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Supplemental Information

TP63-Mediated Enhancer Reprogramming Drives the Squamous Subtype of Pancreatic Ductal Adenocarcinoma

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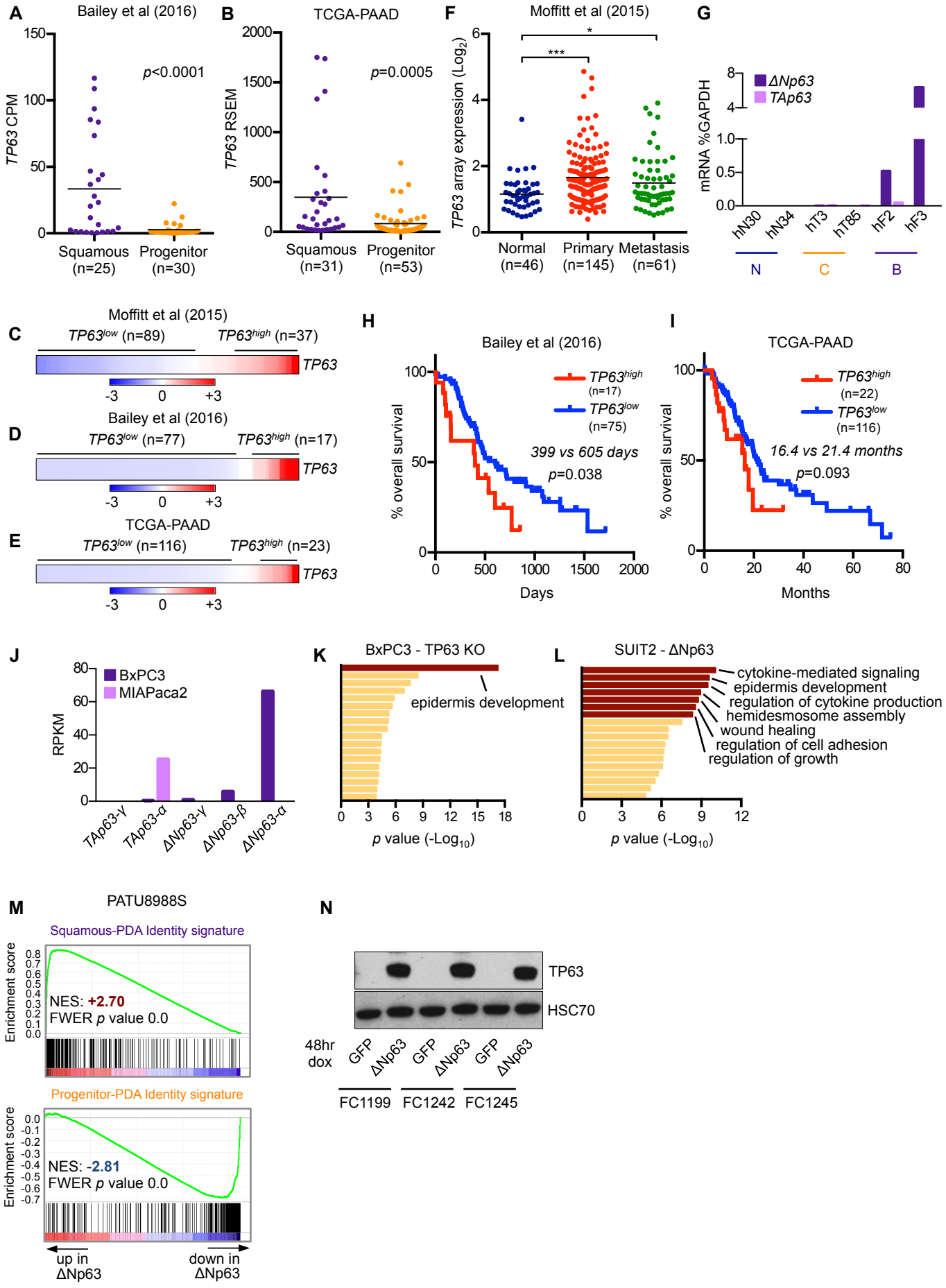
A
Bailey et al (2016)
TP63 CPM
p<0.0001
Squamous (n=25)
Progenitor (n=30)
B
TCGA-PAAD
TP63 RSEM
p=0.0005
Squamous (n=31)
Progenitor (n=53)
F
Moffitt et al (2015)
TP63 array expression (Log2)
***
*
Normal (n=46)
Primary (n=145)
Metastasis (n=61)
G
ΔNp63
TAp63
mRNA %GAPDH
hN30
hN34
hT3
hT85
hF2
hF3
N
C
B
C
Moffitt et al (2015)
TP63low (n=89)
TP63high (n=37)
TP63
D
Bailey et al (2016)
TP63low (n=77)
TP63high (n=17)
TP63
E
TCGA-PAAD
TP63low (n=116)
TP63high (n=23)
TP63
H
Bailey et al (2016)
% overall survival
TP63high (n=17)
TP63low (n=75)
399 vs 605 days
p=0.038
Days
I
TCGA-PAAD
% overall survival
TP63high (n=22)
TP63low (n=116)
16.4 vs 21.4 months
p=0.093
Months
J
BxPC3
MIAPaca2
RPKM
TAp63-γ
TAp63-α
ΔNp63-γ
ΔNp63-β
ΔNp63-α
K
BxPC3 - TP63 KO
epidermis development
p value (-Log10)
L
SUIT2 - ΔNp63
cytokine-mediated signaling
epidermis development
regulation of cytokine production
hemidesmosome assembly
wound healing
regulation of cell adhesion
regulation of growth
p value (-Log10)
M
PATU8988S
Squamous-PDA Identity signature
Enrichment score
NES: +2.70
FWER p value 0.0
Progenitor-PDA Identity signature
Enrichment score
NES: -2.81
FWER p value 0.0
up in ΔNp63
down in ΔNp63
N
TP63
HSC70
48hr dox
GFP
ΔNp63
FC1199
FC1242
FC1245

Figure S1. *TP63* is Aberrantly Expressed in a Subset of Pancreatic Tumors that Display a Squamous-like Transcriptional Signature and is Necessary to Drive Progenitor-to-Squamous Transcriptional Reprogramming in Human PDA cells. Related to Figure 1.

(A-B) Dot plots show *TP63* expression values in the indicated molecular subtypes in the indicated studies. Each dot represents one patient sample. *p* value was calculated using unpaired Student's t test.

(C-E) Heat maps show *TP63* expression across the indicated studies. In each study, samples were designated as $TP63^{high}$ or $TP63^{low}$ based on z-score expression values >0.35 or <0, respectively. Scale bar indicates the standardized expression value.

(F) *TP63* expression in normal pancreas, primary tumor and metastatic PDA samples. Data are from the study by Moffitt et al (2015). $^{*}p$ <0.05, $^{***}p$ <0.0001 by unpaired Student's t test.

(G) Isoform expression of *TP63* in human organoid samples. Bar chart shows RT-qPCR analysis of *ΔNp63* or *TAp63* isoform expression in human organoids derived from normal pancreas 'N' or tumor samples assigned to the classical 'C' or basal-like 'B' molecular subtypes from the study by Tiriac et al (2018).

(H-I) Survival curves of patients from the indicated studies stratified according to high or low *TP63* expression as in (C-E) for which survival information was available. *p* value was calculated using the log rank (Mantel-Cox) test.

(J) Bar chart shows *TP63* isoform expression in the indicated human PDA cell lines.

(K-L) Gene ontology (GO) analysis with Metascape of (K) significantly down regulated genes following TP63 knockout in BxPC3 cells or (L) significantly upregulated genes following ΔNp63 expression in SUIT2 cells. For GO analysis, terms are ranked by their significance (*p* value) and the most significant terms ($-\log_{10} p$ value >8) are highlighted.

(M) GSEA plot evaluating the Squamous-PDA and Progenitor-PDA Identity signatures upon ΔNp63 expression in PATU8988S cells.

(N) Representative Western blot analysis in the indicated murine KPC cells for the indicated proteins. Whole cell lysates were prepared 48 hours following doxycycline administration.

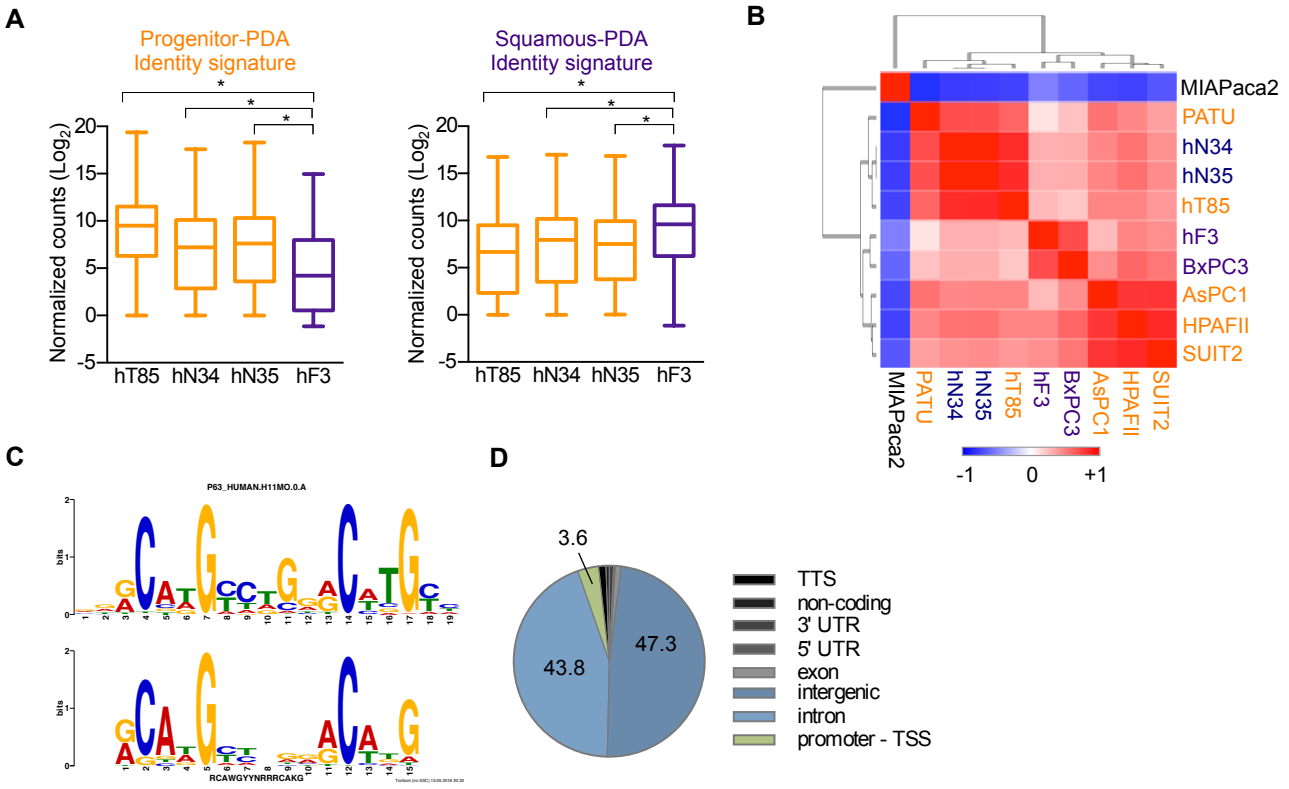

Figure S2. Progenitor-PDA and Squamous-PDA Identity Signature Expression in Human Organoids and a Unique Enhancer Landscape Linked to ΔNp63 Occupancy in PDA. Related to Figure 2.

(A) Box plots show expression levels of Progenitor-PDA Identity genes (left panel) or Squamous-PDA Identity genes (right panel) in the indicated human organoid samples. Data are from Tiriac et al (2018). $^{*}p$ <0.0001 by one-way ANOVA.

(B) Heat map representation of unsupervised hierarchical clustering of ten human cell lines or organoids representing PDA or normal pancreatic ducts based on H3K27ac occupancy at total H3K27ac ChIP-seq peaks. Global H3K27ac profiles were correlated by Pearson correlation and clustered by Euclidean distance with average linkage using Morpheus. Scale bar indicates Pearson correlation coefficient. Normal organoids, hN34, hN35; PDA organoids, hF3, hT85; PATU, PATU8988S.

(C) Position weight matrices for TP63 (top panel) and motif recovered from TP63 ChIP-seq in BxPC3 cells (bottom panel). Motif discovery was performed using MEME.

(D) Pie chart showing the genomic distribution of TP63 according to annotation of H3K27ac peaks by HOMER. TTS, transcription termination site; TSS, transcription start site; UTR, untranslated region.

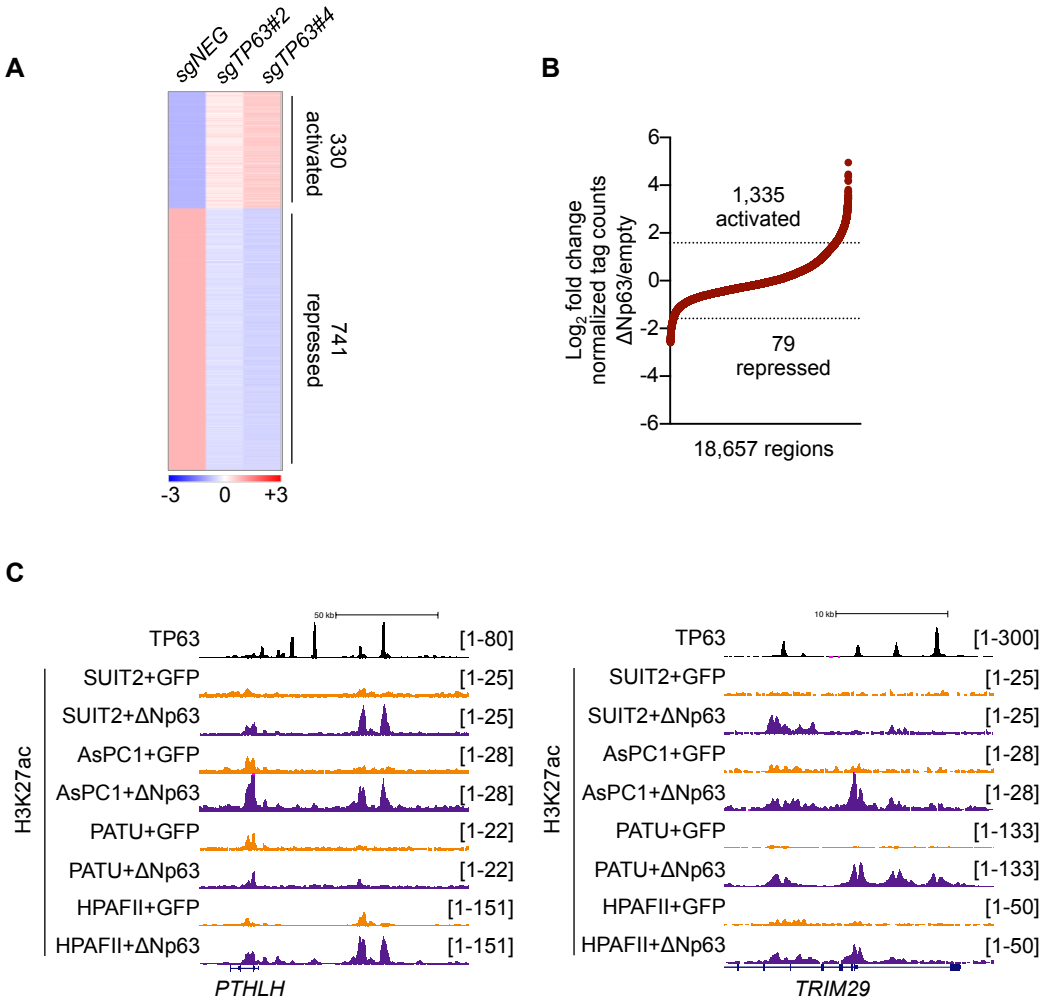

Figure S3. **ΔNp63 Expression is Sufficient to Install and Maintain a Squamous Enhancer Landscape in PDA. Related to Figure 3.**

(A) Heatmap representation of H3K27ac regions with a mean 3 fold change in signal intensity following TP63 knockout in BxPC3-Cas9 cells with two independent sgRNAs compared to those infected with control sgRNAs (sgNEG).

(B) Scatter plow shows H3K27ac regions with a mean 3 fold change in signal intensity following ΔNp63 expression in SUIT2 cells compared to those infected with an empty vector control (empty). Dashed lines indicate 3-fold change value.

(C) ChIP-seq profiles of TP63 (top track) and H3K27ac at representative squamous elements close to *PTHLH* (left panel) and *TRIM29* (right panel) in the indicated cell lines following doxycycline-inducible expression of ΔNp63 or GFP as a control. Cells were cross-linked and prepared for ChIP-seq analysis 48 hours following doxycycline administration.

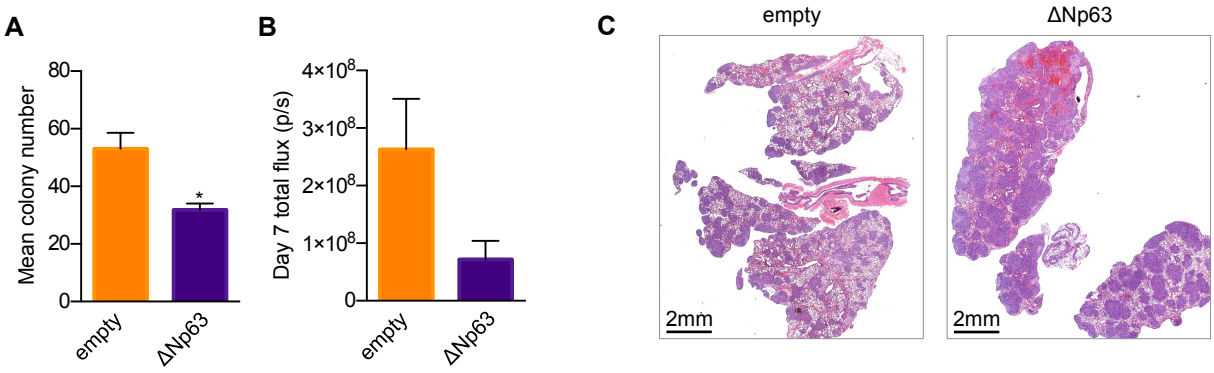

Figure S4. **Phenotypic Consequences of ΔNp63-mediated Squamous Lineage Reprogramming. Related to Figure 4.**
(A) Bar chart showing quantification of colony number per well in 3D Matrigel colony formation assays on day seven post plating, day 14 post viral transduction. Colony number was counted using ImageJ software (NIH). Mean+SEM is shown. n=3. $^{*}p$ <0.0001 by unpaired Student's t test. n=3.
(B) Bar chart showing quantification of bioluminescence signal on day seven post transplantation of cells to the pancreas of immune-deficient mice. Mean+SEM is shown. n=4.
(C) Representative H&E staining of lung tissue for the indicated samples from Figure 4F.

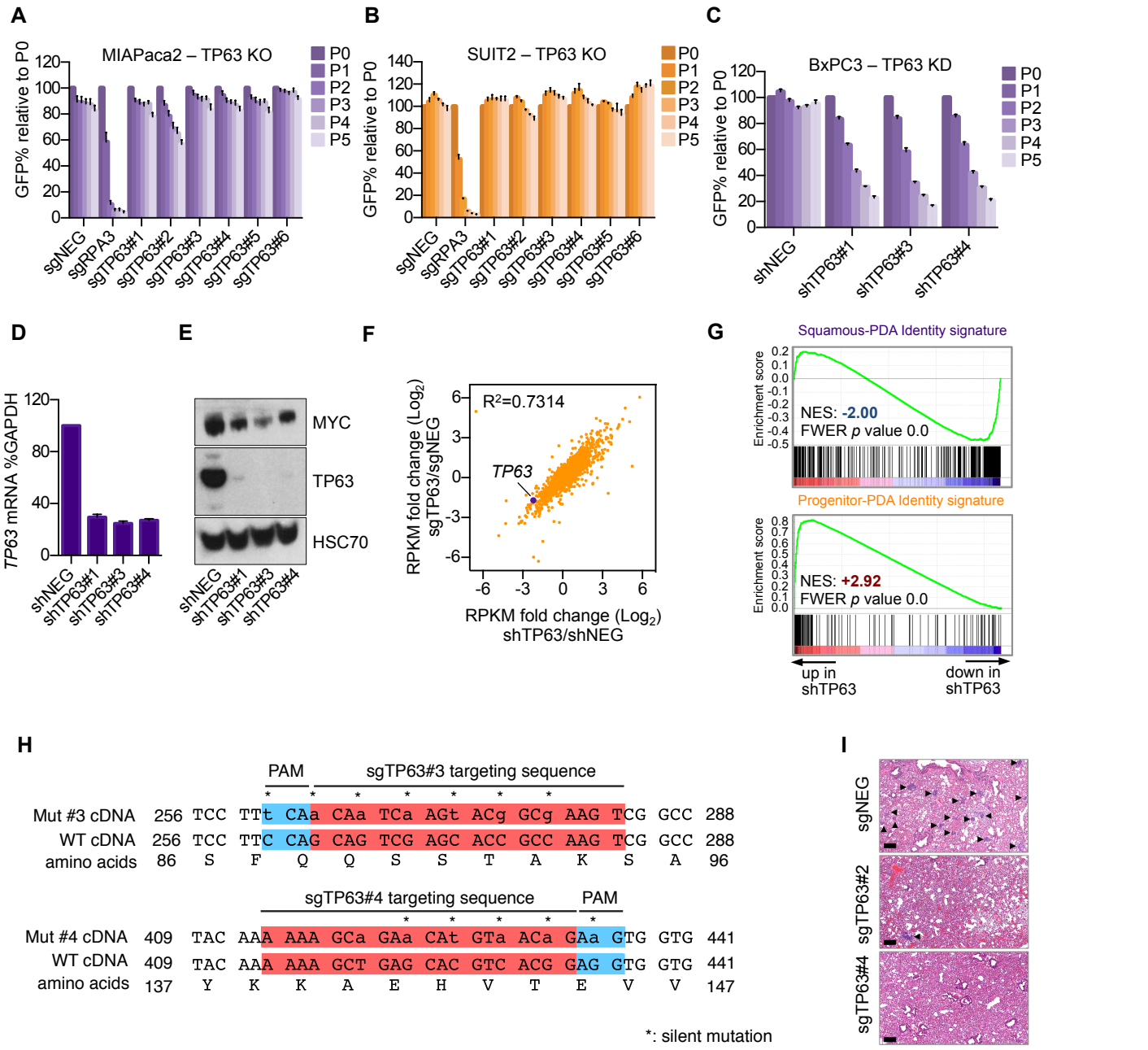

Figure S5. ΔNp63 is a Dependency in Squamous PDA Cells. Related to Figure 5.

(A-C) Competition based proliferation assays in the indicated cells following infection with the indicated sgRNAs (A-B) or shRNAs (C) linked to GFP. CRISPR targeting of *TP63* in SUIT2 cells was performed as a negative control to rule out growth arrest as a non-specific effect of forming double-strand DNA breaks associated with CRISPR-Cas9 genome editing. Mean+SEM is shown. n=3.

(D-F) TP63 knockdown in BxPC3 cells. (D) Bar chart shows RT-qPCR analysis of *TP63* mRNA following infection with the indicated shRNAs. RNA was extracted on day seven post infection with shRNAs, five days post selection with puromycin. Mean+SEM is shown. n=3 technical repeats.

(E) Representative Western blot analysis for the indicated proteins in the indicated conditions. Whole cell lysates were prepared on day five post infection with shRNAs, three days post selection with puromycin. (F) Scatter plot shows the mean fold change in RPKM values of 10,300 expressed genes (RPKM ≥2) comparing two independent shRNAs or sgRNAs targeting TP63 compared to shNEG or sgNEG, respectively.

(G) GSEA plots evaluating the Squamous-PDA and Progenitor-PDA Identity signatures upon TP63 knockdown in hF3 organoids. RNA was extracted on day eight-post infection with shRNAs, five days post selection with puromycin.

(H) Illustration of ΔNp63 cDNA rescue assay. Multiple silent mutations were cloned into the ΔNp63 cDNA to disrupt base-pairing with TP63 sgRNA#3 (Mut#3, top panel) or TP63 sgRNA#4 (Mut #4, bottom panel).

(I) Representation H&E staining of lung tissue for the indicated samples from Figure 5E. Arrowheads indicate tumor lesions. Scale bar indicates 250μm.

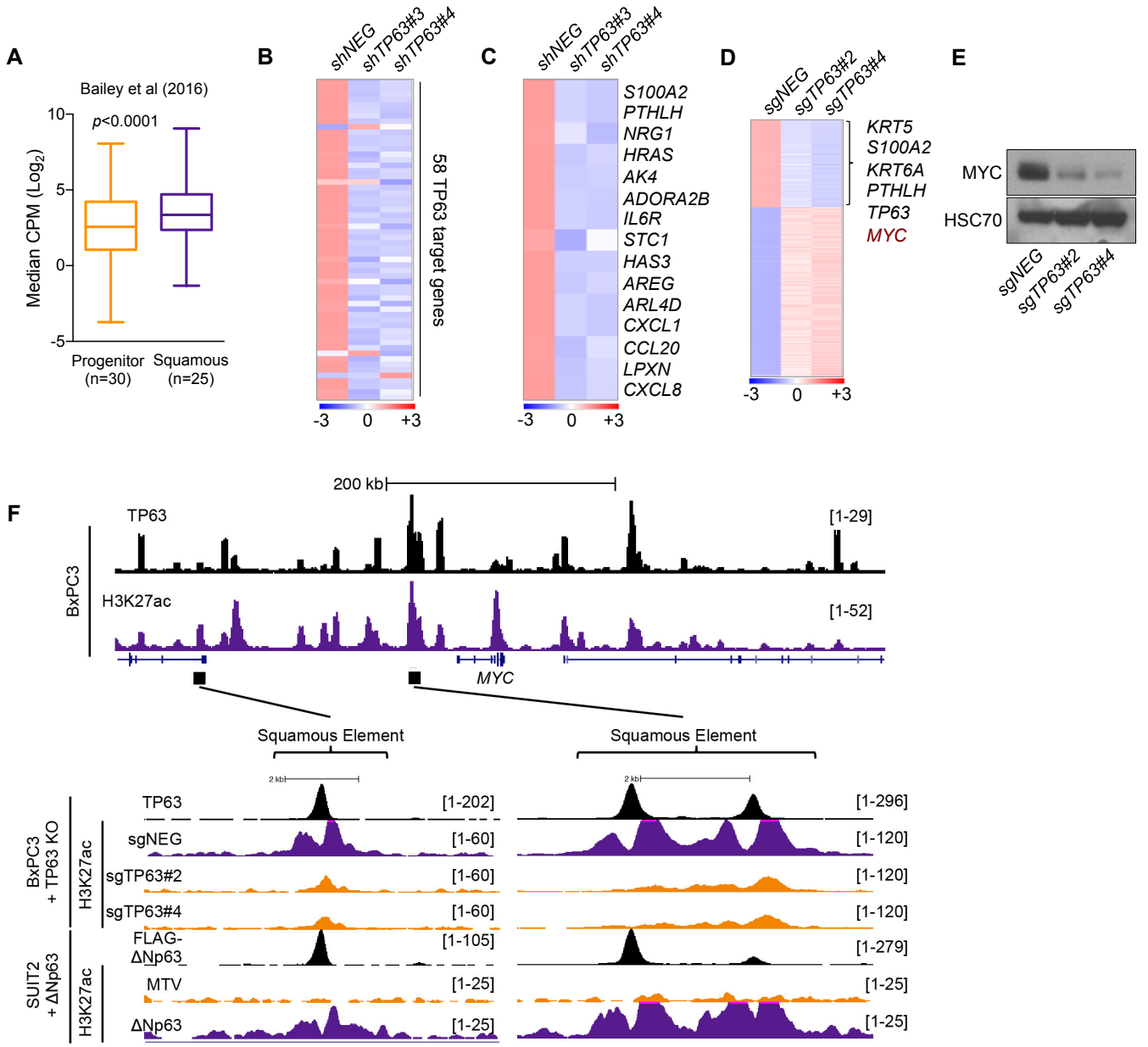

Figure S6. The Core Circuitry of ΔNp63-mediated enhancer Reprogramming in Squamous PDA Cells. Related to Figure 6.
(A) Box plots show median expression values of the 58 ΔNp63 target genes in Progenitor or Squamous PDA tumors.
(B-C) Heatmap representation of the 58 TP63 target genes from Figure 6A (B) or those with oncogenic potential from Figure 6B (C) following TP63 knockdown in hF3 organoids.
(D) Heatmap representation of significantly changed genes following TP63 knockout in BxPC3 cells. Selected genes significantly down regulated following TP63 knockout are highlighted.
(E) MYC expression following TP63 knockout in BxPC3 cells. Representative Western blot analysis for the indicated proteins in the indicated conditions. Whole cell lysates were prepared on day five post infection with sgRNAs, three days post selection with G418.
(F) ChIP-seq profiles of TP63 and H3K27ac surrounding the MYC locus in BxPC3 cells (top panel) and at the indicated Squamous Elements following TP63 knock out or expression in BxPC3-Cas9 cells and SUIT2 cells, respectively (bottom panel).