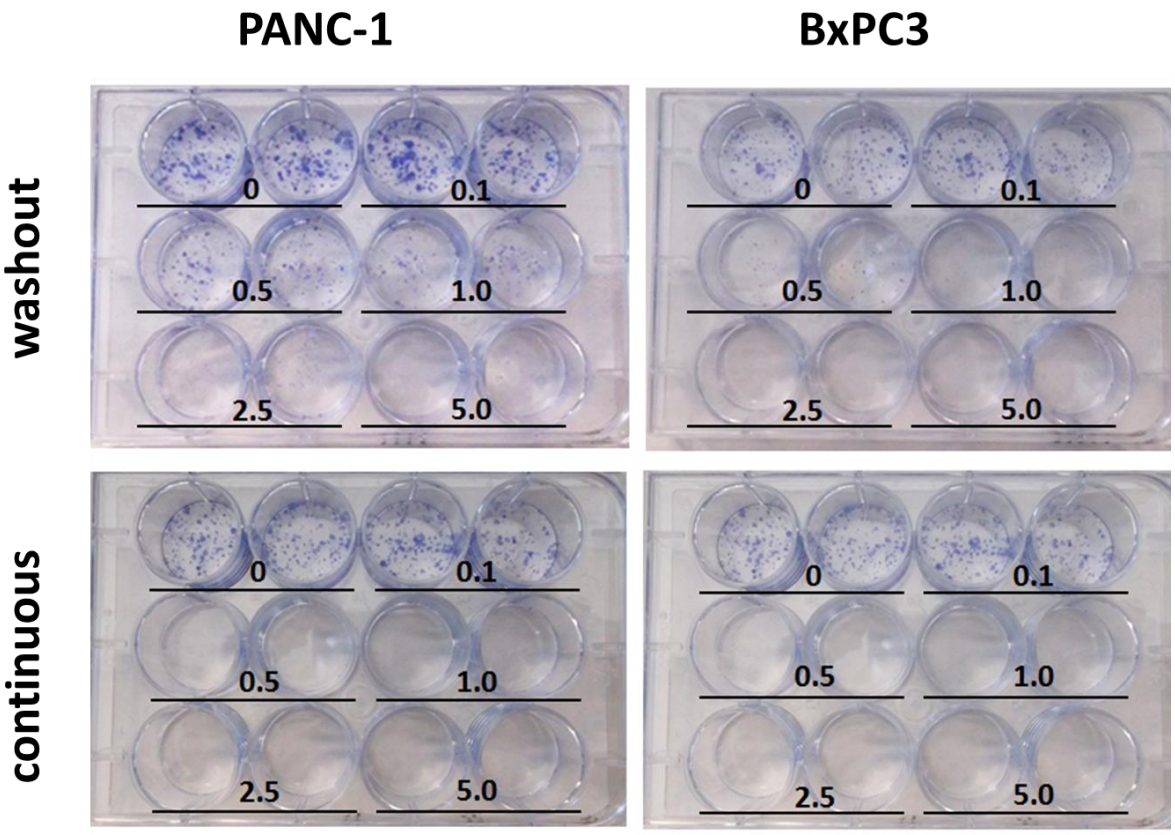

Figure S3 Clonogenic survival of PANC-1 and BxPC3 cells treated with NMS-P715. PANC-1 and BXPC3 cells were plated at 500 and 1000 cells, respectively into each well. 24h later, NMS-P715 was added at the indicated concentration (µmol/L). Control (0) was supplemented with vehicle (DMSO). In the washout experiment, NMS-P715 was removed after 24h and cells were grown in drug free medium for 8 days. For continuous treatment, cells were treated with NMS-P715 for 9 days.

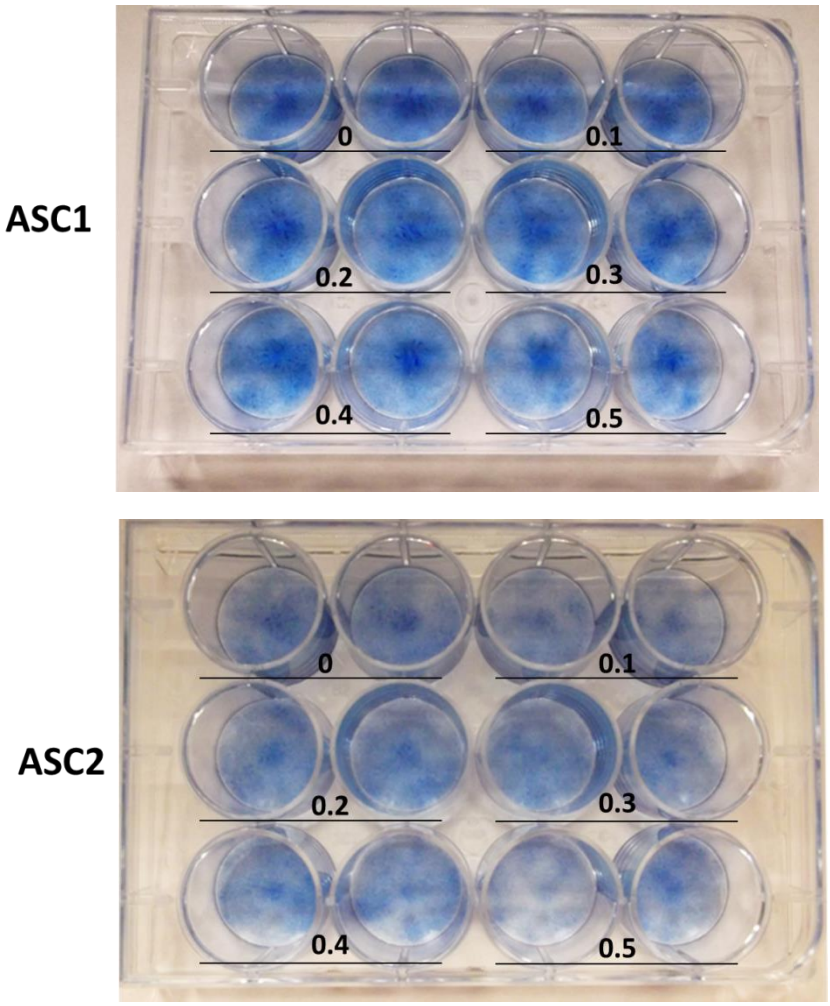

Figure S4 Growth of human adipose stem cells (ASCs) in the presence of NMS-P715. Two ASC isolates (ASC1 and ASC2) cultured in NMS-P715 for 6 days at the indicated concentrations (µmol/L). Proliferation was measured by a colorimetric assay (see Fig. 4).

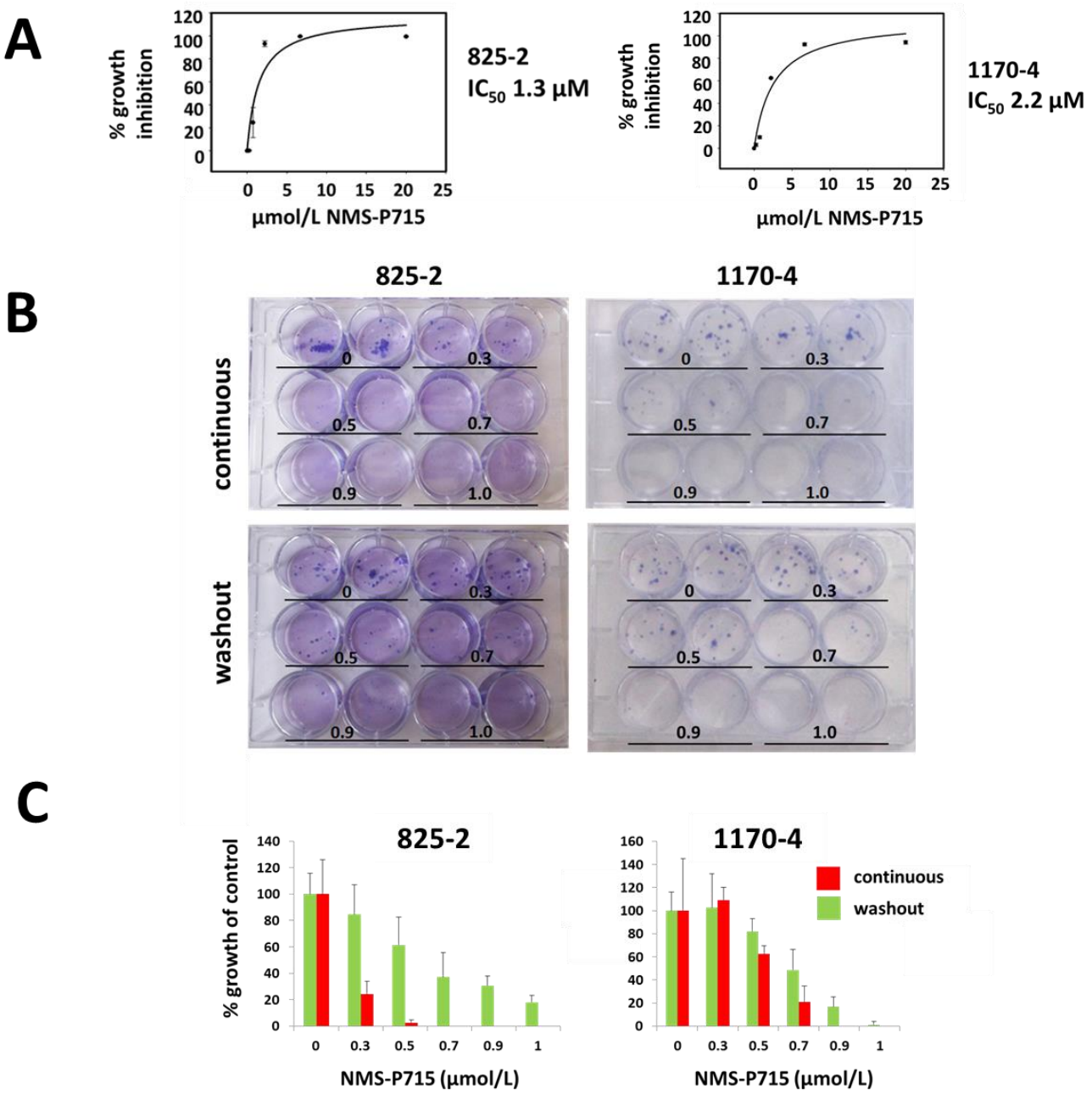

Figure S5 Murine PDAC KRC cell growth is inhibited by NMS-P715. A. Dose effect of NMS-P715 on KRC cell proliferation. KRC cell lines 825-2 and 1170-4 were seeded into 96 well plates. 24h later, NMS-P715 was added at the indicated doses. Cells were incubated for 72h and proliferation measured using colorimetric assays. **B, C.** Clonogenic survival assays: **B.** Murine 825 or 1170-4 cells were seeded at 100 or 50 cells/ well, respectively and allowed to attach for 24h, after which NMS-P715 was added at the indicated concentrations. In the washout, compound was removed after 24h and cells were cultured for a further 4 days in

compound-free medium. In the continuous treatment, cells were cultured for 5 days. **C.** Colonies were stained with giemsa and those with ≥50 cells were counted. The percentage of colonies surviving in the treatment groups was measured relative to the DMSO control. Data in each graph were generated from 2 replicate plates per treatment group.

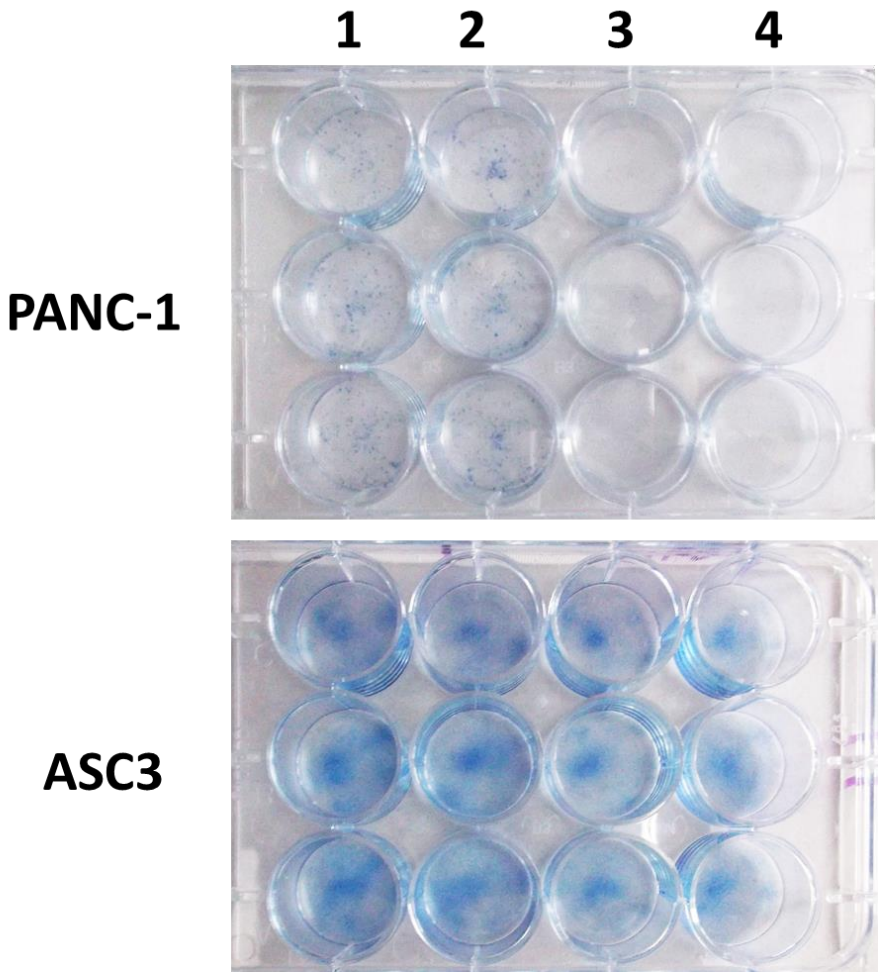

Figure S6 Inhibition of PDAC cell growth after pre-treatment with NMS-P715. PANC-1 and ASC3 cells were cultured with 1 µmol/L NMS-P715 or control medium in duplicate. After 72h, cells were harvested and processed for FISH (Fig. 5A) or counted and plated at 400 cells/ well in triplicate in compound-free medium in a 12 well dish to measure effects on growth (shown above). Columns 1-4 (above) contain cells that were either untreated (1, 2) or treated (3, 4) plated in triplicate and grown for 6 days. ASC4 cell growth was similar to ASC3 in this assay (data not shown). Growth was quantified by a colorimetric assay (Fig. 5B).

Table S1 Chromosome stability in cell lines.

Cell line	Modal chromosome number/ range	Structural aberrations	[4]Basal %MD human chromosome X	[4]Basal %MD human chromosome 17	Average %MD for human chromosomes X and 17	[4]Basal %MD murine chromosome 11
BxPC-3[1]	55 (48-55) [1]	yes[1]	21.4±2.4	10.5±1.27	15.95	n/a
PANC-1[1]	59 (56-60) [1]	yes[1]	13.9 ±2.6	11.9±6.8	12.9	n/a
ASC-3	nd	nd	3.1±1.46	4.25±3.3	3.67	n/a
ASC-4	nd	nd	1.3±1.86	2.1±0.7	1.7	n/a
825-2	62 (56-112)[2]	yes[2]	n/a	n/a	n/a	65 +/-9.9
1170-4	77 (64-83)[2]	yes[2]	n/a	n/a	n/a	49 +/-1.4
hTERT-HPNE	46[3]	1 aberration[3]	4±0	8±0	6	n/a

[1] Chromosome number and structure information are reported in Sirivatanauksorn et al., Non-random chromosomal rearrangements in pancreatic cancer cell lines identified by spectral karyotyping. Int. J. Cancer. 2001;91:350-358. [2]Based on analyses of 10 chromosome spreads/ cell line. See also Fig. S2. Marker chromosomes and Robertsonian fusion chromosomes were observed. [3] Information supplied by ATCC. ~50% of the cells harbored a derivative chromosome 21 with additional material at p12. [4] Basal %MD (% modal deviation) is based on FISH analysis of vehicle control cells presented in Fig.3 and Fig. 5. nd=not done. n/a=not applicable.

Table S2 PDAC CIN25 genes ranked by Cox score[1]

Gene	Cox score	p value
CDC45	20.986	<0.001
NCAPH	18.106	<0.001
TRIP13	15.863	<0.001
ESPL1	14.98	<0.001
KIF20A	14.934	<0.001
KIF4A	14.775	<0.001
[3]PTTG1	14.399	<0.001
[3]AURKB	14.386	<0.001
TPX2	13.577	<0.001
TOP2A	12.219	<0.001
CDC6	12.16	<0.001
RAD51AP1	12.103	<0.001
[3]MPS1	11.845	<0.001
[2]MCM10	10.237	0.001
ASF1B	10.197	0.001
[3]ZWINT	10.066	0.002
[3]CDC20	9.934	0.002
UBE2C	9.742	0.002
PBK	9.701	0.002
CDCA3	9.467	0.002
FEN1	9.356	0.002
[3]ZWILCH	9.162	0.002
RRM2	9.15	0.002
[2]NCAPD2	8.895	0.003
[2]UNG	8.678	0.003

[1] A Wald test was used to calculate the Cox score. [2]These genes did not conform to the proportional hazard assumption of the Cox model. [3]SAC components and associated proteins (see Vleugel M, Hoogendoorn E, Snel B, Kops GJ. Evolution and function of the mitotic checkpoint. Dev Cell 2012;23:239-50).

Supplementary methods for gene expression and survival analyses

Three resected PDAC gene expression microarray datasets were downloaded from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo). GSE17891 comprised 25 primary tumors analyzed on the Affymetrix HGU133 Plus 2.0 platform and normalized by RMA (1). GSE32676 comprised 27 primary tumors analyzed on the Affymetrix HGU133 Plus 2.0 platform and normalized by GCRMA (2). GSE28735 comprised 45 pairs of primary tumor and adjacent normal tissue analyzed on the Affymetrix 1.0 ST platform and normalized by RMA (3). Patient survival data were either available at the Gene Expression Omnibus (GSE17891, GSE28735) or obtained from the authors (GSE32676). Comparison of gene expression in PDAC and normal pancreas was performed on the RMA normalized GSE28735 dataset. For data merging, each dataset was first standardized by converting gene expression values into z scores with mean 0 and standard deviation 1 across samples. For GSE28735, standardization included only tumor samples. Z-score standardization, further described in Chen et al 2008 (4), is a method to permit merging of microarray datasets by mitigating batch effects. Microarray analysis was performed with Genespring 12.5 software (Agilent Technologies). CIN70 and PDAC CIN25 scores were derived by summing the z scores of constituent genes in each sample. For Fig. 1D, MPS1 expression levels in PDAC cell lines (n=3 replicates/ sample) and human pancreatic ductal epithelial cells (n= 6; 2 isolates in triplicate) were derived from the publically available microarray dataset GSE 45765 (5).

Statistical analysis

Univariate Cox proportional hazards and Kaplan Meier analysis was performed in Sigmaplot 12.3 using censored data with disease specific survival as the outcome metric. Statistical significance of differential gene expression was assessed by 2-sided, paired or moderated (6) t-test with Benjamini-Hochberg multiple testing correction in Genespring 12.5.

References for Supplementary Methods

1. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011;17:500-3.

2. Donahue TR, Tran LM, Hill R, Li Y, Kovochich A, Calvopina JH, et al. Integrative survival-based molecular profiling of human pancreatic cancer. Clin Cancer Res. 2012;18:1352-63.

3. Zhang G, Schetter A, He P, Funamizu N, Gaedcke J, Ghadimi BM, et al. DPEP1 inhibits tumor cell invasiveness, enhances chemosensitivity and predicts clinical outcome in pancreatic ductal adenocarcinoma. PLoS One. 2012;7:e31507.

4. Chen QR, Song YK, Wei JS, Bilke S, Asgharzadeh S, Seeger RC, et al. An integrated cross-platform prognosis study on neuroblastoma patients. Genomics. 2008;92:195-203.

5. Gysin S, Paquette J, McMahon M. Analysis of mRNA profiles after MEK1/2 inhibition in human pancreatic cancer cell lines reveals pathways involved in drug sensitivity. Mol Cancer Res. 2012;10:1607-19.

6. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol. 2004;3:Article3.