



qRT-PCR of selected genes validate the results of RNA-seq analysis. To confirm the expression profile of genes in RNA-Seq results, 13 genes with known involvement in clinical PDAC progression were selected. Extra-cellular matrix (ECM) and Cancer stem cell-related (CSC) genes were up-regulated in PDAC as compared to healthy pancreas as per the RNA-seq analysis. Methodology: pancreata from KPT mice **1.** Untreated with TAM (normal whole pancreas control; N=3); and **2.** At 5 months post-TAM (frank PDAC, N=3) were harvested, minced into fragments of approximately 2mm³ using sterile scalpels and stored in RNeasy lysis buffer (QIAGEN) at -80°C until isolation. RNA was isolated using RNeasy extraction kit (QIAGEN). One microgram of RNA was converted to cDNA using the RevertAid First Strand cDNA Synthesis kit from Life Technologies using random hexamer primers. All reactions were carried out according to manufacturer's protocols. Gene expression analyses were performed in triplicate using the QuantStudio 3 Real-Time PCR system (Life Technologies) with SYBR Green (Applied Biosystems, Life Technologies). Target genes were amplified using the following conditions: 50°C for 2 minutes, 95°C for 10 seconds, followed by 40 cycles of 95°C for 15 seconds; 60°C for 1 minute. 18S rRNA was used as a house keeping gene to normalize for cDNA input. Results from N=3 mice/group were combined and expressed as fold change compared to whole normal pancreas control. Statistical analysis of relative gene expression was performed using the comparative $\Delta\Delta CT$ method and significance determined by using ANOVA followed with Bonferroni correction on Excel. Primers were purchased from Integrated DNA Technologies (IDT). Primer sequences used are listed in the table below:

Target gene	Primer sequence 5'-3'
<i>Aldh1a3</i>	F: GGGTCACACTGGAGCTAGGA
	R: CTGGCCTCTTCTTGGCGAA
<i>Dclk1</i>	F: TCCACCGGAATTGAACTCGG
	R: GGGAGCGAACAGTCTCAGA
<i>Lgr5</i>	F: CCTACTCGAAGACTTACCCAGT
	R: GCATTGGGGTGAATGATAGCA
<i>Shh</i>	F: AAAGCTGACCCCTTTAGCCTA
	R: TTCGGAGTTTCTTGTGATCTTCC
<i>Tspan8</i>	F:TCTGGGTATGTGGTACACTGAT
	R: AGGGGTTCGTGCTAGAGTCTC
<i>Wnt2</i>	F: CTCGGTGGAAATCTGGCTCTG
	R: CACATTGTACACATCACCCCT
<i>Has1</i>	F: GGCGAGCACTCACGATCATC
	R: AGGAGTCCATAGCGATCTGAAG
<i>Has2</i>	F: TGTGAGAGGTTTCTATGTGTCCT
	R: ACCGTACAGTCCAAATGAGAAGT
<i>Has3</i>	F: GTGGGCACCAGTCTGTTTG
	R: CCACTGAACGCGACCTCTG
<i>CD44v1</i>	F: CACCATTGCCTCAACTGTGC
	R: TTGTGGGCTCCTGAGTCTGA
<i>CD44v3</i>	F: TCGATTTGAATGTAACCTGCCG
	R: CAGTCCGGGAGATACTGTAGC
<i>CD44v5</i>	F: TCTGCCATCTAGCACTAAGAGC
	R: GTCTGGGTATTGAAAGGTGTAGC
<i>Hmnr</i>	F: CCTTGCTTGCTTCGGCTAAAA
	R: CTGCTGCATTGAGCTTTGCT