Supplementary Materials and Method

Immunostaining and Western Blot Analysis

For immunofluorescence staining, mouse and human cells were fixed with 4% paraformaldehyde-PBS for 15 min. Following Triton-X100 permeabilization and blocking, cells were incubated with primary antibodies overnight at 4°C following with Alexa 594-conjugated secondary antibodies at 4°C for 1 hour (Thermo Fisher Scientific, 1:1000). Samples were mounted using VECTASHIELD Antifade Mounting Medium with DAPI (Vector Laboratories) and immunofluorescence was detected using Olympus confocal microscopy. For western blot analysis, cells were lysed on ice using RIPA buffer supplemented with protease and phosphatase inhibitors (Sigma).

Primary Antibodies for Immunostaining and Western Blot Analysis: Yap (14074, Cell Signaling), pYAP (4911, Cell Signaling), Lats1 (3477, Cell Signaling), pLats1(8654, Cell Signaling), Wnt5a (2530, Cell Signaling), cleaved Caspase-3 (9661, Cell Signaling), Ki-67 (VP-K451, Vector Laboratories), Cyr61 (sc-13100, Santa Cruz Biotechnology), CTGF (sc-14939, Santa Cruz Biotechnology), AXL (8661, Cell Signaling), pErk (4376, Cell Signaling), pMEK (4376, Cell Signaling), Ck-19 (16858-1-AP, Proteintech), Actin (A2228, Sigma Aldrich), Vinculin (V4139, Sigma Aldrich), Kras (sc-30, Santa Cruz Biotechnology).

Ectopic expression of YAP1 and WNT5A in mouse and human cells

To generate $YAP1^{S127A}$-expressing stable Pa04C cells, Pa04C cells were transfected with a linearized pcDNA3.1 plasmid with or without YAP1 cDNA containing S127A substitution. Two days post-transfection using Lipofectamine1000, cultures were selected in G418 (Sigma) and single clones were picked and expanded for further analysis. Overexpression of YAPS127A or WNT5A in human or mouse cells other than Pa04C were acheieved with lentivral infection. Briefly, lentivirus infection was performed by transfecting 293T cells with either GFP control,

YAP1^{S127A}, or WNT5A cloned in pHAGE lentivirus vector {EF1α promoter-GW-IRES-eGFP (GW: Gateway modified)}. The virus was concentrated using ultracentrifuge and added to target cells in a 6-well plate containing 10ug/ml of polybrene (Millipore). 48 hours after infection GFP positive cells were selected by flow sorting.

Lentivirus Mediated shRNA Knockdown

The clone IDs for shRNA are as follows: sh_mouse Yap1-1 (TRCN0000238436), sh mouse Yap1-2 (TRCN0000095864), sh_huYap1-1 (TRCN0000107265), sh_huYap1-2 (TRCN0000107266), sh_huWnt5a-1 (TRCN0000062717), sh_huWnt5a-2 (TRCN0000288987), sh_hu Kras-1 (TRCN0000033260), sh_hu Kras-2 (TRCN0000033262).

Crispr-Cas9 Mediated Gene Knockout

Sequences for *Wnt5a* sgRNA are as follows:

sgRNA-1 F: CTTGAGAAAGTCCTGCCAGT; R: ACTGGCAGGACTTTCTCAAG.

sgRNA-2 F: GAAACTCTGCCACTTGTATC; R: GATACAAGTGGCAGAGTTTC.

sgRNA-3 F: TATACTTCTGACATCTGAAC; R: GTTCAGATGTCAGAAGTATA.

sgRNA-4 F: ACAGCCTCTCTGCAGCCAAC, R: GTTGGCTGCAGAGAGGCTGT.

Sequences for *Yap1* sgRNA are as follows:

sgRNA-1 F: ACCAGGTCGTGCACGTCCGC; R: GCGGACGTGCACGACCTGGT.

sgRNA-2 F: CCCCGCGGACGTGCACGACC; R: GGTCGTGCACGTCCGCGGGG.

Xenograft Studies

For orthotopic xenografts, 5×10^5 cells were injected pancreatically into NCr nude mice (Taconic) and tumor growth was monitored with bioluminescent imaging as described[1]. For Sub-Q xenografts, 1×10^6 cells (mouse tumor cells) or 3×10^6 cells (human PDAC or PDX cells) were injected subcutaneously into the lower flank of NCr nude mice. Tumor volumes were measured

every 7 days starting from Day 7 post injection and calculated using the formula, volume = length × width^2/2.

Immunoprecipitation Assay

Immunoprecipitation of YAP1 complexes was performed by lysing cells with 1% NP40 lysis buffer containing phosphatase and protease inhibitor cocktails on ice for 45 minutes. 1 mg of lysate was incubated overnight at 4^{o}C with primary antibodies followed with protein A/G Plus-agarose (sc-2003, Santa Cruz) for 3 hours at 4^{o}C. Immunoprecipitates were washed three times with lysis buffer, then re-suspended with 2X sample buffer boiled for 5 min and detected by western blot analyses.

Quantitative Real-time Polymerase Chain Reaction Analysis

RNA from cell lines and pancreas tissues was isolated using RNeasy Mini Kit (Qiagen) and first-strand cDNA was synthesized from 2 μg total RNA using random primers and Omniscript® Reverse Transkriptase Kit (Qiagen). Actin was used as housekeeping gene. Real-time polymerase chain reaction experiments were performed in triplicates and are displayed ± SD.

Clonogenic assay

500-2000 cells were seeded into each well of 6-well plate in triplicates and incubated to allow colony formation for 10-20 days. The colonies were stained with 0.2% crystal violet in 80% methanol for 30 minutes at room temperature and de-stained upon which they were scanned and colonies counted using Image J (http://rsb.info.nih.gov/ij/).

Gene Expression and PDAC-Subtype Analysis

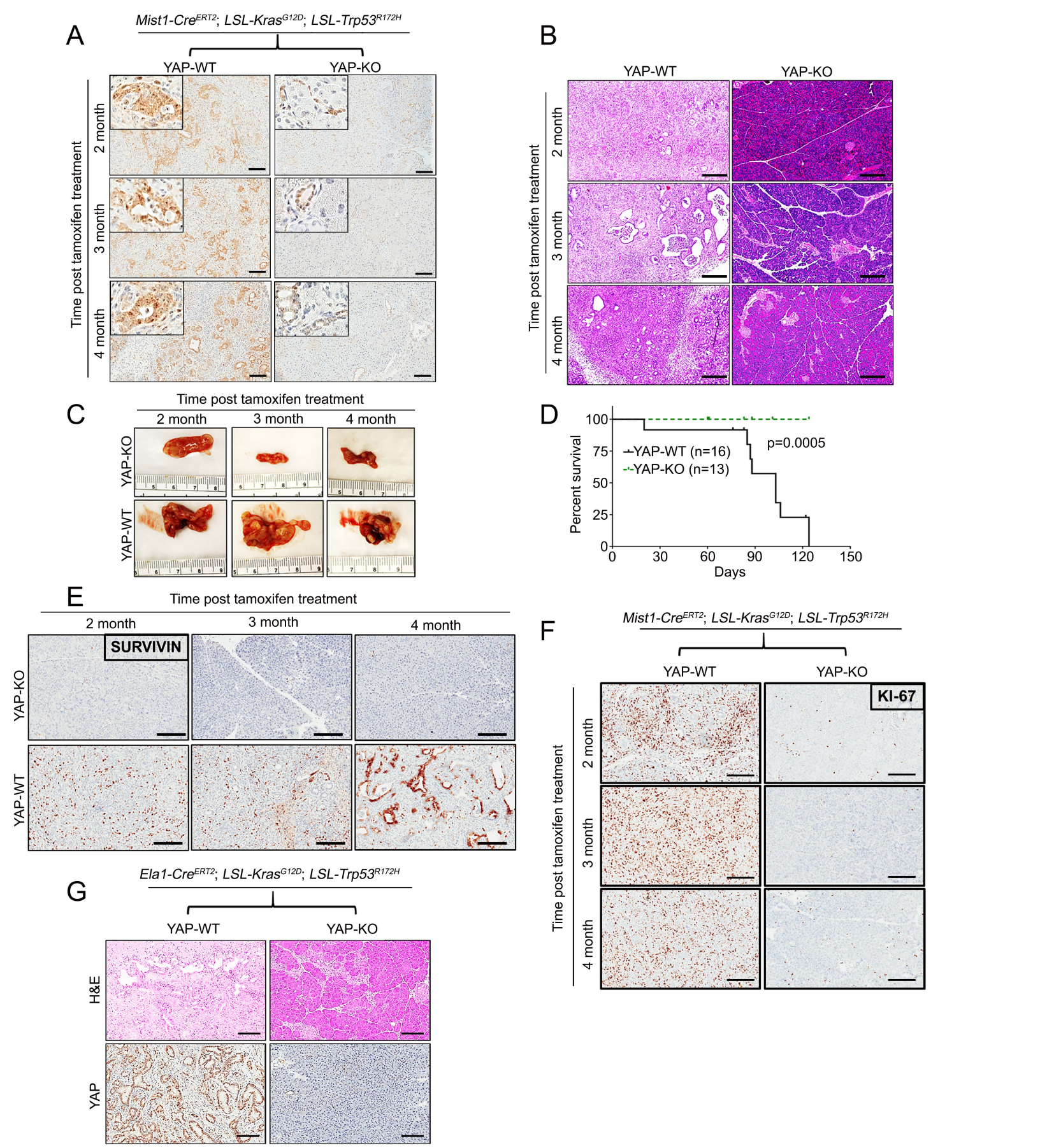
mRNA expression profiling on Illumina microarrays were performed according to the manufacture's protocol. Raw data was processed using Genome Studio (GSGX Version 1.6.0) and analysis was done using group quantile normalization with background subtraction. Complete

profiles are available at GEO at GSE135754. The software package LIMMA (Linear Models for Microarray Data) was applied to detect significantly differentially expressed probes using Benjamini-Hochberg adjusted p-values. For GSEA analysis, gene sets collection from MSigDB 3.0 and Kyoto Encyclopedia of Genes and Genomes (KEGG) were included in the analysis.

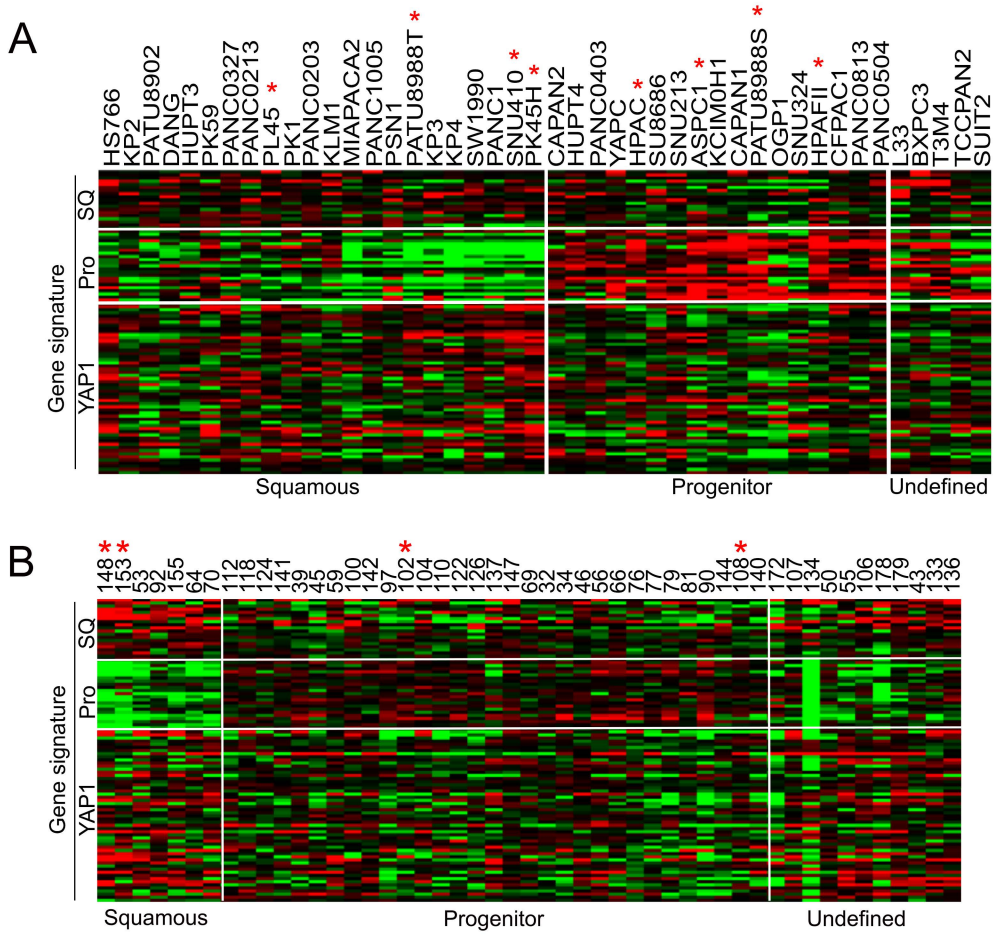
For molecular subtype analysis, we combined subtype specific genes from Collisson et al and Bailey et al studies to construct a "NanoString signature" comprised of 32, 23, and 17 subtype specific genes for squamous, progenitor, and ADEX subtypes, respectively (see supplementary table 2). To call PDAC subtypes in human PDAC cell lines or PDXs, we used the following algorithm. First, subtype signature scores were calculated by summing up Z scores from subtype specific genes which were ceiled at 2.5 and bottomed at -0.5. Kmeans two separation were then done to call high and low groups within each subtype. SQ/Pro subtypes are then called as squamous gene high/progenitor gene low and vice versa. ADEX subtype is called as ADEX gene high and SQ/Pro gene low. The remaining tumors are undefined. The nanoString call results are further confirmed by heatmap visualiation using Collission genes (data not shown)[2]. The clusters were viewed using Java TreeView (version 1.1.6r4) [3].

Supplementary Reference

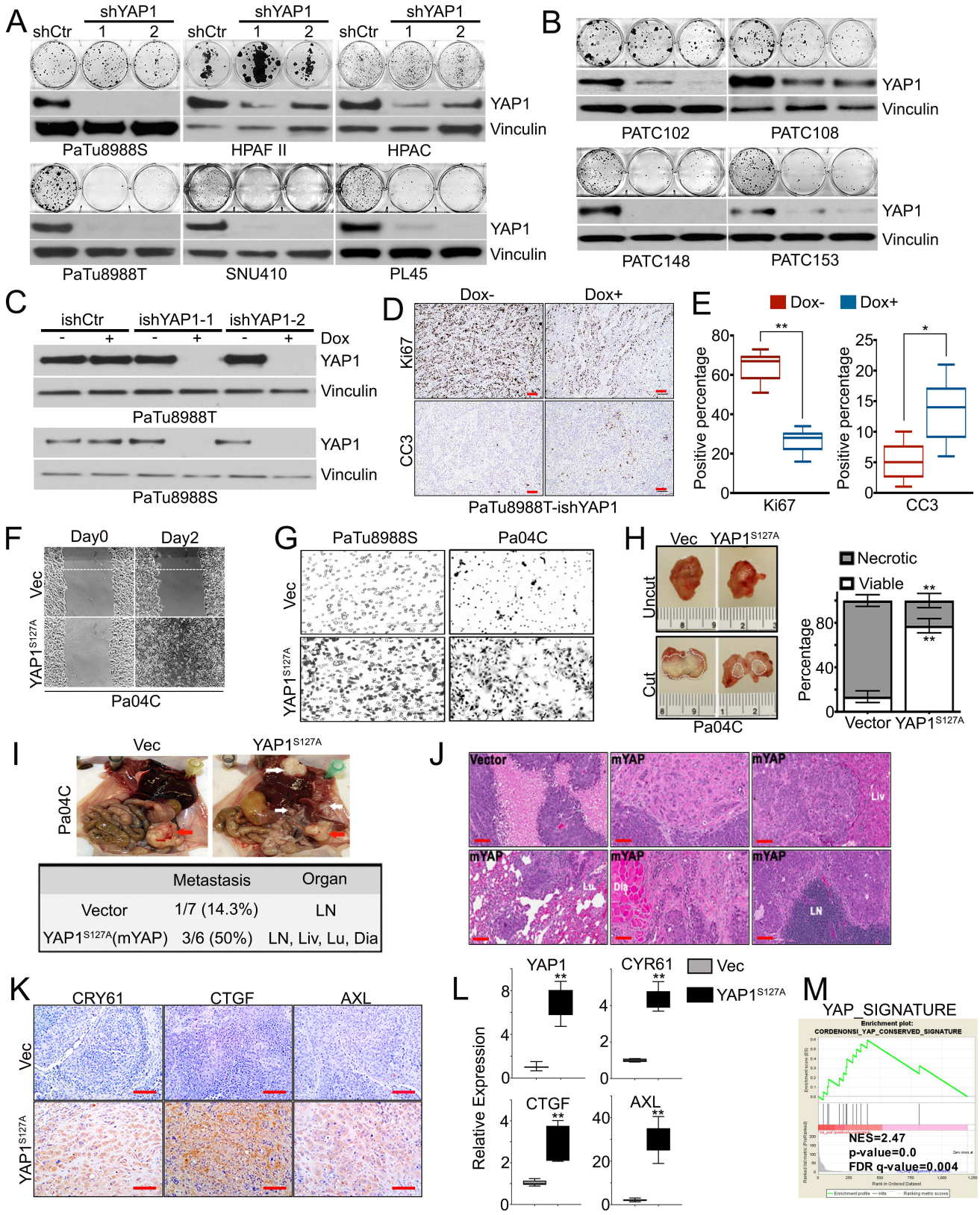
1. Kapoor A, Yao W, Ying H, et al. Yap1 activation enables bypass of oncogenic Kras addiction in pancreatic cancer. *Cell* 2014;158(1):185-97. doi: 10.1016/j.cell.2014.06.003 [published Online First: 2014/06/24]
2. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* 2011;17(4):500-3. doi: 10.1038/nm.2344 [published Online First: 2011/04/05]
3. Saldanha AJ. Java Treeview--extensible visualization of microarray data. *Bioinformatics* 2004;20(17):3246-8. doi: 10.1093/bioinformatics/bth349



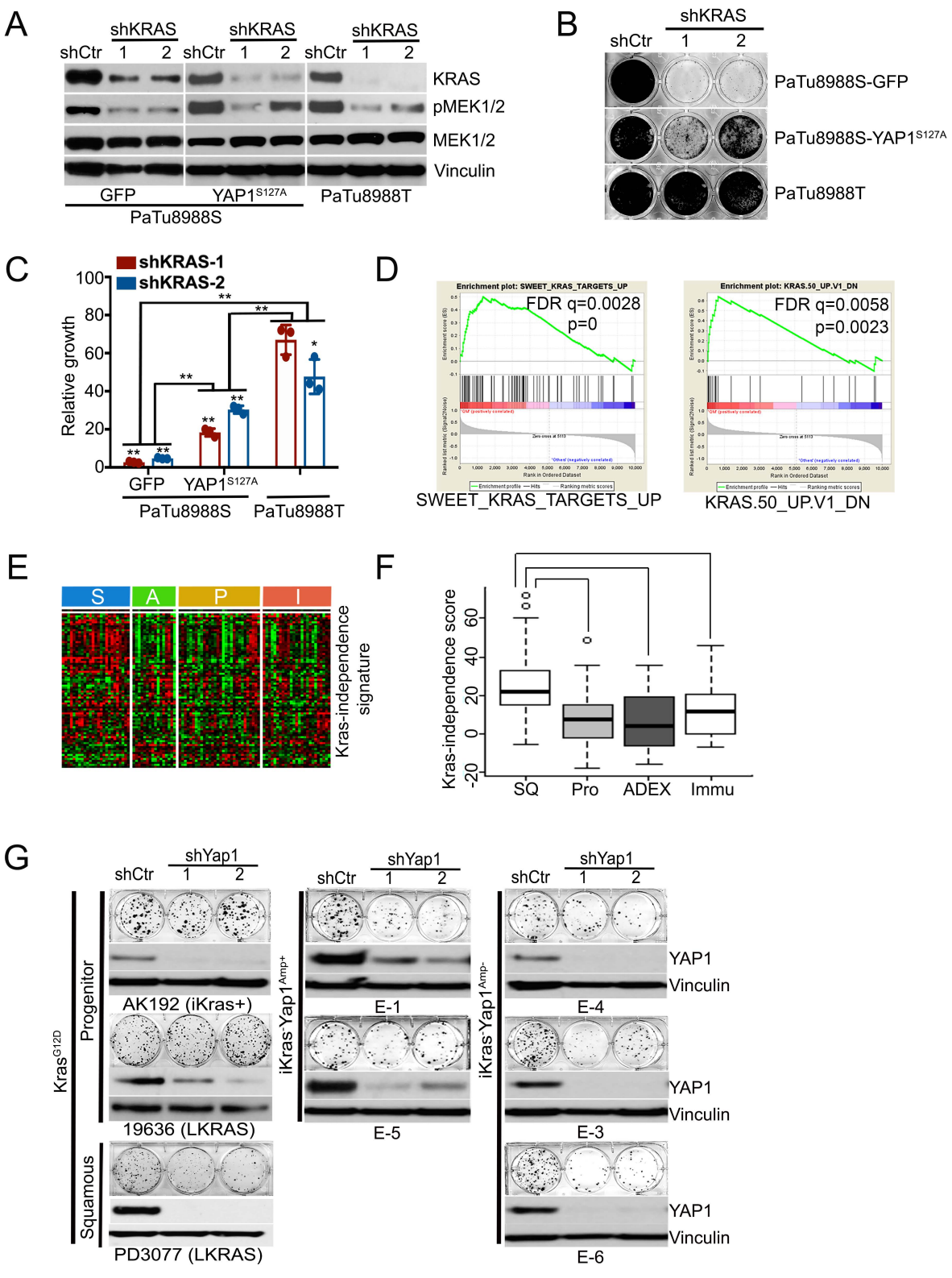
Supplementary Figure 1. YAP1 is essential for PDAC development. (**A**) *Yap1* wild-type (WT) or knockout (KO) MKP mice driven by tamoxifen-inducible Mist1-CreERT2. Pancreata were collected at indicated time points, and YAP1 staining shows YAP1 activation in PDAC tumors of MKP mice. Scale bar: 50 μm. (**B-C**) Gross images of pancreata (**B**) and H&E staining (**C**) show block of tumor development in *Yap1*-KO mice. Scale bar: 100 μm. (**D**) Kaplan-Meier overall survival analysis for *Yap1*-WT and *Yap1*-KO MKP mice. The *P* value for survival analysis was calculated with the log rank test. (**E-F**) Pancreata from *Yap1* WT or KO MKP mice were collected at indicated time points and stained for Survivin (**E**) or Ki67 (**F**). For each time point (age group), at least 4 mice per genotype were analyzed. Scale bar: 100 μm. (**G**) *Yap1* wild-type (YAP-WT) or knockout (YAP-KO) *Kras*^{G12D};*Trp53*^{R172H} mice driven by *Elastase-Cre*^{ERT2} upon tamoxifen injection. Pacreata collected at 4 months post-injection and stained for H&E (Top) and YAP1 (Bottom) Scale bar: 100μm.



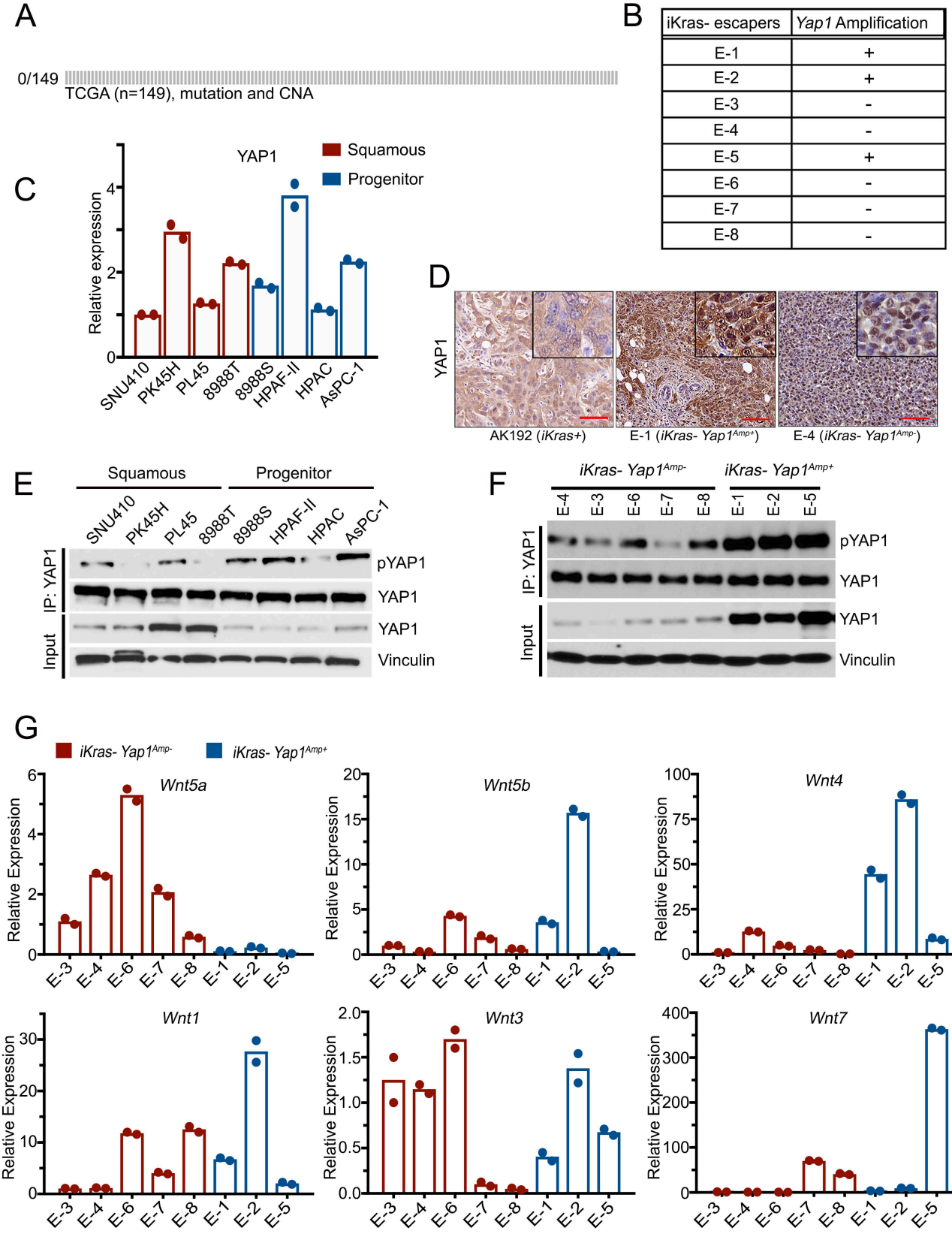
Supplementary Figure 2. YAP1 is activated in the squamous subtype of human PDAC. (**A-B**) Clustering of human PDAC cell lines (**A**) and human PDXs (**B**) based on subtype-specific gene signatures. Heatmap of squamous (SQ), progenitor (PRO), and YAP1 activation signature is shown. Asterisk: cell lines used in this study.



Supplementary Figure 3. YAP1 is essential for the maintenance of squamous subtype of PDACs. (**A-B**) Representative images of the colony formation assay in human PDAC cell lines (**A**) or PDX cells (**B**) infected with YAP1 shRNAs or non-targeting shRNA (shCtr). The YAP1 knockdown efficiency was detected by Western blot. (**C**) Western blot analysis for YAP1 in PaTu8988S and PaTu8988T cells engineered with inducible shRNA targeting YAP1 in the presence or absence of doxycycline for 72 hours. Inducible non-targeting shRNA (ishCtr) was used as control. (**D**) Subcutaneous xenograft tumors from inducible shYAP1-infected PaTu8988T cells were treated with vehicle or doxycycline for 1 week. Tumors were collected and stained for Ki67 or cleaved Caspase3 (CC3). Scale bar: 100 μm. Percentage of positive cells is shown in (**E**). Error bars represent ±SD (n=6 fields, 250 cells/field). *: p<0.05; **: p<0.01. (**F**) In vitro wound healing assay for Pa04C cells expressing YAP1^{S127A} or control vector (Vec). (**G**) Ectopic expression of YAP1^{S127A} in PaTu8988S and Pa04C cells promotes cell invasion in a Boyden chamber assay. (**H**) Representative gross images of orthotopic tumors from Pa04C-Vector (Vec) or Pa04C-YAP^{S127A} cells. Left panel shows uncut tumors (top) and tumors cut in half to reveal necrotic area (bottom). Right panel shows quantification of necrotic area. **: $P < 0.01$. (**I**) Orthotopic xenograft tumors from Pa04C-Vector or -YAP1^{S127A} cells (top). Red arrows indicate primary tumors and white arrows indicate metastatic tumors grown on peritoneum, lymph node and liver. Metastasis rate and involved organs are summarized in bottom panel. (**J**) H&E staining of orthotopic primary and metastatic tumors show metastases of Pa04C-YAP1^{S127A} (mYAP) cells in liver (Liv), lung (Lu), diaphragm (Dia) and lymph node (LN). Scale bar: 100μm. (**K**) Orthotopic xenograft tumors from Pa04C cells expressing vector (Vec) or YAP1^{S127A} were stained for CYR61, CTGF and AXL. Scale bar: 100μm. (**L**) Relative mRNA levels of YAP1 and indicated YAP1-target genes in Pa04C cells expressing vector (Vec) or YAP1^{S127A}. Error bars indicate ±SD of triplicates. **: $P < 0.01$. (**M**) GSEA plot of YAP1 activation signature based on the gene expression profiles of vector- vs YAP1^{S127A}-expressing Pa04C cells. NES denotes normalized enrichment score.

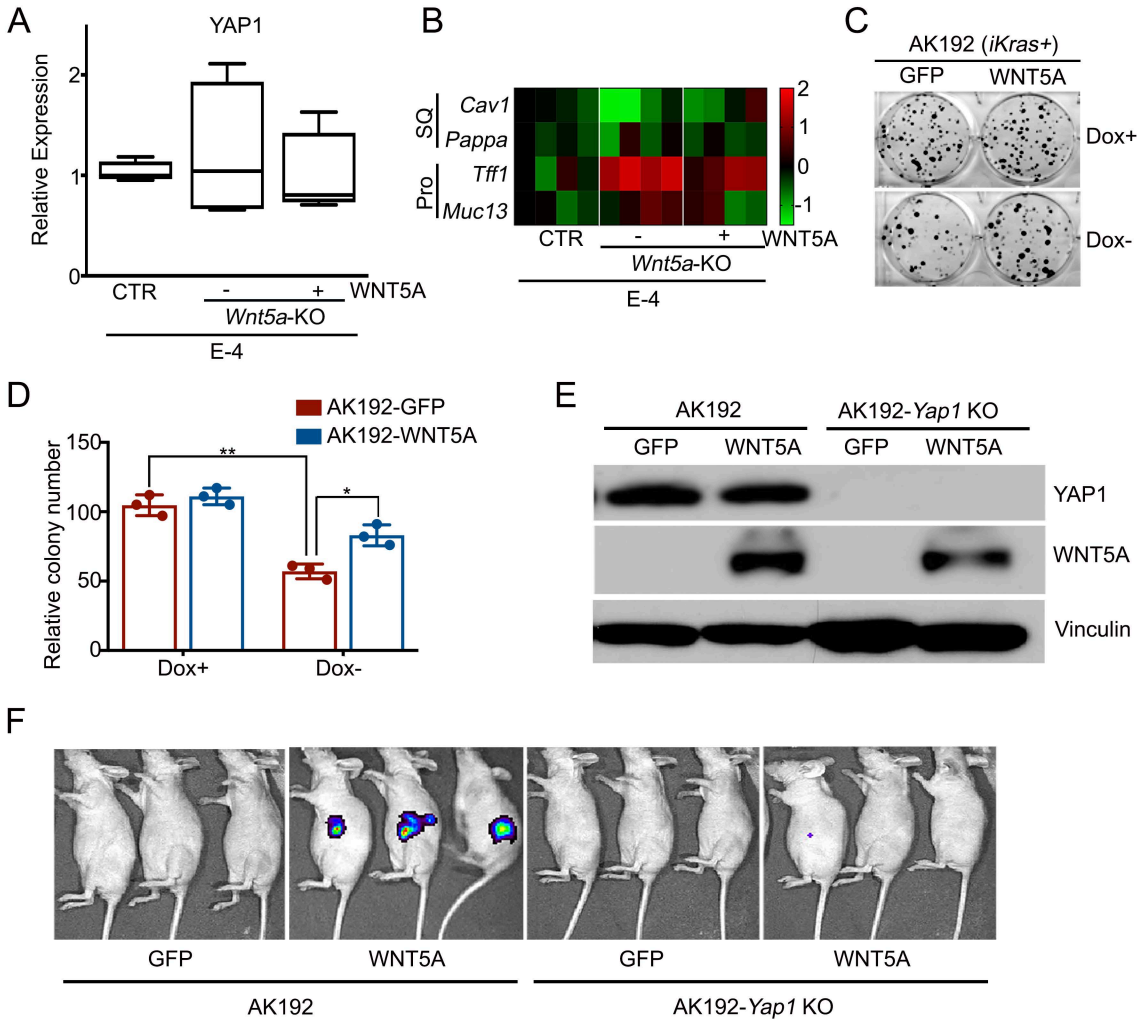


Supplementary Figure 4. YAP1 bypasses KRAS-dependency in PDAC. (**A**) Western blot analysis for KRAS, phosphor-MEK1/2 and MEK1/2 in PaTu8988S cells expressing GFP or YAP1^{S127A} and PaTu8988T cells upon knockdown of KRAS with two independent shRNAs. Non-targeting shRNA (shCtr) was used as control. (**B**) Representative images of the cell growth assay in human PDAC cell lines infected with KRAS shRNAs or non-targeting shRNA (shCtr). Quantification from triplicates is shown in (**C**) and is presented as relative growth upon normalization to shCtr group. Error bars indicate ±SD. *: $P < 0.05$; **: $P < 0.01$. (**D**) GSEA plots show genes induced upon KRAS knockdown or suppressed upon KRAS expression are enriched in squamous subtype human PDAC. (**E**) Heatmap shows the expression of KRAS-independence gene signature among human PDAC subtypes in PDAC TCGA dataset with quantification shown in (**F**). S: squamous subtype; A: ADEX subtype; P: progenitor subtype; I: immunogenic subtype. (**G**) Representative images of the colony formation assay in mouse PDAC cells infected with Yap1 shRNAs or non-targeting shRNA (shCtr). The YAP1 knockdown efficiency was detected by Western blot.



iKras- escapers	*Yap1* Amplification
E-1	+
E-2	+
E-3	-
E-4	-
E-5	+
E-6	-
E-7	-
E-8	-

Supplementary Figure 5. YAP1 activation in PDAC is mediated by WNT5A overexpression. (**A**) Mutation and copy number alteration (CAN) requency of *YAP1* in human PDAC from TCGA datasets. (**B**) *Yap1* gene amplification status in *iKras*- tumors. (**C**) mRNA expression level of YAP1 in human PDAC cell lines (n=2). (**D**) *iKras+*, *iKras- Yap1*$^{Amp-}$ and *iKras- Yap1*$^{Amp+}$ tumors were stained for YAP1. (**E-F**) Equal amount of YAP1 protein was immunoprecipitated from human PDAC cell lines (**E**) or mouse iKras- PDAC cells (**F**) and subjected to western blot analysis for phosphor-YAP1 (S127) and YAP1 (top). Input shows the western blot analysis for YAP1 in whole cell lysates (bottom). (**G**) Relative mRNA levels of indicated WNT ligands in mouse iKras- PDAC cells (n=2).



Supplementary Figure 6. WNT5A overexpression enables tumor maintenance and bypass of KRAS dependence. (**A**) Expression of Yap1 or subtype-specific genes (**B**) in subcutaneous xenograft tumors generated with E-4 (*iKras- Yap1*$^{Amp-}$) and E4-*Wnt5a* KO cells infected with GFP or WNT5A. (**C**) Representative images of the colony formation assay in mouse AK192 (*iKras+*) cells infected with GFP or WNT5A grown in the presence (Dox+) or absence (Dox-) of doxycycline. Quantification of colony formation from triplicates is shown in (**D**). Error bars from all panels indicate ±SD. *P* value was corrected with Dunnett's method. *: $P < 0.05$; **: $P < 0.01$. (**E**) Western blot analysis for YAP1 and WNT5A in AK192 or AK192-*Yap1* KO cells infected with GFP or WNT5A. (**F**) AK192 or AK192-*Yap1* KO cells expressing luciferase were infected with lentivirus expressing GFP or WNT5A and orthotopically injected into nude mice pancreas in the presence of doxycycline. Animals were withdrawn from doxycycline four days later and tumor growth was visualized by bioluminescent imaging at 6 weeks.