Reconstitution of human PDAC using primary cells reveals oncogenic transcriptomic features at tumor onset

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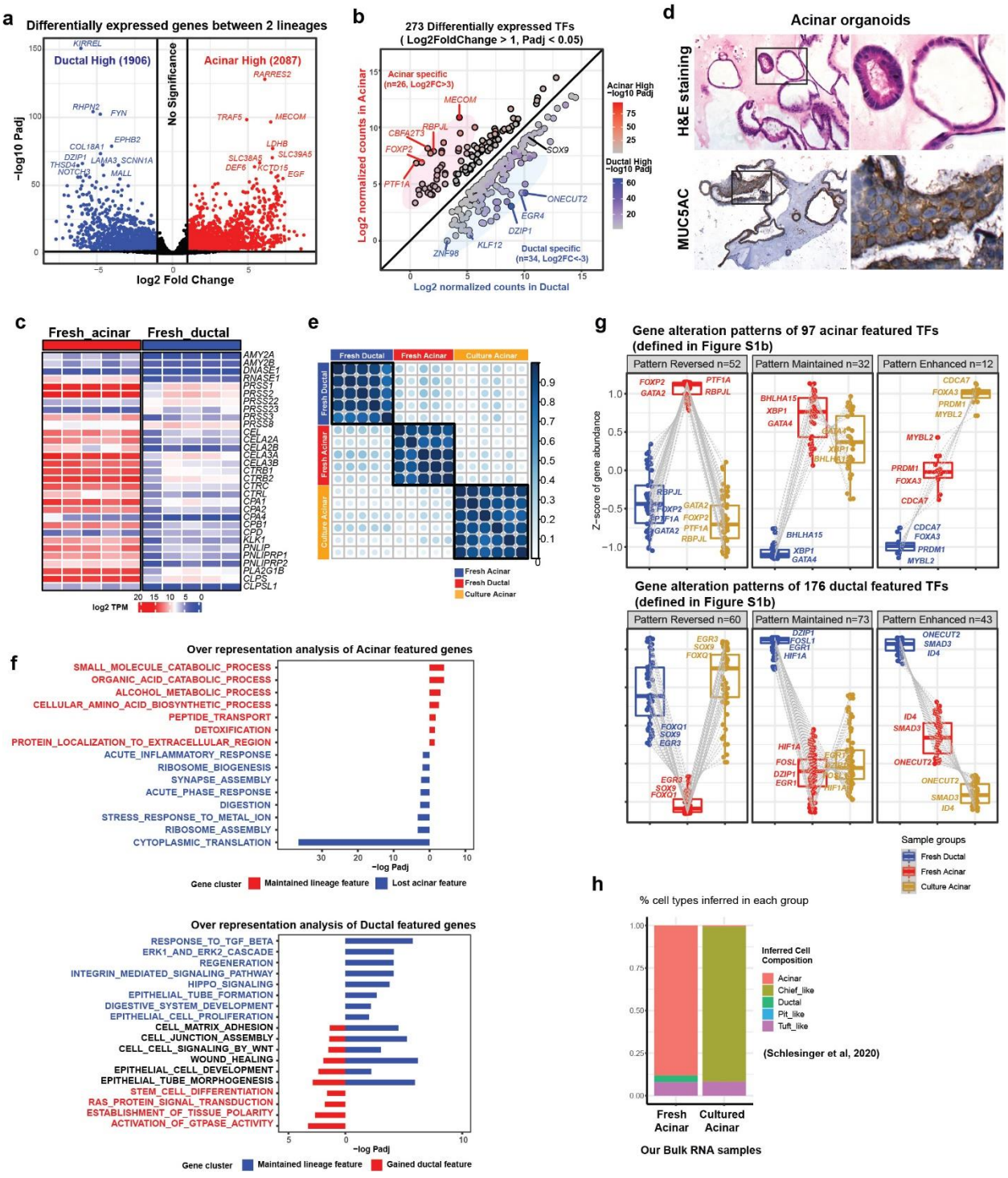


Figure S1. Molecular profiling of fresh and cultured human primary pancreatic exocrine cells. a. Volcano plot of the differentially expressed genes in fresh acinar (n=5) vs fresh ductal cells (n=5, fold change > 2, adj.p < 0.05, by negative binomial

Wald test using DESeq2 package in R software). **b.** Mean normalized expression of 273 differentially expressed transcription factors in sorted fresh acinar and ductal cells (fold change > 2, adj.p < 0.05). The shaded area includes the most significantly differentially expressed TFs in each lineage (log2 fold change > 3). **c.** Expression heatmap of genes encoding known pancreatic digestive enzymes in sorted fresh cells. **d.** H&E staining and MUC5AC staining in cultured acinar organoids. Each staining was performed in sections from 3 independent donor tissues with 8 fields of views for each. Scale bar: 50 μm. **e.** Pearson correlation plot of gene expression in fresh acinar cells (n=5), fresh ductal cells (n=5), and cultured acinar organoids (n=6). **f.** Gene ontology overrepresentation analysis of the two gene subgroups within the acinar signature gene cluster and ductal signature gene cluster described in Figure 1d. The analysis was performed using the clusterProfiler 4.4.4 package in R software with default settings. A significant enrichment was considered with multiple-test adjusted p-value < 0.05. **g.** Expression patterns of 97 acinar specific and 176 ductal specific transcription factors in fresh acinar, fresh ductal and cultured acinar cells. Each dot represents the mean expression of a transcription factor in the indicated group of samples. The middle line of box represents the median value of the expression of a group of transcription factors, the bounds of box represent the IQR, and the whiskers extend to 1.5 × IQR. **h.** Bar plot of inferred cell type composition in our fresh (n=5) and cultured acinar (n=6) samples using a previously characterized scRNA seq dataset as a reference. The bulk RNA sample deconvolution was performed by using a Multi-Subject Single Cell deconvolution method.

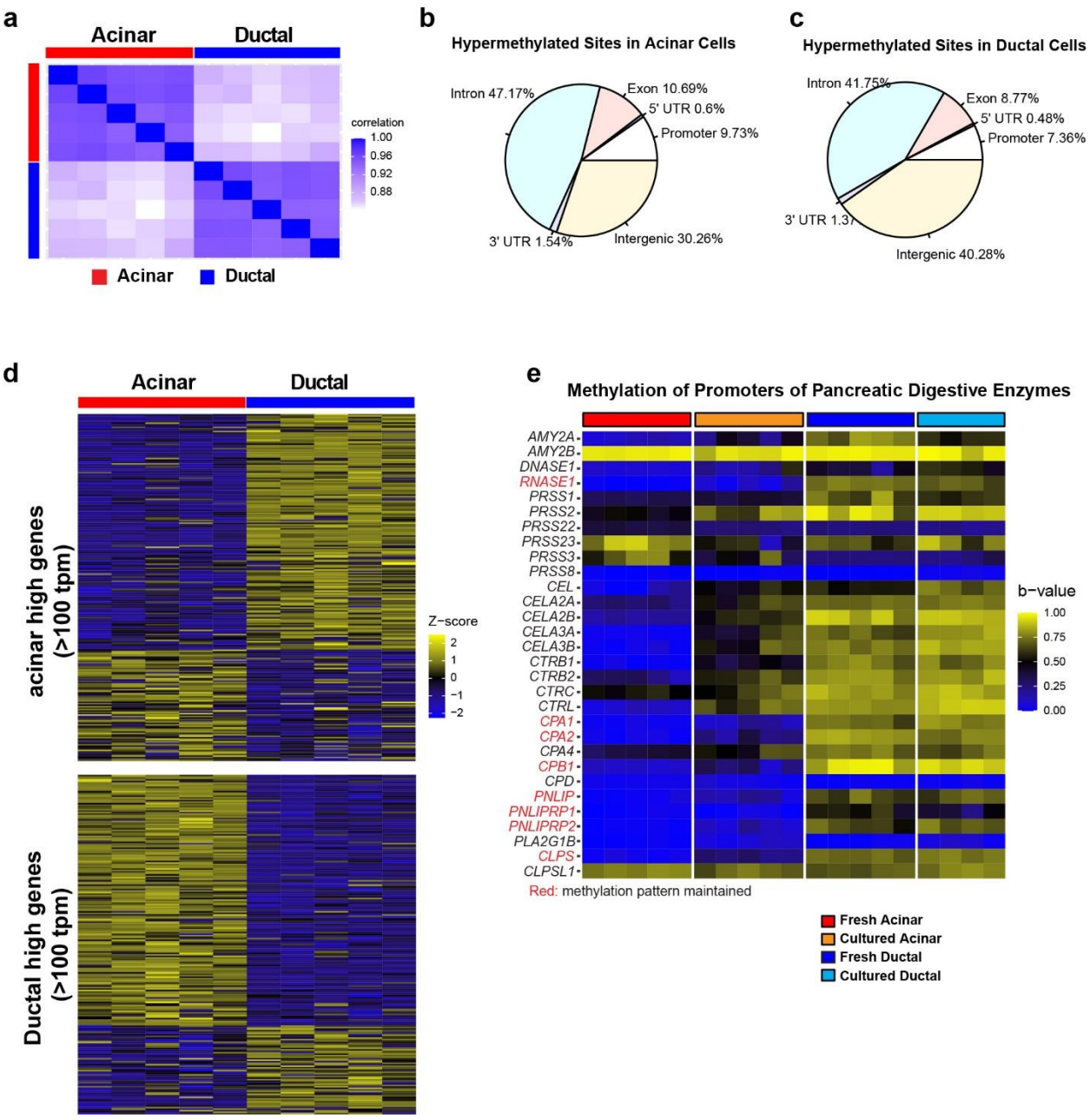


Figure S2. DNA methylation status of fresh and cultured acinar and ductal cells.

a. Correlation of DNA methylation of fresh acinar (n=5) and ductal cells (n=5) by all surveyed methylation sites. **b-c.** Distribution of lineage-specific differentially methylated CpG sites with reference to genomic features. **d.** Heatmap of promoter methylation status of top differentially expressed genes (identified in Figure 1) in fresh acinar and ductal samples. **e.** Heatmap of DNA methylation status in the promoter regions of genes

encoding pancreatic digestive enzymes of fresh acinar (n=5), cultured acinar (n=5), fresh ductal (n=5) and cultured ductal cells (n=4). Genes in red are associated with a lineage-specific methylation pattern which is maintained after long term culture.

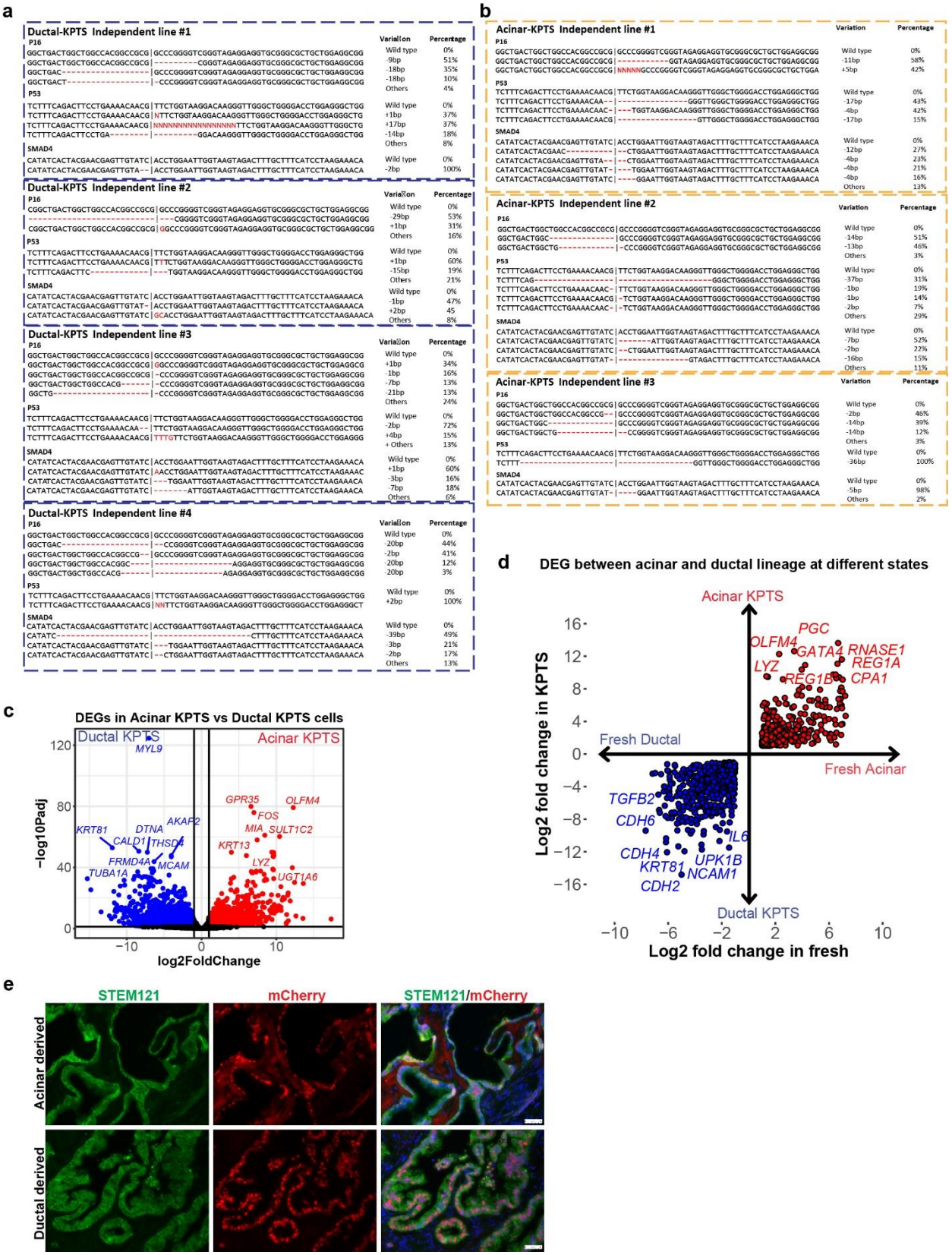

a
Ductal-KPTS Independent line #1
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGACTGGCTGGCCACGGCCGCG|----------CGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -9bp 51%
GGCTGAC----------|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -18bp 35%
GGCTGACT----------|--CCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -18bp 10%
Others 4%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAGACTTCCTGAAAACAACG|NTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTG +1bp 37%
TCTTTCAGACTTCCTGAAAACAACG|NNNNNNNNNNNNNNNNTTCTGGTAAGGACAAGGGTTGGGCTG +17bp 37%
TCTTTCAGACTTCCTGA--------|----------GGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -14bp 18%
Others 8%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAACGAGTTGTA--|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -2bp 100%
Ductal-KPTS Independent line #2
P16
CGGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
----------|---CGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -29bp 53%
CGGCTGACTGGCTGGCCACGGCCGCG|GGCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG +1bp 31%
Others 16%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAGACTTCCTGAAAACAACG|TTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTG +1bp 60%
TCTTTCAGACTTC----------|---TGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -15bp 19%
Others 21%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAACGAGTTGTAT-|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -1bp 47%
CATATCACTACGAACGAGTTGTATC|GCACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA +2bp 45
Others 8%
Ductal-KPTS Independent line #3
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGACTGGCTGGCCACGGCCGCG|GGCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCG +1bp 34%
GGCTGACTGGCTGGCCACGGCCGCG|-CCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -1bp 16%
GGCTGACTGGCTGGCCACG------|-CCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -7bp 13%
GGCTG----------|-CCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -21bp 13%
Others 24%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAGACTTCCTGAAAACAA--|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -2bp 72%
TCTTTCAGACTTCCTGAAAACAACG|TTTGTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGG +4bp 15%
+ Others 13%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAACGAGTTGTATC|AACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAAC +1bp 60%
CATATCACTACGAACGAGTTGTATC|----TGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -3bp 16%
CATATCACTACGAACGAGTTGTATC|-------ATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -7bp 18%
Others 6%
Ductal-KPTS Independent line #4
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGAC----------|--CCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -20bp 44%
GGCTGACTGGCTGGCCACGGCCG--|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -2bp 41%
GGCTGACTGGCTGGCCACGGC----|----------AGGAGGTGCGGGCGCTGCTGGAGGCGG -20bp 12%
GGCTGACTGGCTGGCCACG------|----------AGAGGAGGTGCGGGCGCTGCTGGAGGCGG -20bp 3%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAGACTTCCTGAAAACAACG|NNTTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCT +2bp 100%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATC----------|----------CTTTGCTTTCATCCTAAGAAACA -39bp 49%
CATATCACTACGAACGAGTTGTATC|---TGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -3bp 21%
CATATCACTACGAACGAGTTGTATC|--CTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -2bp 17%
Others 13%
b
Acinar-KPTS Independent line #1
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGACTGGCTGGCCACGGCCGCG|----------GGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -11bp 58%
GGCTGACTGGCTGGCCACGGCCGCG|NNNNNGCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGA +5bp 42%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAGACTTCCTGAAAACAA--|----------GGGTTGGGCTGGGGACCTGGAGGGCTGG -17bp 43%
TCTTTCAGACTTCCTGAAAACAAC-|---TGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -4bp 42%
TCTTTCAGACTTCCTGAAAACAACG|----------GTTGGGCTGGGGACCTGGAGGGCTGG -17bp 15%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAAC----------|--CTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -12bp 27%
CATATCACTACGAACGAGTTGTA--|--CTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -4bp 23%
CATATCACTACGAACGAGTTGTAT-|---TGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -4bp 21%
CATATCACTACGAACGAGTTGTATC|----GGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -4bp 16%
Others 13%
Acinar-KPTS Independent line #2
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGACTGGC----------|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -14bp 51%
GGCTGACTGGCTG----------|-CCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -13bp 46%
Others 3%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTTCAG----------|----------GGGCTGGGGACCTGGAGGGCTGG -37bp 31%
TCTTTCAGACTTCCTGAAAACAAC-|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -1bp 13%
TCTTTCAGACTTCCTGAAAACAACG|-TCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -1bp 14%
TCTTTCAGACTTCCTGAAAACAAC-|-TCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG -2bp 7%
Others 29%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAACGAGTTGTATC|-------ATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -7bp 52%
CATATCACTACGAACGAGTTGTATC|--CTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -2bp 22%
CATATCACTACGAACGAGTTGTAT-|----------GTAGACTTTGCTTTCATCCTAAGAAACA -16bp 15%
Others 11%
Acinar-KPTS Independent line #3
P16
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG Wild type 0%
GGCTGACTGGCTGGCCACGGCCGCG|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -2bp 46%
GGCTGACTGGC----------|GCCCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -14bp 39%
GGCTGACTGGCTG----------|--CCGGGGTCGGGTAGAGGAGGTGCGGGCGCTGCTGGAGGCGG -14bp 12%
Others 3%
P53
TCTTTCAGACTTCCTGAAAACAACG|TTCTGGTAAGGACAAGGGTTGGGCTGGGGACCTGGAGGGCTGG Wild type 0%
TCTTT----------|----------GGTTGGGCTGGGGACCTGGAGGGCTGG -36bp 100%
SMAD4
CATATCACTACGAACGAGTTGTATC|ACCTGGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA Wild type 0%
CATATCACTACGAACGAGTTGTAT-|----GGAATTGGTAAGTAGACTTTGCTTTCATCCTAAGAAACA -5bp 98%
Others 2%
c
DEGs in Acinar KPTS vs Ductal KPTS cells
Ductal KPTS
Acinar KPTS
MYL9
GPR35
OLFM4
FOS
MIA
SULT1C2
KRT13
LYZ
UGT1A6
KRT81
DTNA
AKAP2
CALD1
THSD4
FRMD4A
MCAM
TUBA1A
−log10Padj
log2FoldChange
d
DEG between acinar and ductal lineage at different states
Acinar KPTS
Ductal KPTS
Fresh Ductal
Fresh Acinar
PGC
OLFM4
GATA4
RNASE1
REG1A
LYZ
REG1B
CPA1
TGFB2
CDH6
IL6
CDH4
KRT81
UPK1B
NCAM1
CDH2
Log2 fold change in KPTS
Log2 fold change in fresh
e
STEM121
mCherry
STEM121/mCherry
Acinar derived
Ductal derived

Figure S3. Genetical engineering primary acinar and ductal cells to oncogenic KPTS cells. a-b. Genotyping of *p16*, *p53* and *SMAD4* mutation status of four ductal-KPTS 2D cultures (a) and three acianr-3D cultures (b). **c.** Volcano plot of the differentially expressed genes in acinar-KPTS (n=3) versus ductal-KPTS cells (n=4, fold change > 2, adj.p < 0.05, by negative binomial Wald test using DESeq2 package in R software). **d.** Scatter plot of genes which were significantly highly expressed in both fresh and KPTS cells of each lineage compared with the other (log2 fold change > 1, adj.p < 0.05). Acinar high genes are colored in red, ductal high genes are colored in blue. X axis represents log2 fold change between fresh acinar and fresh ductal, y axis represents log2 fold change between acinar-KPTS and ductal-KPTS. **e.** Immunofluorescence staining of human specific STEM121 and mCherry of acinar-derived and ductal-derived tumor sections. The staining was performed in 6 independent tumor tissues with 3 sections for each. Scale bar: 50 μm.

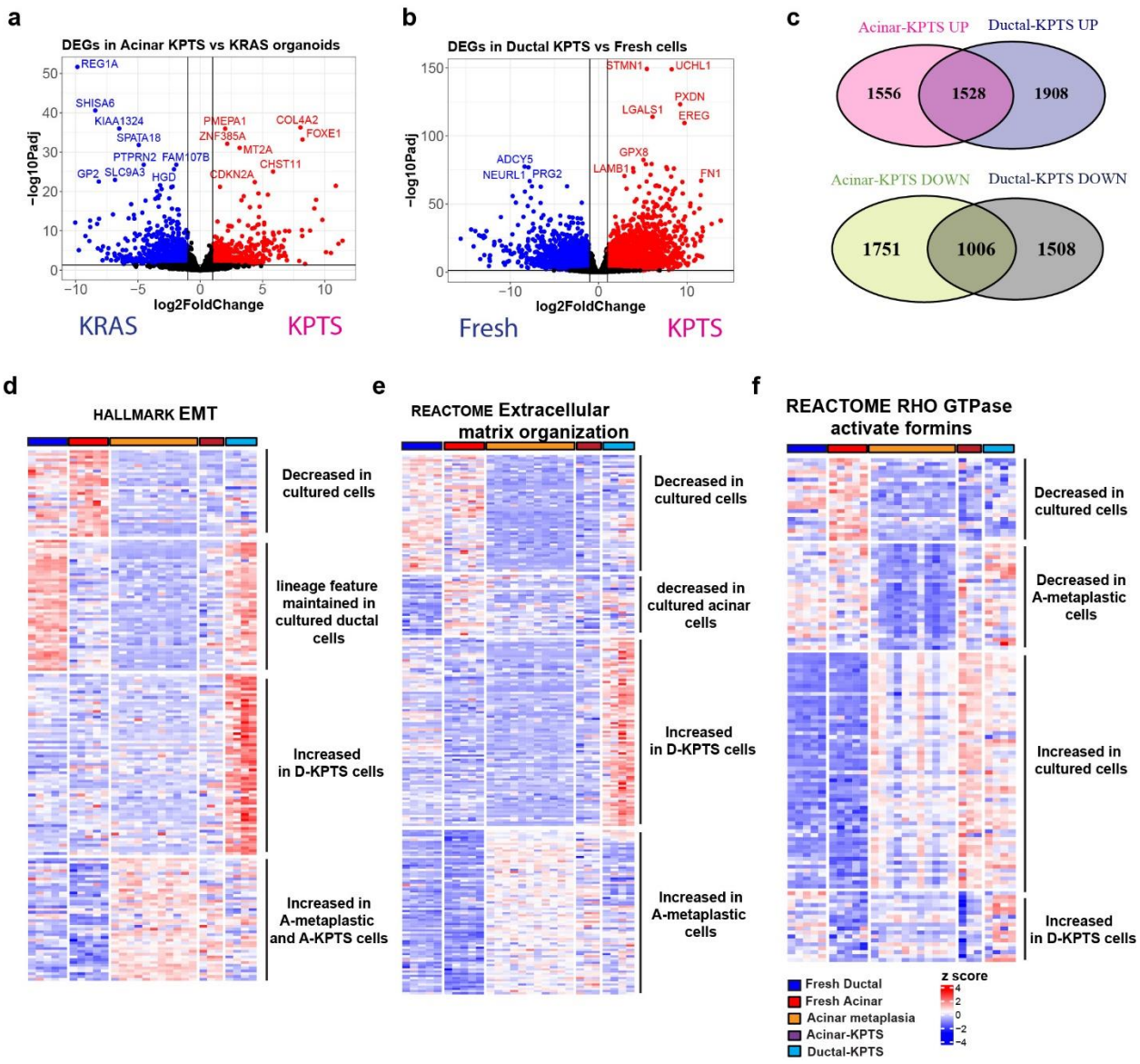


Figure S4. Molecular profiling oncogenic KPTS cells of acinar and ductal lineages. a. Volcano plot of the differentially expressed genes in acinar-KPTS (n=3) versus acinar-KRAS organoids (n=5). DEGs were identified by negative binomial Wald test using DESeq2 1.36.0 in R software with fold change > 2 and adjusted p-value < 0.05. **b.** Volcano plot of the DEGs in ductal-KPTS (n=4) versus fresh ductal cells (n=5). DEGs were identified by negative binomial Wald test using DESeq2 with fold change > 2 and adjusted p-value < 0.05. **c.** Intersection of DEGs (described in a and b) in acinar-

KPTS and ductal-KPTS cells. **d-f.** Expression heatmaps of genes in indicated gene sets in fresh, metaplastic, and KPTS cells. K means clustering was performed to identify different expression patterns across all groups of samples.

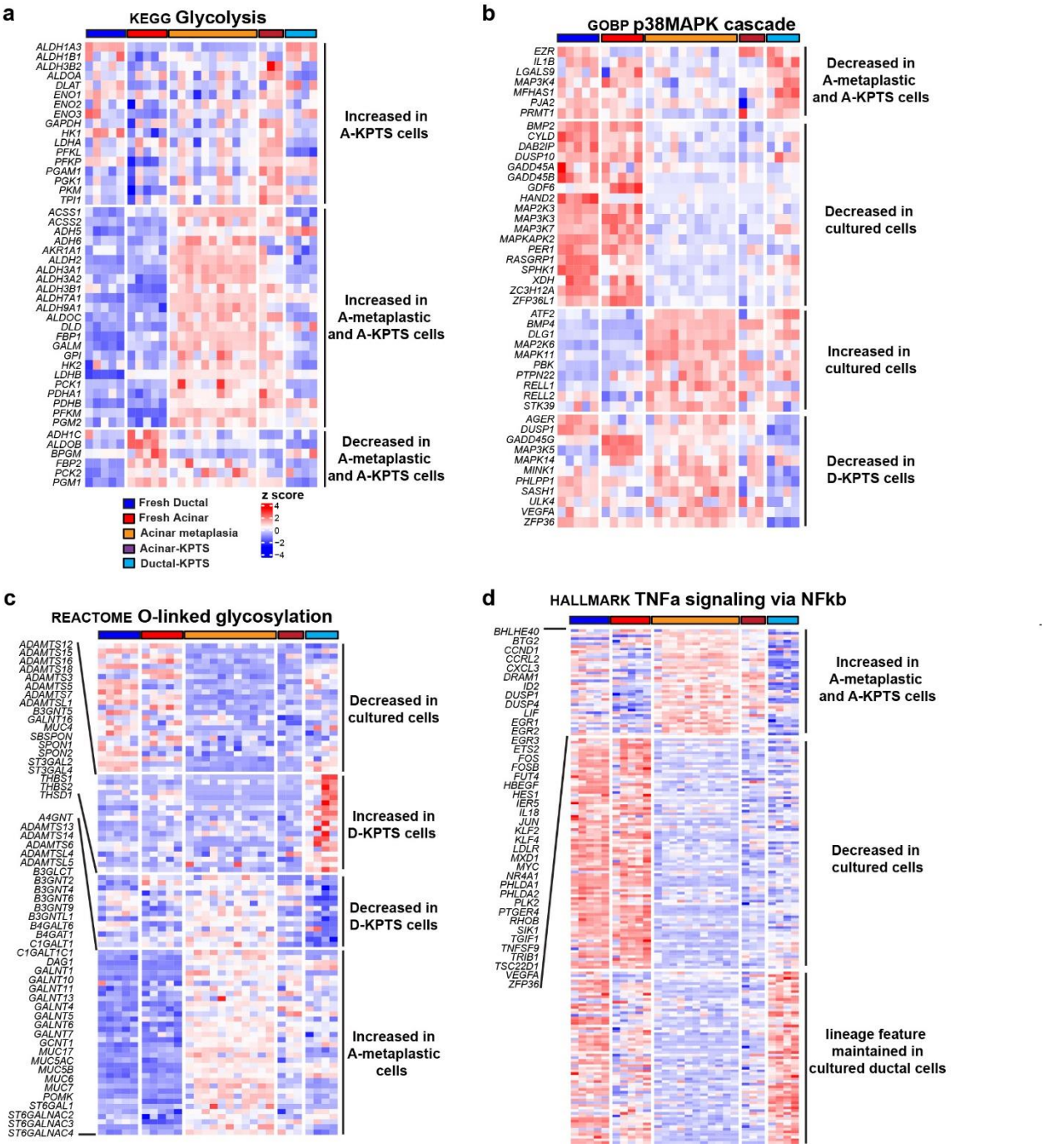
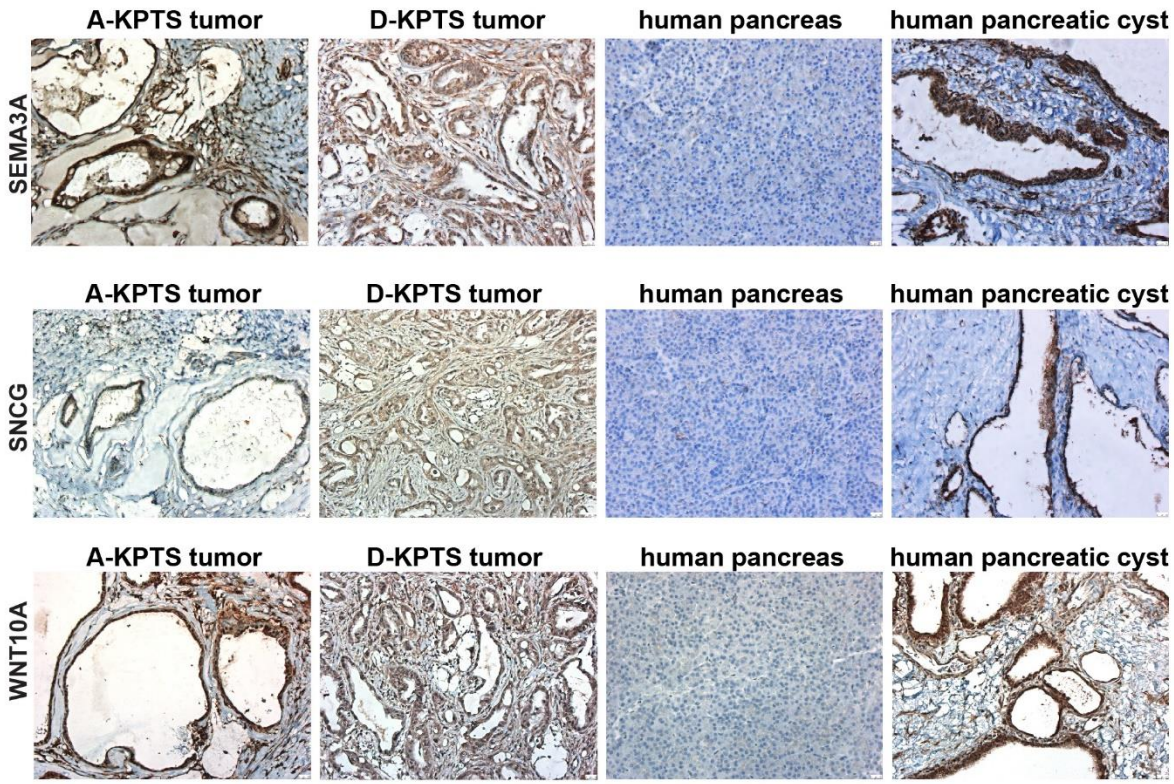


Figure S5. Altered transcriptomic programs during oncogenic transition. a-d.

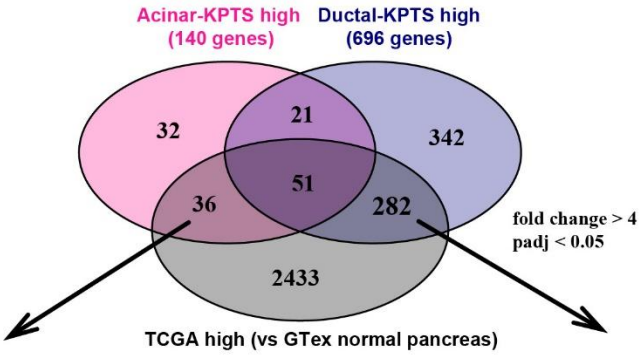
Expression heatmaps of genes in indicated gene sets in fresh, metaplastic, and KPTS cells. Fresh ductal n = 5, fresh acinar n = 5, acinar-metaplastic n = 11, acinar-KPTS n =

3, ductal-KPTS n = 4. K means clustering was performed to identify different expression patterns across all groups of samples.

a



b



Acinar-KPTS high & TCGA high (36 genes)

	Extracellular space and Plasma membrane	Transcription Factor and others
Associated with PDAC known function	*CSTB, IL10, NTSR1, *PSCA, S100A4,*	*CDC25C,SIX1, *UBE2C*
Associated with PDAC unclear function	*ITGB2, LYPD3, SLC16A3*	*ACSL5, SDR16C5, TBX15*
Unknown implication in PDAC	*CD22, CILP2, FAM3D, FIBCD1, LGR6, NPFFR1*	*ACTL8, ALDH3B2, FNDC11, DMBX1, FABP6, GREB1L, GSDMA, HPGD, KRT79, PTTG1, S100A5, ST8SIA6, TPRG1*

Ductal-KPTS high & TCGA high (38 out of 282 genes)

	Extracellular space	Transcription Factor
Associated with PDAC known function	*BDNF, DKK2, FGF1, FGF19, FGF5, GREM1, INHBA, LGALS1, LTBP1, MDK, *NOG, NRP2, PTHLH, SERPINB2, SERPINE1, TGFB2, THBS1, THBS2, VEGFC, WNT7A,WNT7B*	*E2F7, GATA3, ISL2, RUNX2, HMGA2, HOXB8, HOXB9, *LOXL2*
Associated with PDAC unclear function	*BMP3, CFH, LTBP2*	*SIM2, BASP1,*
Unknown implication in PDAC	*GDF5*	*HES2, PRRX2, ZFHX4*

*** : Prognostic genes from TCGA PAAD**

Figure S6. Identified genes upregulated in early and established PDAC. a. Immunohistochemistry staining of SEMA3A, SNCG and WNT10A proteins in the KPTS tumors generated in this work (n = 2 with 8 field of views for each), and a human pancreatic cystic neoplasm sample as well as adjacent normal pancreas (8 field of views for each). Scale bar: 25 μm. **b.** Annotation of 36 genes highly expressed in both acinar-KPTS cells and TCGA PDAC samples (left), as well as selected genes highly expressed in both ductal-KPTS cells and TCGA PDAC samples (right).