# Kallikrein-Related Peptidase 6 Is Associated with the Tumour Microenvironment of Pancreatic Ductal Adenocarcinoma

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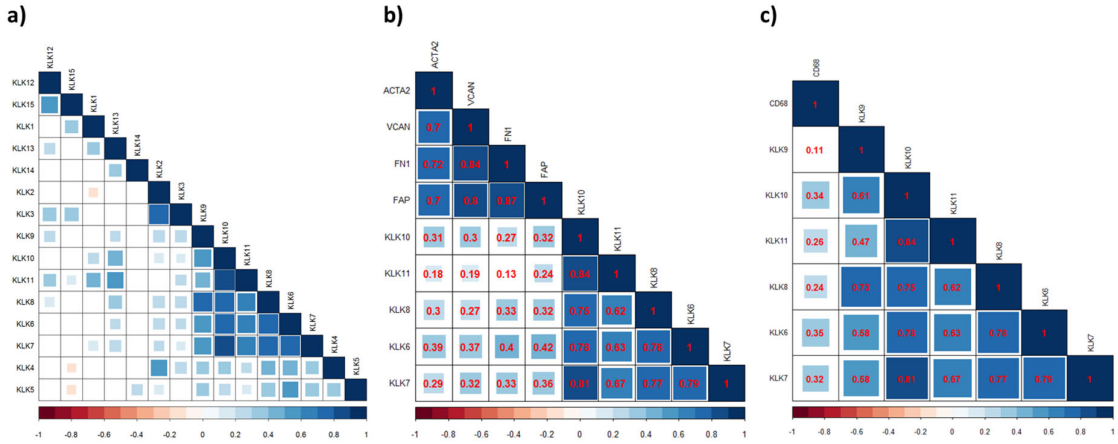


**Figure S1.** KLK co-expression using the TCGA PDAC dataset. (**a**) Correlation analysis of the 15 members of the KLK family indicating the PDAC-specific KLK cluster that includes KLK6, KLK7, KLK8, KLK10 and KLK11. (**b**) Out of the PDAC-specific KLK cluster, KLK6 correlated with tumour-associated genes present in the stroma. (**c**) Out of the PDAC-specific KLK cluster, KLK6 correlated with the tumour-associated macrophage marker CD68.

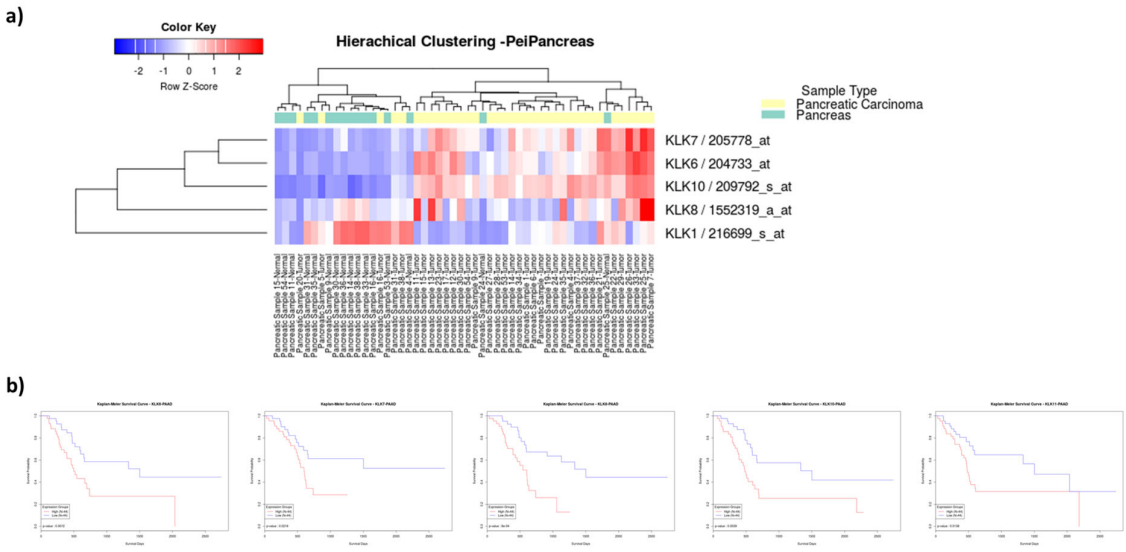


**Figure S2.** KLK co-expression using the KLK-CANMAP. (**a**) KLK6, KLK7, KLK8 and KLK10 were identified as highly upregulated in PDAC compared to normal pancreas tissues, which showed an upregulation of KLK1. (**b**) Kaplan-Meier analysis indicated that high KLK6 ($p$ = 0.0072), KLK7 ($p$ =

0.0216), KLK8 ($p$ = 0.0008), KLK10 ($p$ = 0.0039) and KLK11 ($p$ = 0.0138) expression levels were significantly associated with a shorter 5-year survival than low expression levels.

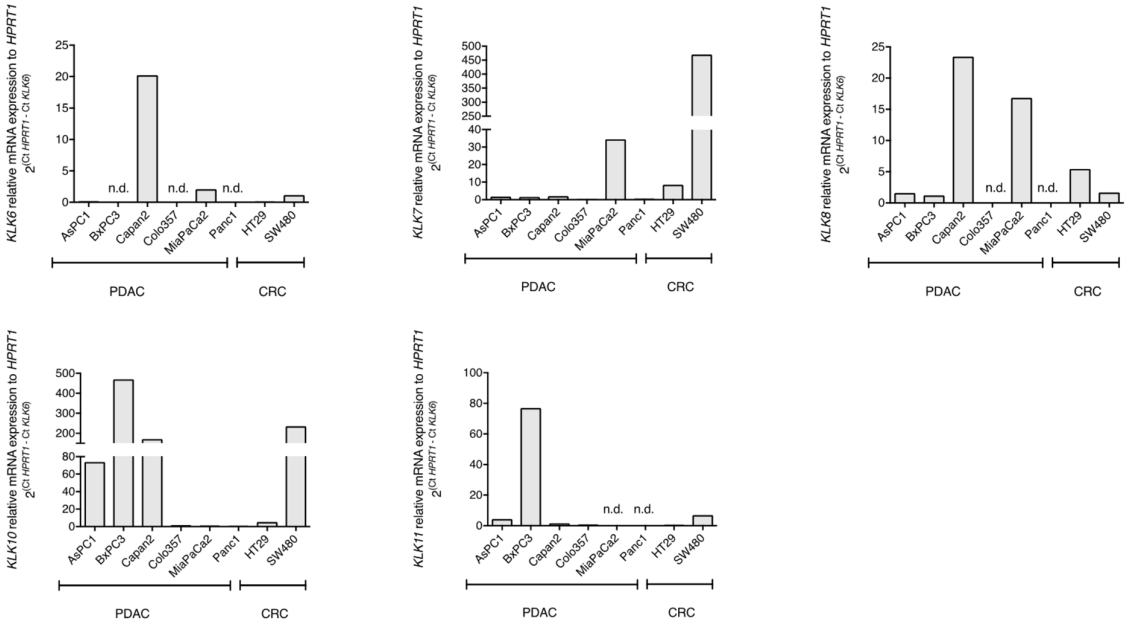


**Figure S3.** KLK mRNA expression in human cancer cells. Gene expression analysis of KLK6, KLK7, KLK8, KLK10 and KLK11 in human pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC) cells. n.d., not determined.

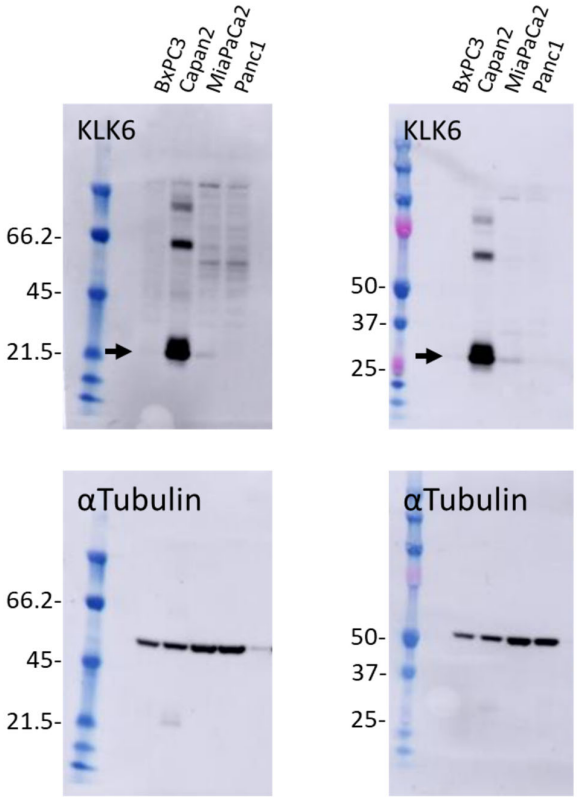


**Figure S4.** KLK6 protein expression in human pancreatic ductal adenocarcinoma cells. Protein expression analysis of KLK6 (arrows; 26.8 kDa) in whole-cell lysates using two different molecular weight ladders.

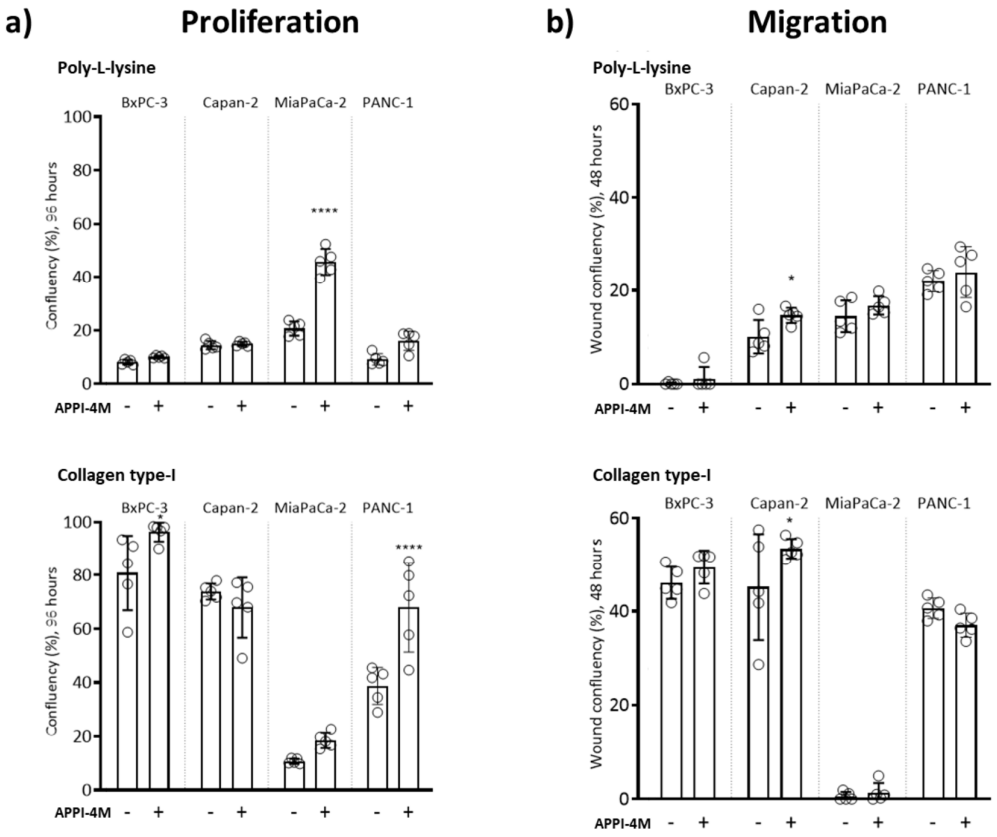


**Figure S5**. Effect of the APPI-4M KLK6 inhibitor on tumour cell proliferation and migration. (**a**) Incucyte proliferation analysis of human pancreatic ductal adenocarcinoma cells using poly-L-lysine and collagen type-I-coated plates after 96 hours ± KLK6 inhibition. (**b**) Incucyte migration analysis of human pancreatic ductal adenocarcinoma cells using poly-L-lysine and collagen type-I-coated plates after 48 hours ± KLK6 inhibition.

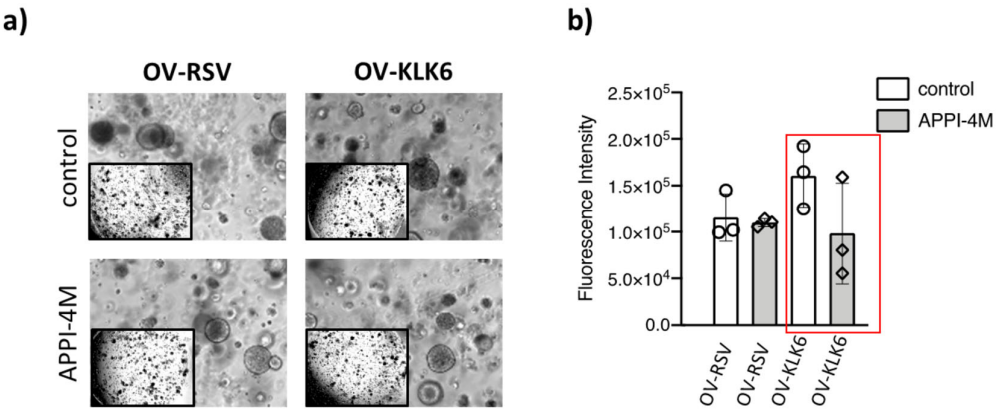


**Figure S6.** Effect of the APPI-4M KLK6 inhibitor on tumour spheroids. (**a**) Representative brightfield micrographs of ovarian cancer cells grown in polyethylene glycol-based hydrogels over 14 days ± KLK6 inhibition; magnification 20×, insert 4×. (**b**) KLK6 inhibition reduced the metabolic activity of KLK6-expressing tumour spheroids (OV-KLK6; red box) compared to vector controls (OV-RSV).

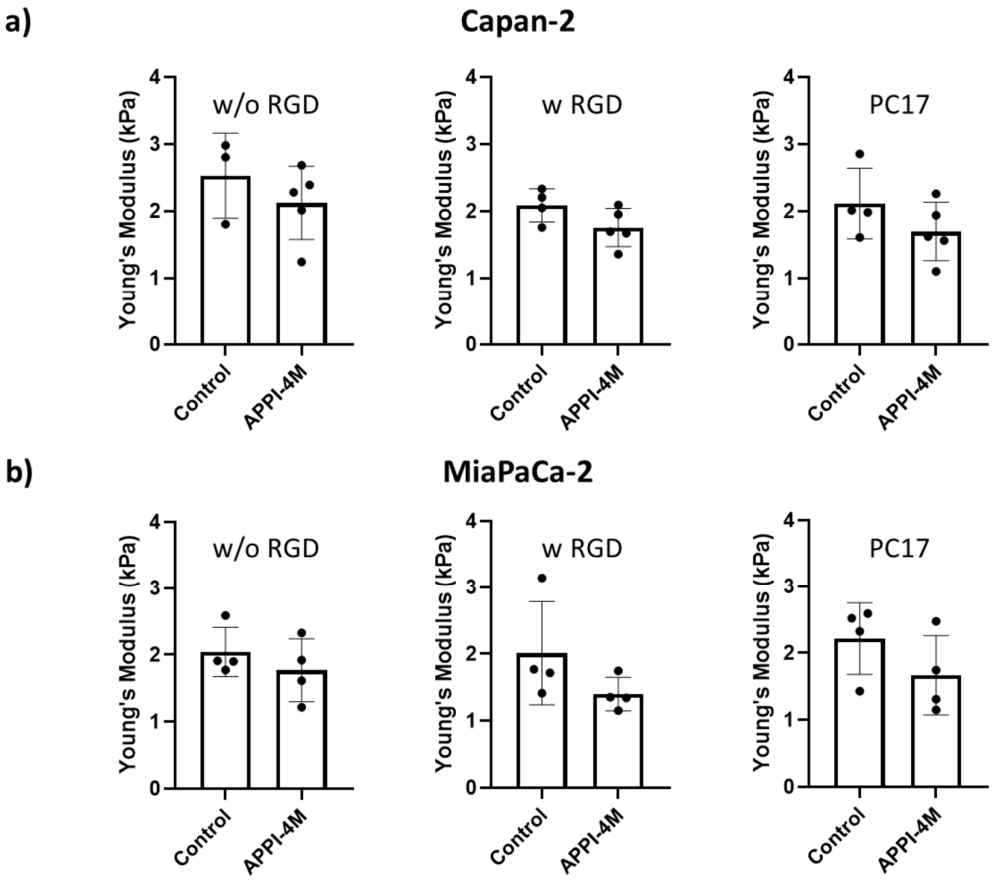


**Figure S7.** Mechanical properties of cell-containing hydrogels. (**a**) Young's modulus of Capan2-containing hydrogels of varying composition (without RGD motif, RGD-functionalised, PC17) ± KLK6 inhibition. (**b**) Young's modulus of MiaPaCa2-containing hydrogels of varying composition (without RGD motif, RGD-functionalised, PC17) ± KLK6 inhibition.

**Table S1.** Details of individuals diagnosed with pancreatic adenocarcinoma following the grading and staging of the World Health Organization criteria and specimen used to generate our tissue microarray. T, tumour; N, nodes.

| Patient | Gender | Age | Size (mm) | T stage | N Stage | Stage |
|---|---|---|---|---|---|---|
| 1 | Female | 77 | 30 | pT2 | pN0 | IB |
| 2 | Male | 68 | 22 | pT2 | pN0 | IB |
| 3 | Male | 41 | 30 | pT2 | pN1 | IIB |
| 4 | Male | 56 | 23 | pT2 | pN1 | IIB |
| 5 | Male | 54 | 19 | pT1c | pN1 | IIB |
| 6 | Male | 62 | 23 | pT2 | pN0 | IB |
| 7 | Female | 74 | 39 | pT2 | pN1 | IIB |
| 8 | Female | 68 | 20 | pT1c | pN2 | III |
| 9 | Female | 75 | 34 | pT4 | pN1 | III |
| 10 | Male | 83 | 11.5 | pT1c | pN0 | IB |
| 11 | Male | 70 | | cT4 | | III |
| 12 | Female | 66 | 28 | pT2 | pN1 | IIB |
| 13 | Female | 74 | 27 | pT2 | pN1 | IIB |
| 14 | Male | 68 | 30 | pT2 | pN1 | IIB |
| 15 | Female | 62 | 25 | pT2 | pN1 | IIB |
| 16 | Male | 75 | 28 | pT2 | pN0 | IB |
| 17 | Female | 74 | 29 | pT2 | pN0 | IB |
| 18 | Female | 63 | 16 | pT1c | pN0 | IA |
| 19 | Male | 56 | 26 | pT2 | pN1 | IIB |
| 20 | Female | 70 | 26 | pT2 | pN1 | IIB |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21 | Female | 70 | | cT4 | pN1 | III |
| 22 | Male | 75 | 39 | pT4 | pN1 | III |
| 23 | Male | 64 | 11 | pT1a | pN0 | I |
| 24 | Male | 81 | 25 | pT2 | pN2 | III |
| 25 | Female | 76 | 17 | pT1c | pN0 | IA |
| 26 | Male | 69 | 55 | pT3 | pN2 | III |
| 27 | Female | 62 | 24 | pT2 | pN0 | IB |
| 28 | Male | 60 | 26 | pT2 | pN1 | IIB |
| 29 | Female | 81 | 19 | pT1c | pN0 | IA |
| 30 | Male | 82 | 20 | pT1c | pN1 | IIB |
| 31 | Male | 64 | 68 | pT3 | pN1 | IIB |
| 32 | Male | 62 | 27 | pT2 | pN1 | IIB |
| 33 | Male | 63 | 32 | pT2 | pN1 | IIB |
| 34 | Male | 41 | 28 | pT2 | pN1 | IIB |
| 35 | Male | 81 | 25 | pT3 | pN2 | IIIB |
| 36 | Male | 74 | 24 | pT3b | pN0 | IIB |
| 37 | Male | 74 | 28 | pT3 | pN0 | IB |
| 38 | Male | 77 | 33 | pT2 | pN2 | III |
| 39 | Female | 73 | 55 | pT3 | pN1 | IIB |
| 40 | Female | 70 | 27 | pT2 | pN2 | III |
| 41 | Male | 81 | | pT4 | | III |

**Table S2.** Comparison of the study by Rückert et al and our findings. APGI, Australian Pancreatic Cancer Genome Initiative; Col-I, collagen type-I; KLK, kallikrein-related peptidase; PDAC, pancreatic ductal adenocarcinoma; TCGA, The Cancer Genome Atlas.

| | **Rückert et al. 2008** | **Our study** |
|---|---|---|
| investigated the KLK network in PDAC using publicly available datasets | high-density DNA microarray $n = 19$ | TCGA dataset $n = 178$, APGI dataset $n = 456$ |
| investigated own PDAC patient cohort | $n = 54$ | $n = 262$ (German cohort), $n = 41$ (Spanish cohort) |
| co-expression | KLK6 and KLK10 | KLK6, KLK7, KLK8, KLK10 and KLK11 |
| associated with shorter survival | co-expression of KLK6 and KLK10 | high KLK6 levels in tumour and CD68+ cells, high KLK7 level, high KLK10 level, high KLK11 level |
| KLK6 mRNA expression | cell lines | tissues and cell lines, in tumour, stromal and CD68+ cells |
| KLK6 protein expression | tissues and cell lines | tissues and cell lines, in tumour, stromal and immune cells |
| KLK6 serum expression | patient-derived samples | |
| cell proliferation | | 3 out of 4 cell lines increased on Col-I |
| cell migration | reduced in KLK10 knockout cells | 3 out of 4 cell lines increased on Col-I |
| tumour spheroid formation | | hydrogel-based assay |
| investigated KLK6 interaction partners | in silico analysis | human protease array |
| tested KLK6 inhibition | | used the proteolysis-resistant KLK6 inhibitor APPI-4M |
| effects of KLK6 inhibition | | reduced mRNA expression, reduced cell metabolic activity, reduced KLK6 secretion, increased secretion of urokinase plasminogen activator and cathepsin D |