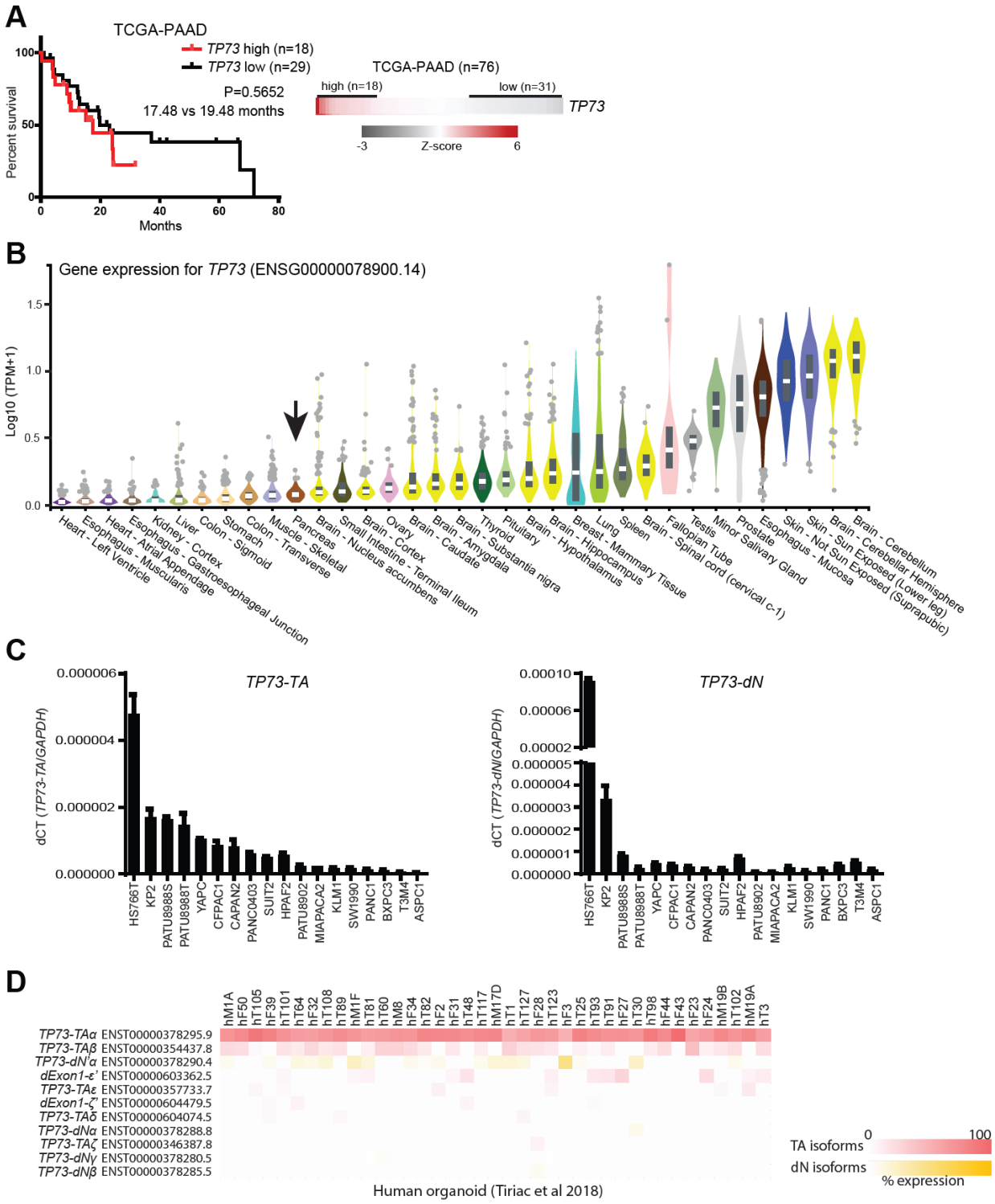


Supplementary Figure 1. *TP73* is prognostic and expresses multiple isoforms; *TP73-TA* is predominantly expressed in PDAC. Related to Figure 1.

(*A*) Survival curve of patients stratified by *TP73* expression. Log-rank (Mantel-Cox) test used to assess the median survival values and p-values. (*B*) Expression of *TP73* in normal tissues from

the GTEx project (The GTEx Consortium, 2015). Arrow points to pancreas tissue. (*C*) Expression of *TP73* isoforms in PDAC cell lines analyzed by RT-qPCR using *TP73-TA* specific primers (left) or *TP73-dN*-specific primers (right). Expression levels of *TP73* isoforms were measured relative to the expression level of *GAPDH*, and presented as dCT (i.e. 2 ^ -dCT) values. Mean ± SEM shown (n=3). (*D*) *TP73* isoform expression in PDAC organoids derived from primary tumors (hT), metastases (hM), or fine needle biopsies of primary or metastatic lesions (hF) (Tiriac et al., 2018). For the heatmap, organoids are listed in descending order (from left to right) of total *TP73* isoform expression. See figure legend for Figure. 1 E for additional details.

A

Bailey et al

	high	low	high (%)	low (%)
Grade 1-well differentiated	0	0	0	0
Grade 2-moderately differentiated	6	27	28.6	67.5
Grade 3-poorly differentiated	15	12	71.4	30.0
Grade 4-undifferentiated	0	1	0	2.5
# Total patient	21	40		

AJCC tumor stage	high	low	high (%)	low (%)
IA	0	3	0	7.5
IB	3	1	13.6	2.5
IIA	4	10	18.2	25
IIB	14	26	63.6	65
III	0	0	0	0
IV	1	0	4.5	0
# Total patient	22	40		

	high	low	high (%)	low (%)
KRAS	20	35	90.9	87.5
TP53	19	25	86.4	62.5
SMAD4	5	6	22.7	15
CDKN2A	3	9	13.6	22.5
# Total patient	22	40		

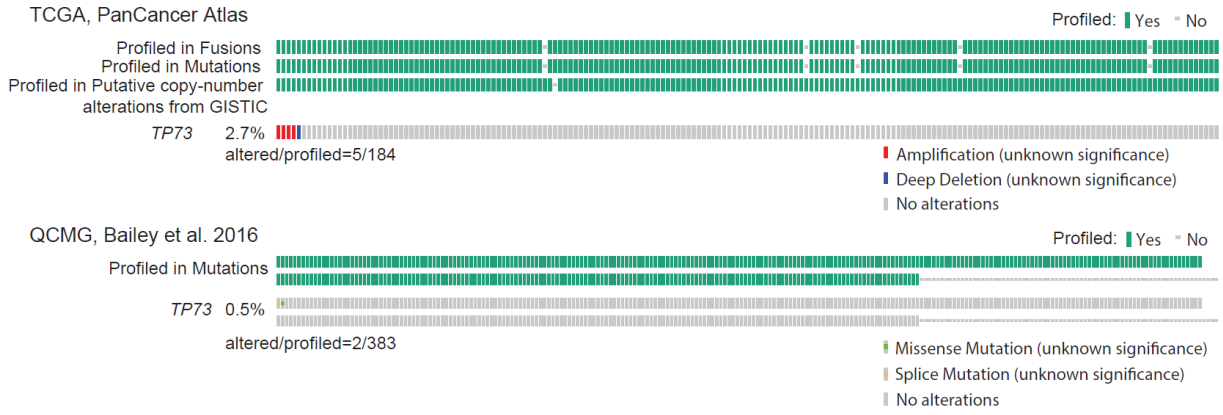
TCGA-PAAD

	high	low	high (%)	low (%)
Grade1-well differentiated	2	1	11.1	3.2
Grade2-moderately differentiated	7	18	38.9	58.1
Grade3-poorly differentiated	9	12	50	38.7
# Total patient	18	31		

AJCC tumor grade	high	low	high (%)	low (%)
IA	0	0	0	0
IB	0	0	0	0
IIA	7	12	38.9	40
IIB	11	17	61.1	56.7
III	0	1	0	3.3
IV	0	0	0	0
# Total patient	18	30		

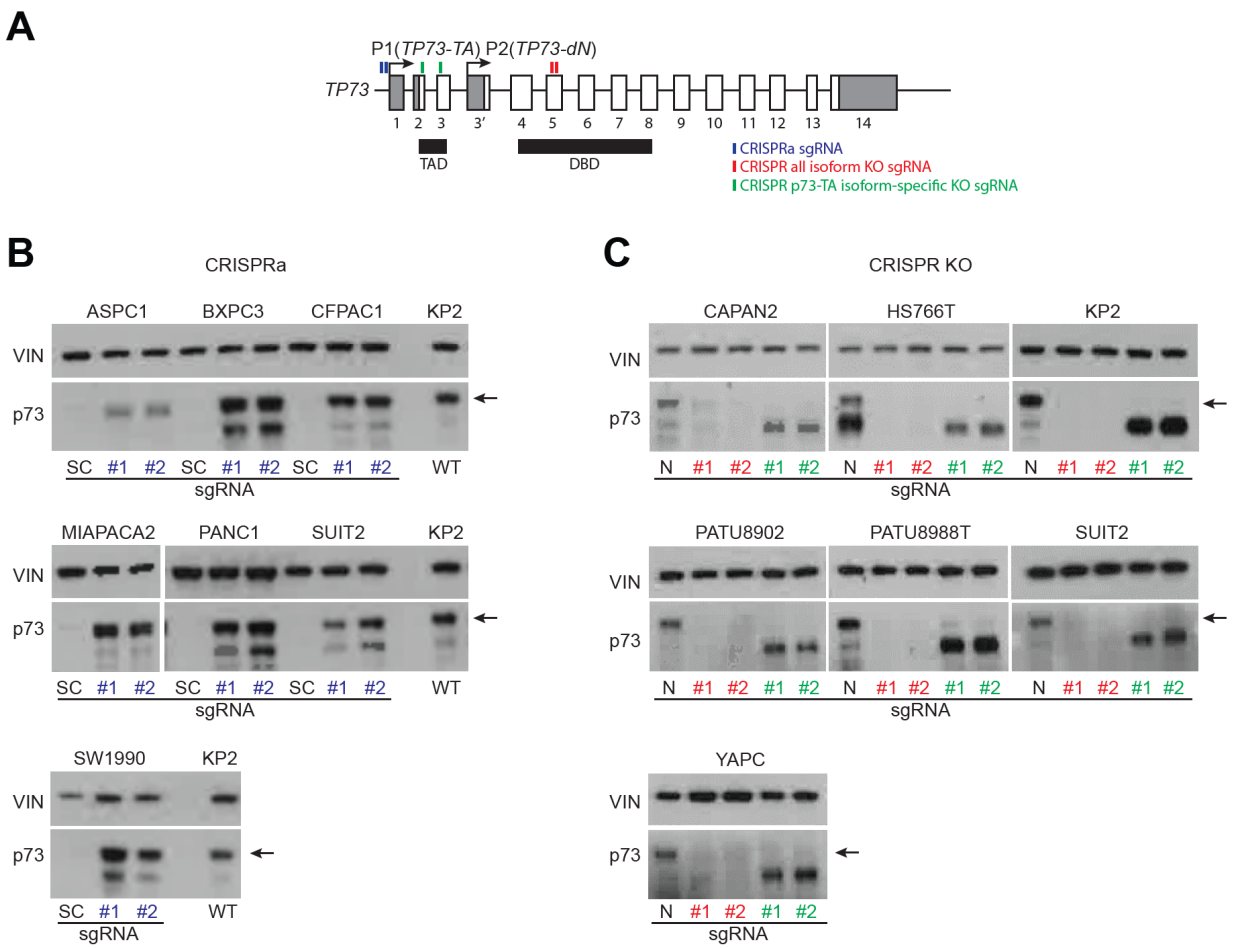
	high	low	high (%)	low (%)
KRAS	17	27	94.4	90
TP53	15	15	83.3	50
SMAD4	7	11	38.9	36.7
CDKN2A	6	2	33.3	6.7
# Total patient	18	31		

B



Supplementary Figure 2. *TP73*^{high} tumors are poorly differentiated and present more frequent *TP53* mutations compared to the *TP73*^{low} tumors, while *TP73* is rarely mutated in PDAC. Related to Figure 1.

(*A*) Comparison between *TP73*^{high} (high) and *TP73*^{low} (low) tumors from the indicated studies, regarding tumor histology, tumor stage/grade, and commonly mutated genes in PDAC. (*B*) *TP73* mutational analysis from the indicated studies downloaded from the cBioPortal (Cerami et al., 2012).



Supplementary Figure 3. CRISPR-based activation and knock-out of p73. Related to Figure 2 and 3.

(*A*) Schematic of the human *TP73* gene and regions targeted by sgRNAs used in this study. Exons (white boxes), UTRs (gray boxes), and introns (thin lines) are depicted. Exons are numbered. Transactivation domain (TAD) and dna-binding domain (DBD) are indicated with black boxes. sgRNAs used for CRISPRa-based induction of p73-TA (blue), CRISPR-based knock-out of all isoforms of p73 (red), CRISPR-based knock-out of p73-TA (green) are illustrated. (*B* and *C*) Western blot analysis of p73 using a pan-p73 antibody following activation or ablation p73 in a panel of PDAC cell lines. Bands corresponding to the molecular weight of the longest p73 isoform (i.e. p73-TAα) indicated with arrows. VINCULIN (VIN) shown as a loading control. (*B*) CRISPRa-based induction of p73-TA using a scramble control (SC) or two independent sgRNAs targeting upstream of P1 promoter (#1 and #2, blue). Wild-type (WT) KP2 cell line included as a reference for endogenous p73 expression. (*C*) CRISPR-based knock-out of p73 using a non-targeting sgRNA (N), two independent sgRNAs targeting DBD (#1 and #2, red) or two independent sgRNAs targeting TAD (#1 and #2, green).

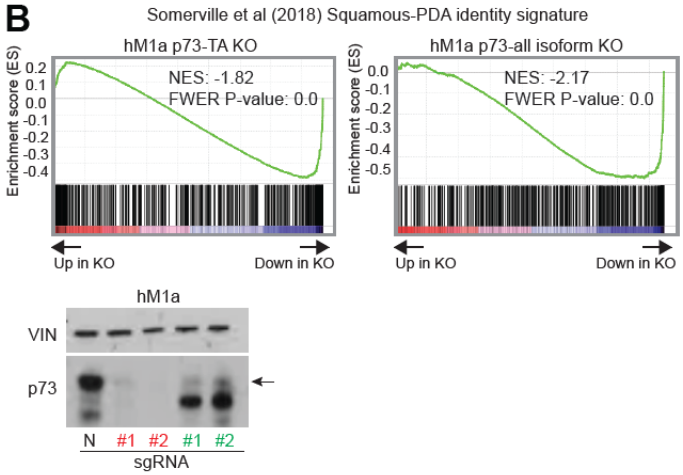
A

Somerville et al (2018) Squamous-PDA identity signature

p73-all isoform KO

Cell line	NES	FWER P-value
SUIT2	-2.18	0
KP2	-1.64	0
YAPC	-1.39	0.005
CAPAN2	-1.15	0.069
HS766T	-1.09	0.198
PATU8902	-1.02	0.348
PATU8988T	1.06	0.129

B



C

Chan-Seng-Yue et al (2020) signature 2 and signature 10 GSEA analysis

p73-TA CRISPRa

Signature 2

Cell line	NES	FWER P-value
SUIT2	2.43	0
SW1990	2.41	0
CFPAC1	2.32	0
BXPC3	2.23	0
PANC1	2.08	0
MIAPACA2	2.05	0
ASPC1	1.72	0

Signature 10

Cell line	NES	FWER P-value
CFPAC1	1.88	0
PANC1	1.7	0
ASPC1	1.42	0.006
MIAPACA2	1.02	0.342
SW1990	-1.39	0.005
SUIT2	-1.59	0
BXPC3	-1.98	0

p73-TA isoform-specific KO

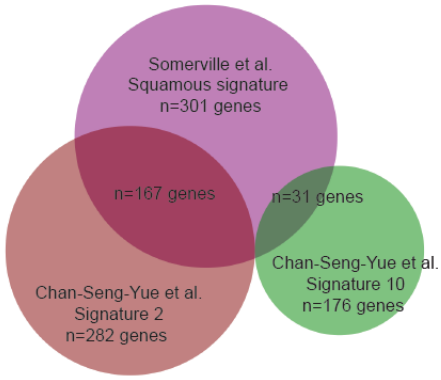
Signature 2

Cell line	NES	FWER P-value
HS766T	-2.20	0
KP2	-2.11	0
PATU8902	-1.58	0.008
YAPC	-1.55	0.018
PATU8988T	-1.47	0.023
SUIT2	-1.44	0.072
CAPAN2	-1.19	0.231

Signature 10

Cell line	NES	FWER P-value
PATU8902	-2.44	0
YAPC	-2.44	0
SUIT2	-2.21	0
KP2	-1.94	0
CAPAN2	-1.84	0
HS766T	-1.39	0.004
PATU8988T	0.8	0.383

D



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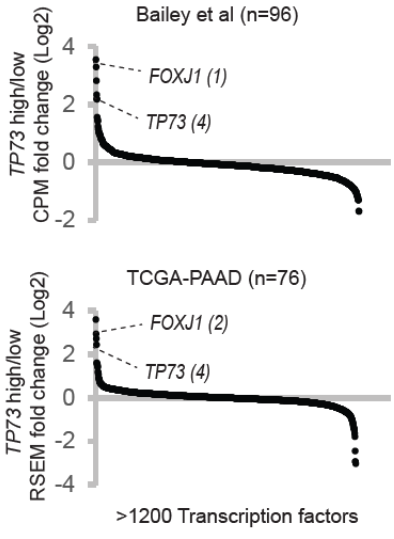
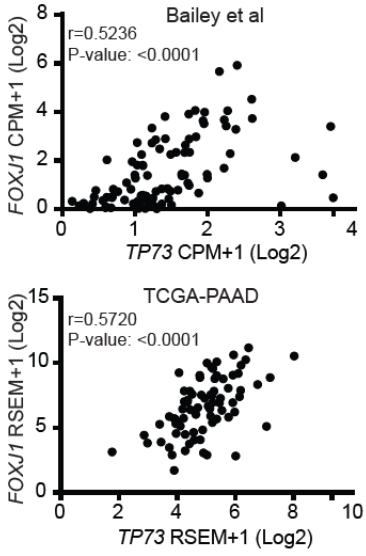


F



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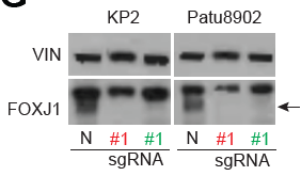
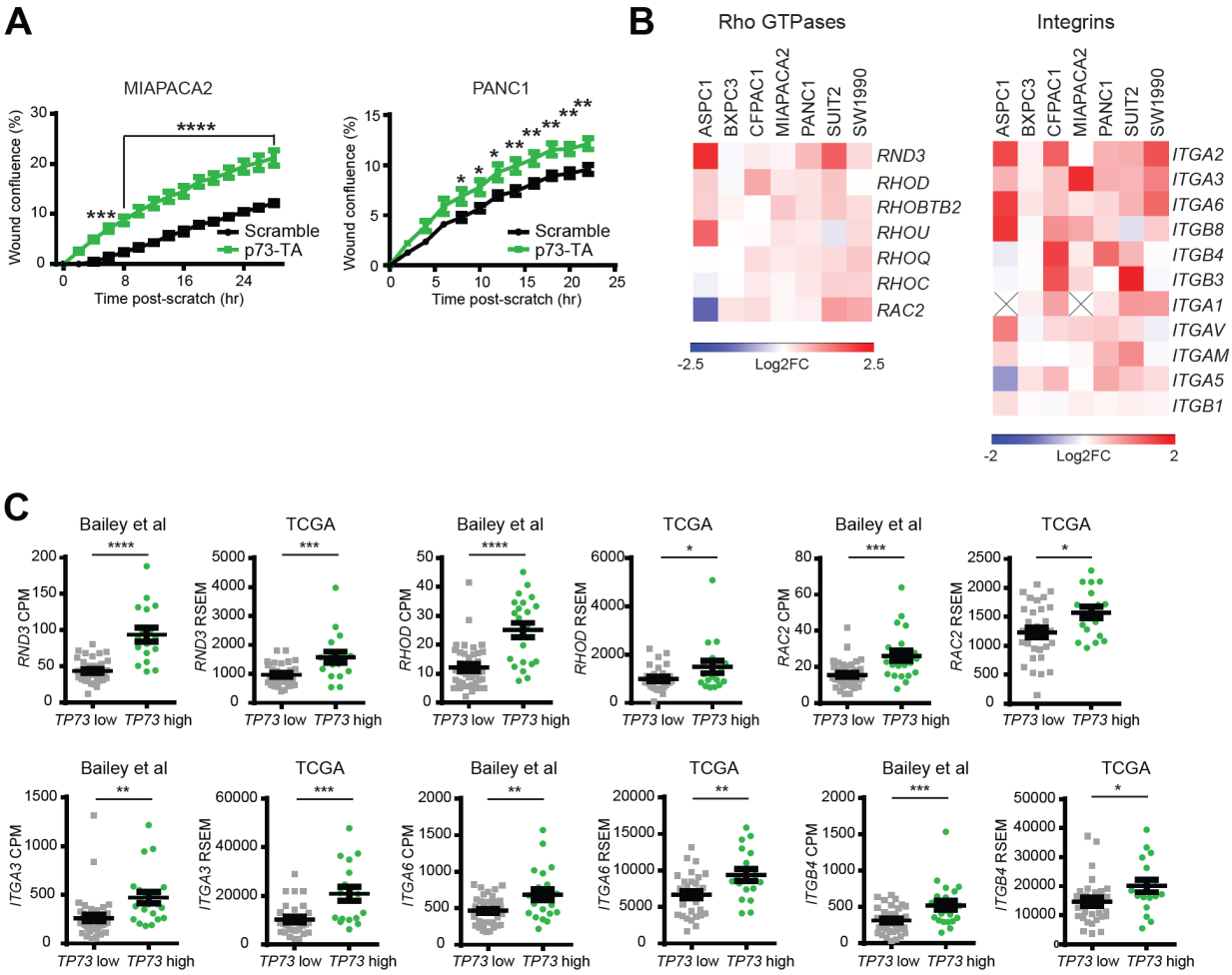


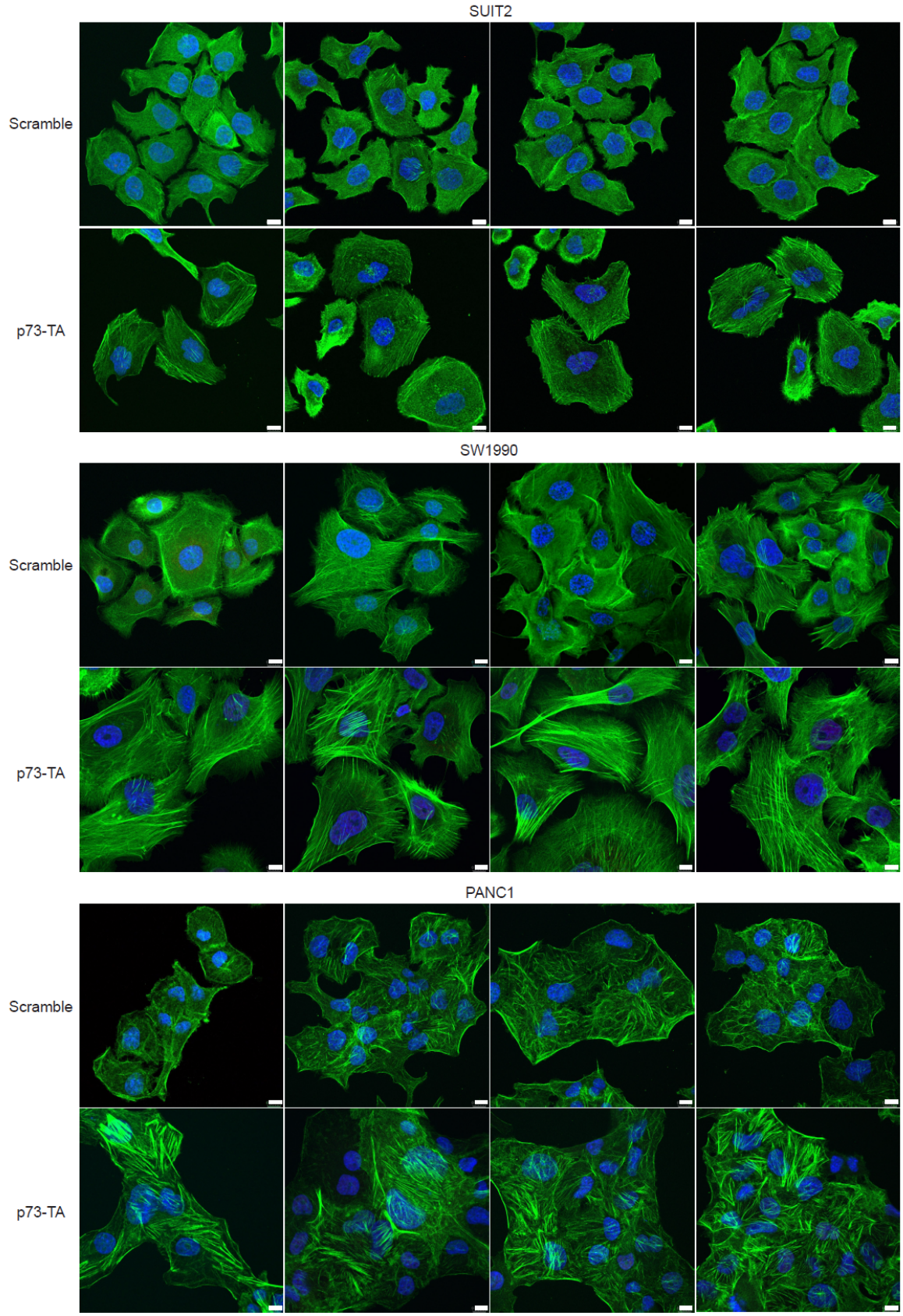
Supplementary Figure 4. p73-TA promotes squamous program and maintains transcriptional program associated with a newly described 'Basal B' subtype of PDAC. Related to Figure 3.

(*A*) RNA-seq analysis following ablation of p73 in PDAC cell lines. Table summarizing GSEA analysis of the squamous subtype PDAC signature (Somerville et al., 2018) subsequent to knocking-out all isoforms of p73. (*B*) Western blot and RNA-seq analysis following p73 knock-out in hM1a PDAC organoid. GSEA plot evaluating the squamous subtype PDAC signature (Somerville et al., 2018) after knocking-out p73-TA (left) or all isoforms of p73 (right). For western blot, sgRNAs targeting all isoforms of p73 (#1and #2, red), sgRNAs targeting p73-TA (#1 and #2, green), and non-targeting sgRNA (N) are shown. Bands corresponding to the molecular weight of the longest p73 isoform (i.e. p73-TAα) indicated with an arrow. VINCULIN (VIN) shown as a loading control. (*C*) Tables summarizing GSEA analysis of signature 2 and signature 10 (Chan-Seng-Yue et al., 2020) after activating (top) or knocking-out (bottom) p73-TA in a panel of PDAC cell lines. (*D*) Venn diagram showing number of genes overlapping between Somerville et al. squamous signature (Somerville et al., 2018), and signature 2 and signature 10 from Chan-Seng-Yue et al. (Chan-Seng-Yue et al., 2020). (*E*) Expression of transcription factors in $TP73^{high}$ vs $TP73^{low}$ tumors. Transcription factors are ranked by their mean log2 fold-change in expression levels in $TP73^{high}$ vs. $TP73^{low}$ tumors. Rank of each gene is written inside parentheses. (*F*) Pearson correlation analysis of expression levels between *TP73* and *FOXJ1*. Pearson correlation coefficient (r) and P-value shown. (*G*) Western blot analysis of FOXJ1 subsequent to knocking-out p73. sgRNA targeting all isoforms of p73 (#1, red), sgRNA targeting p73-TA (#1, green), and non-targeting sgRNA (N) are shown. Bands corresponding to the molecular weight of FOXJ1 indicated with an arrow. VINCULIN (VIN) shown as a loading control.



Supplementary Figure 5. p73-TA promotes cell migration and invasion in PDAC cells and upregulates Rho GTPases and integrins. Related to Figure 4.

(*A*) Scratch assays following activation of p73-TA compared to the control (Scramble). Quantification of % wound confluence plotted at the indicated time points post-scratch. P-values versus Scramble calculated using two-way ANOVA with Sidak's multiple comparisons test. Mean ± SEM shown (n=3). *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. (*B*) RNA-seq analysis following p73-TA activation in a panel of PDAC cell lines. Heatmaps show log2 fold-change in the expression level of a subset of RhoGTPases and integrins that are significantly upregulated in two or more cell lines. *ITGA1* is expressed at low levels in ASPC1 and MIAPACA2 regardless of the p73-TA activation status, thus not depicted and marked as X. (*C*) A subset of RhoGTPases and integrins that are expressed at higher levels in *TP73*^{high} tumors compared to the *TP73*^{low} tumors in both of the indicated data sets. P-values calculated using unpaired t-test. Mean ± SEM shown. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.



Supplementary Figure 6. Phalloidin staining to visualize F-actin. F-actin (green) and nuclei (stained with DAPI, blue) shown in control (Scramble) and p73-TA activated cells (p73-TA). Scale bar indicates 10 µm. Related to Figure 4.

Supplementary Information:

Supplementary Table 1. Sequences of sgRNAs and RT-qPCR primers used in this study

Supplementary Table 2. List of genes depicted in the heatmap in Fig.2 C, in the same order as it appears on the heatmap.