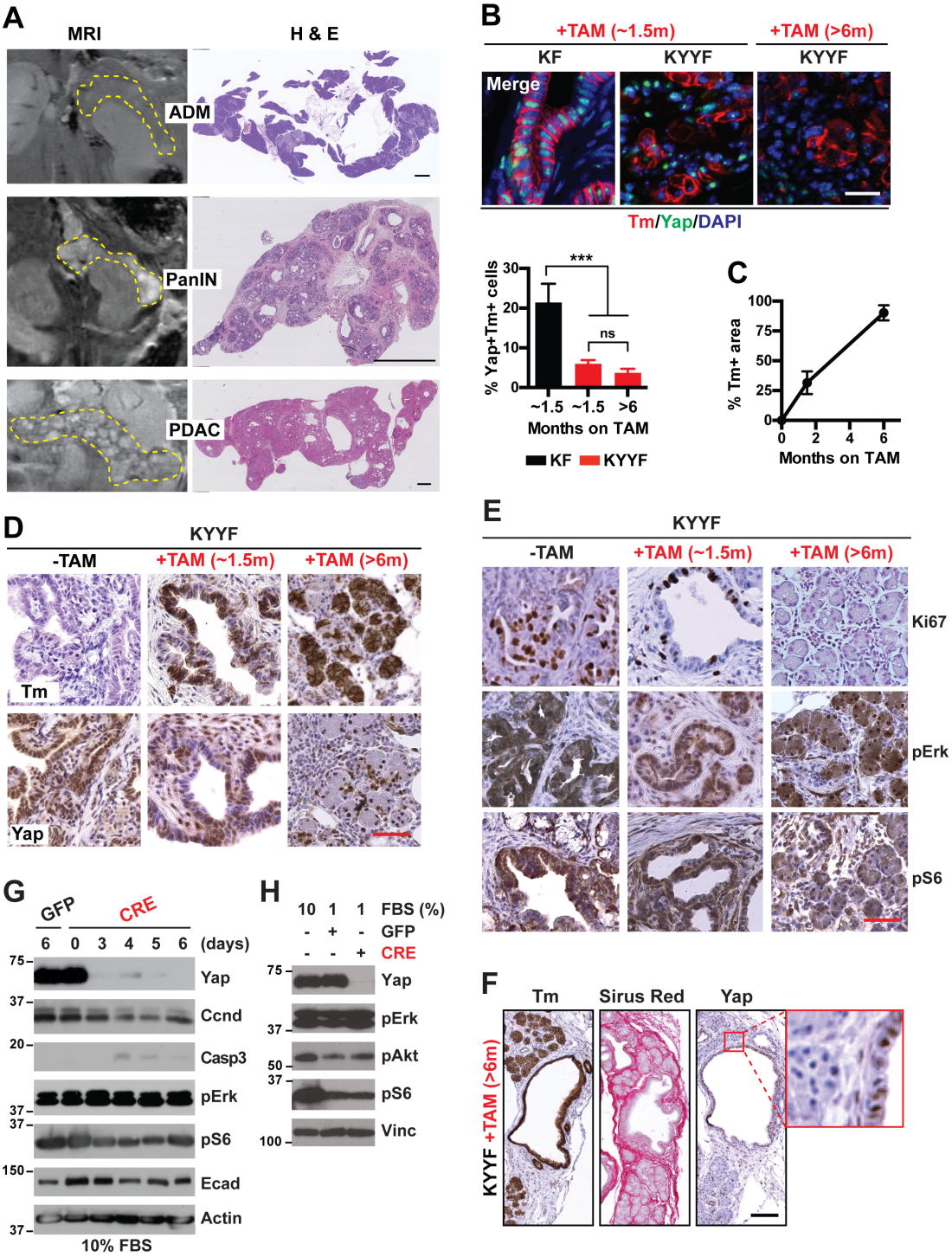


Murakami *et al*. Figure S1

Figure S1. Related to Figure 1; TAM treatment induces mosaic and progressive *Yap* loss in pancreatic tumors without affecting canonical Kras signaling.

(A) Representative MRI and corresponding H&E images of pancreatic lesions at different stages of disease progression. Scale bar = 1 mm. Yellow dotted line marks the pancreas in each MRI image.

(B) Representative images and quantification of IF staining for tdTomato (Tm, red), Yap (green) and DAPI (blue) in KF and KYYF pancreata after ~1.5 months and >6 months of TAM treatment. Scale bar = 100 μm. $n = 5$.

(C) Quantification of percent of Tm-positive area before or after 1.5 months or 6 months of TAM treatment.

(D) Representative IHC images of Tm and Yap in KYYF pancreata before or after ~1.5 months or >6 months of TAM treatment. Scale bar = 50 μm.

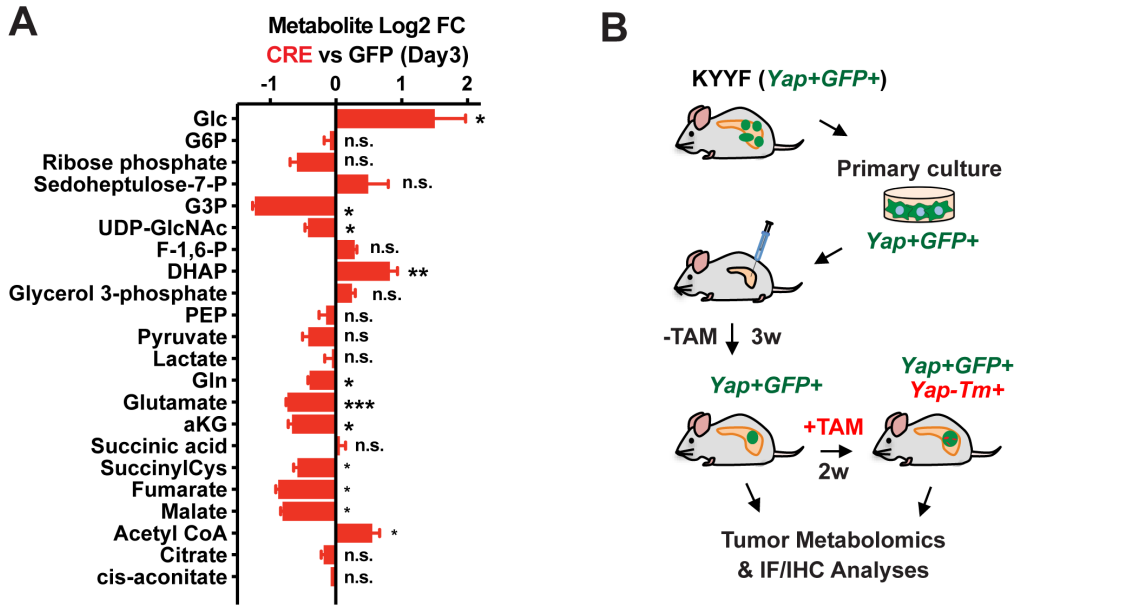
(E) Representative IHC images of Ki67, pErk, and pS6 in KYYF pancreata before or after ~1.5 months or >6 months of TAM treatment. Scale bar = 50 μm.

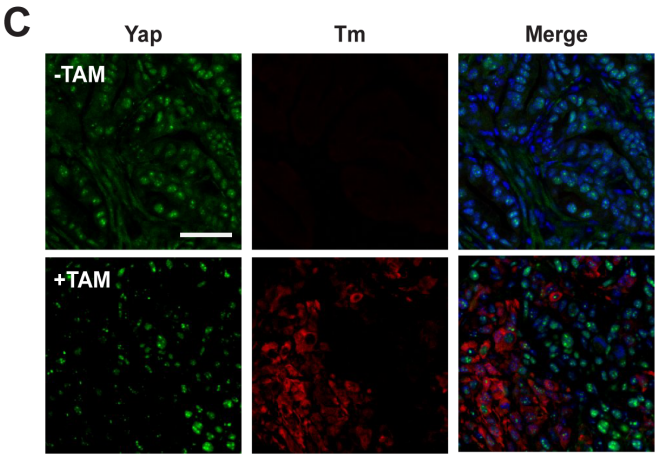
(F) IHC images of Tm, Sirius Red, and Yap in a matched region containing residual ductal lesions of a KYYF pancreas treated for >6 months with TAM. Scale bar = 100 μm.

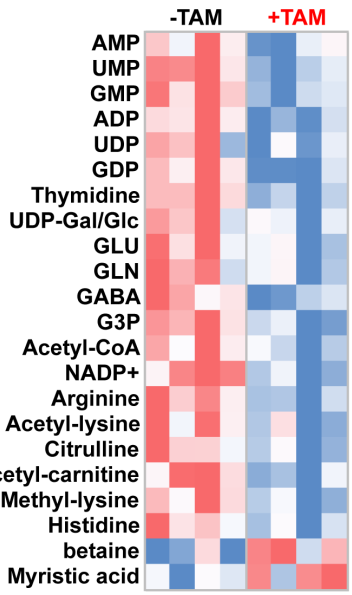
(G) Western blot analysis of indicated proteins in KYYF cells at different days post GFP or CRE treatment in growth medium containing 10% FBS. Actin was used as the loading control. Shown is representative of at least three independent experiments.

(H) Western blot analysis of indicated proteins in KYYF cells at different 5 days post GFP or CRE treatment in growth medium containing 1% FBS or 10% FBS. Vinculin (Vinc) was used as the loading control. Shown is representative of at least three independent experiments.

***$P < 0.0005$. ns: not significant. Error bars indicate s.d.







E

Pathways downregulated in TAM-treated tumors	*P value*
Nitrogen metabolism	2.23E-04
Arginine and proline metabolism	3.22E-04
Purine metabolism	2.45E-03
Pyrimidine metabolism	2.53E-03
Butanoate metabolism	3.61E-03

Murakami *et al*. Figure S2

Figure S2. Related to Figure 2; Yap loss induces global metabolic changes in vitro and in vivo.

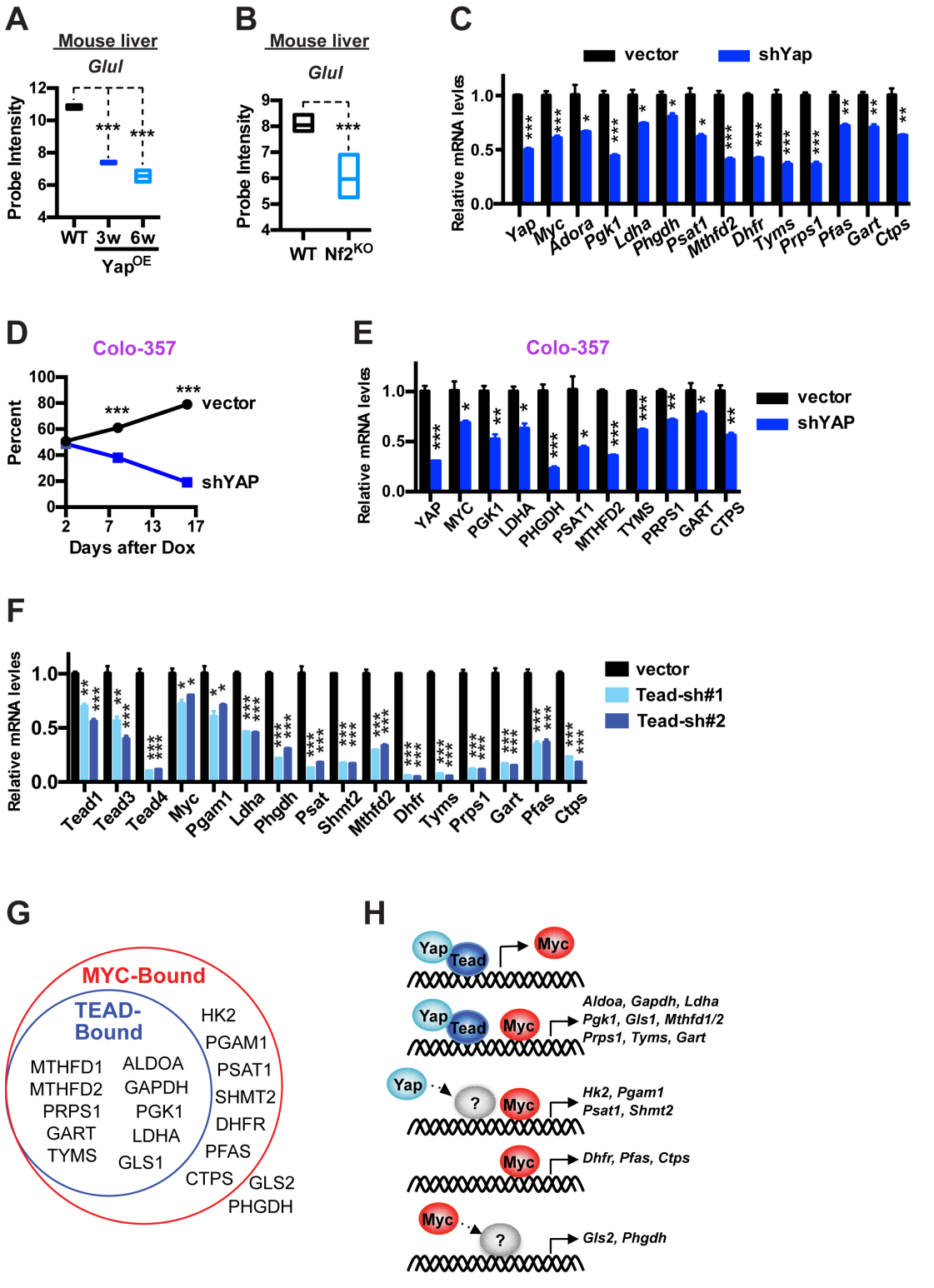
(A) Log2 FC in the levels of indicated metabolites related to glucose/glutamine metabolism as measured by LC-MS/MS. *P < 0.05. **P < 0.005. ***P < 0.0005. ns: not significant. Error bars indicate s.d.

(B) Illustration of the experimental design of orthotopic tumor studies. Primary pancreatic tumor cells were isolated from a tumor-bearing KYYF mouse that was not subjected to TAM treatment, labeled with a luciferase-GFP reporter in vitro, and injected orthotopically into nude mice. Once the tumor bioluminescence signal reaches to a pre-determined level at approximately three weeks post injection, tumor-bearing mice were either sacrificed for tumor harvesting or switched to a TAM-containing diet for two additional weeks to induce *Yap* deletion. Resected tumors were divided for metabolomics, IF and IHC analyses.

(C) Representative images of IF staining for Yap (green), Tm (red) and DAPI (blue) in KYYF pancreata after 15 days of TAM treatment. Scale bar = 100 μm. n = 5.

(D) Heatmap showing metabolites significantly changed between TAM-treated (+TAM) and untreated (-TAM) orthotopic pancreatic tumors as measured by LC-MS/MS. n = 4.

(E) P-value ranking of metabolic pathways significantly downregulated in TAM-treated versus untreated orthotopic pancreatic tumors in targeted LC-MS/MS metabolic analysis. n = 4.



Murakami *et al.* Figure S3

Figure S3. Related to Figure 3; Yap and Teads control the expression of Myc and metabolic genes in both mouse and human pancreatic tumor cells.

(A-B) Probe intensities of *Glul* in published microarray analysis comparing WT livers to livers induced to overexpress Yap for 3 or 6 weeks (Yap^{OE} 3w, or Yap^{OE} 6w) **(A)** or to *Nf2* knockout livers **(B)**.

(C) Relative mRNA levels of indicated genes in Kras mutant primary mouse pancreatic tumor cells expressing vector control or shYap. $n = 3$.

(D) Changes in the ratios of human Colo-357 PDAC cells expressing vector control or shYAP co-cultured over indicated time and analyzed periodically by flow cytometry. n = 3.

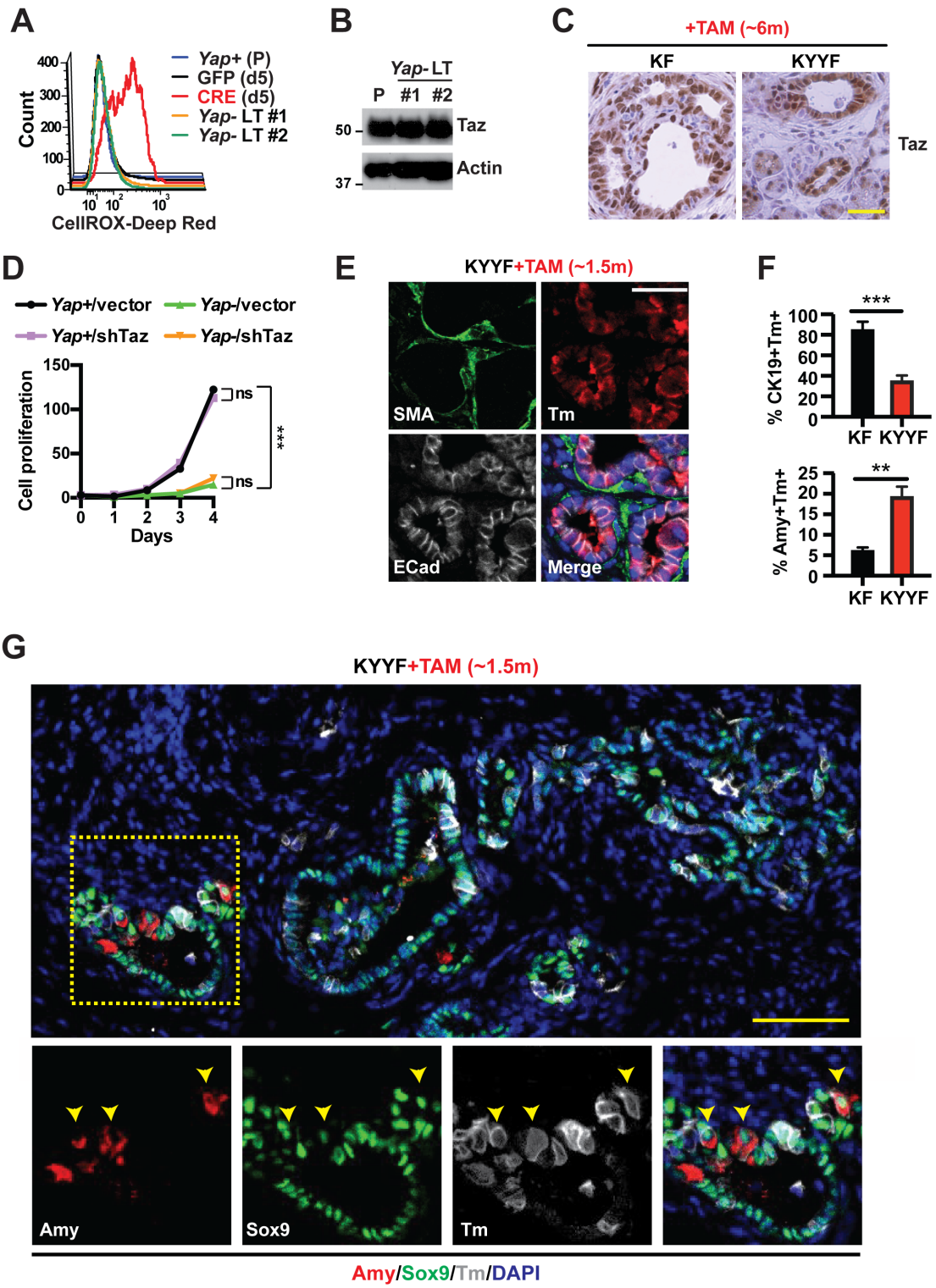
(E) Relative mRNA levels of indicated genes in Colo-357 expressing vector control or shYAP. $n = 3$.

(F) Relative mRNA levels of indicated genes in mouse pancreatic tumor cells expressing vector control, or two independent shTeads. $n = 3$.

(G) Venn diagram and representative enrichment peaks of H3K27ac, MYC, and TEAD4 along the promoters of Yap-regulated metabolic genes illustrating the statuses of TEAD4 or MYC binding based on matched published ChIP-seq datasets from HepG2, HCT-116, A549 and K562 cells.

(H) Schematic illustrating the different types of transcription control of various metabolic enzymes by Yap/Tead and/or Myc and possibly additional factors.

$*P < 0.05$. $**P < 0.005$. $***P < 0.0005$. Error bars indicate s.d.



Murakami *et al*. Figure S4

Figure S4. Related to Figure 4; Yap loss does not induce compensatory Taz upregulation or overt EMT in Kras mutant pancreatic tumor cells.

(A) Representative flow cytometry plot of CellROX-stained *Yap*+ parental (P) KYYF cells, KYYF cells at 5 days post GFP or CRE treament, or two long-term *Yap*-deleted KYYF lines (*Yap*- LT #1 and #2).

(B) Western blot analysis of TAZ in *Yap*+ parental (P) and two long-term *Yap*-deleted KYYF lines (*Yap*- LT #1 and #2). Actin was used as the loading control. Shown is representative of at least three independent experiments.

(C) Representative IHC images of Taz in KF and KYYF pancreata after ~6 months of TAM treatment. Scale bar =100 μm.

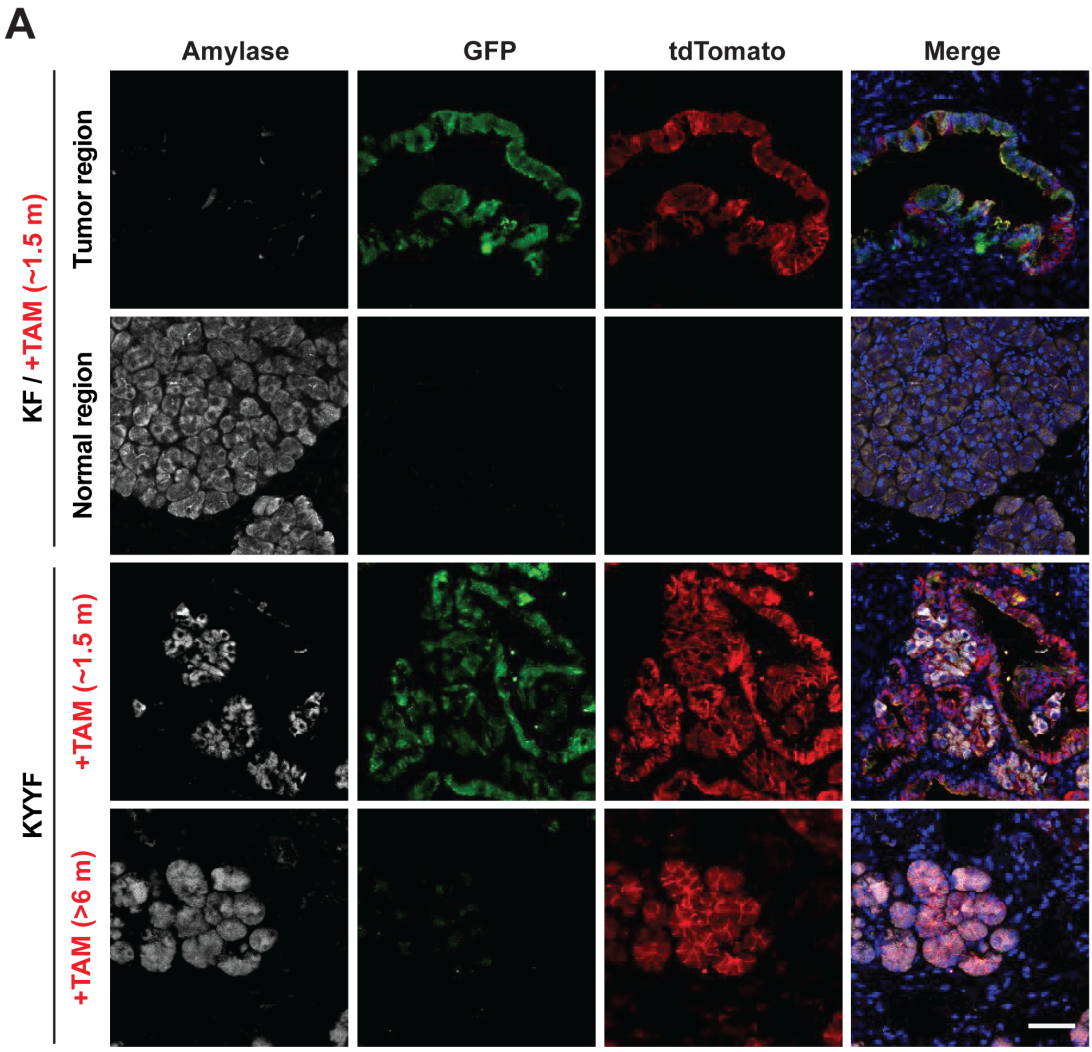
(D) Growth curve of Yap^+ and Yap^- mouse pancreatic tumor cells expressing vector control or shTaz. $n = 3$.

(E) Representative images of IF staining for SMA (green), tdTomato (red), E-Cad (grey) and DAPI (blue) in KYYF pancreata after ~1.5 months of TAM treatment. Scale bar=100 μm.

(F) Quantification of IF staining for Amy, CK19 and Tm in KF and KYYF pancreata after ~1.5 months of TAM treatment.

(G) Representative images of IF staining for Amy (red), Sox9 (green), Tm (gray) and DAPI (blue) in the KYYF pancreata after ~1.5 months of TAM treatment. Scale bar = 100 μm.

$*P < 0.05$. $**P < 0.005$. $***P < 0.0005$. ns: not significant. Error bars indicate s.d.

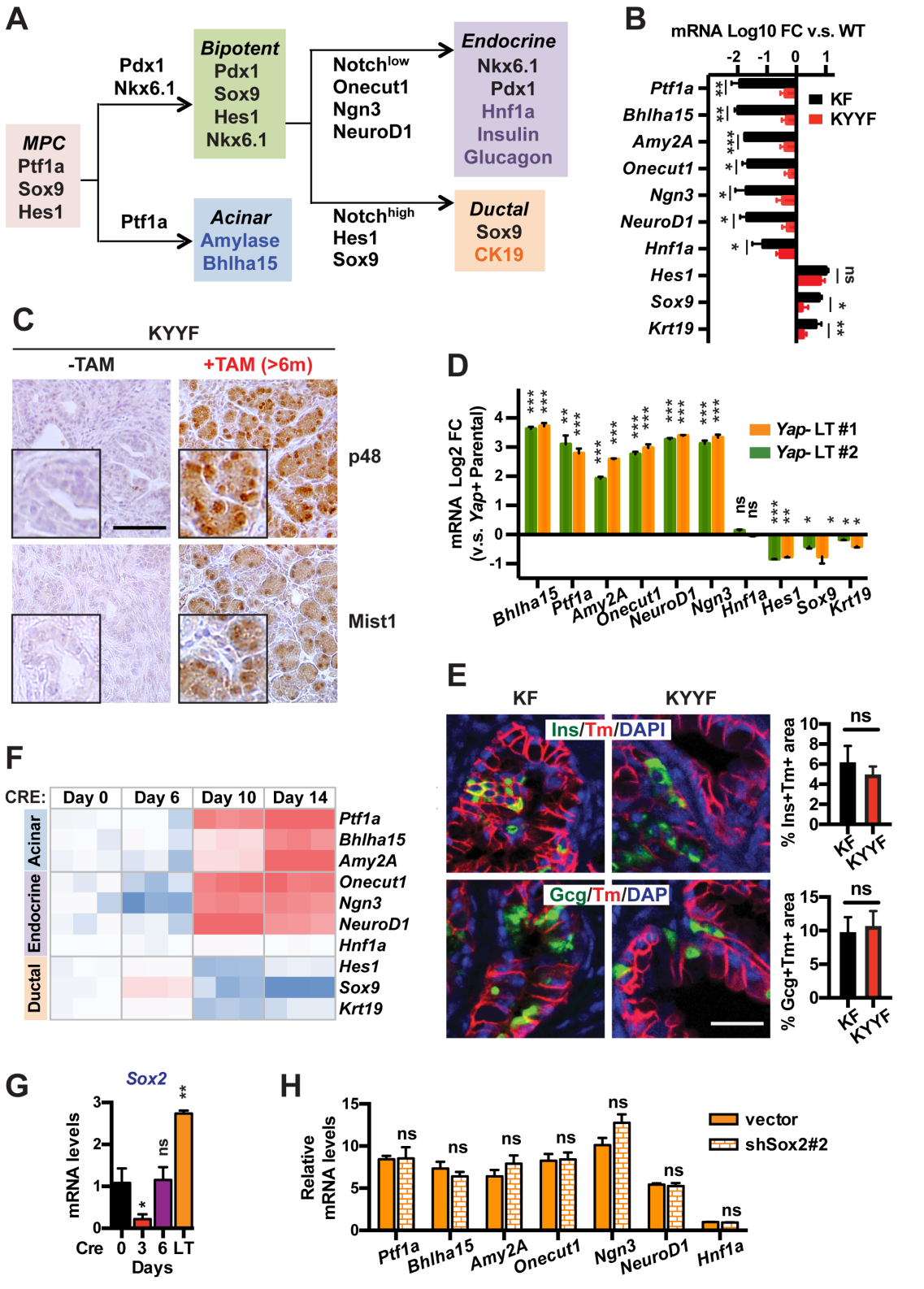


Murakami *et al*. Figure S5

Figure S5. Related to Figure 5; Persistent *Yap* loss induces re-expression of Amylase in spontaneous and orthotopic Kras mutant pancreatic tumors.

(A) Representative images of IF staining for Amylase (gray), GFP (green) or tdTomato (red) and DAPI (blue) in KF and KYYF pancreata after ~1.5 months or >6 months of TAM treatment. Scale bar indicates 100 μm.

(B) Representative IF images of Amylase (green), tdTomato (red), and GFP (gray) in untreated (-TAM) or TAM-treated (+TAM) orthotopic KYY tumors. Scale bar represents 40 μm.



Murakami *et al*. Figure S6

Figure S6. Related to Figure 6; Effects of Yap or Sox2 ablation on the expression of pancreatic lineage genes in vitro and in vivo.

(A) Schematic illustrating the major steps and key transcriptional factors during lineage commitment in normal pancreatic development.

(B) Relative mRNA levels of indicated pancreatic lineage genes in TAM-treated KF and KYYF pancreata normalized to WT pancreata. $n = 3$.

(C) Representative IHC images of p48/*Ptf1a* and Mist1/*Bhlha15a* in KYYF pancreata that were either untreated (-TAM) or treated for >6 months with TAM. Scale bar indicates 50 μm.

(D) Log2 FC of relative mRNA levels of indicated genes in two independent *Yap*- KYYF lines relative to *Yap*+ parental KYYF cells. $n = 3$.

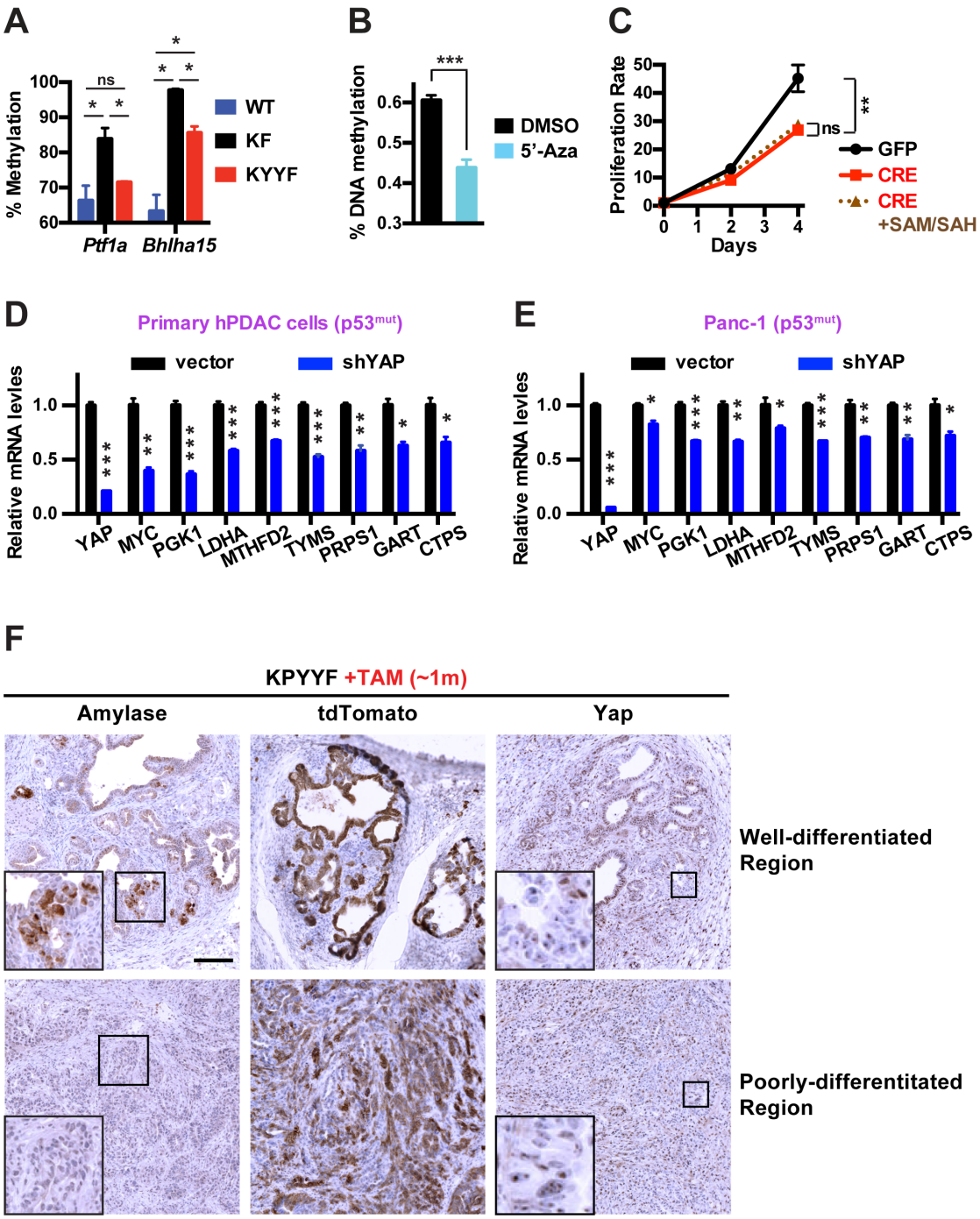
(E) Representative images and quantification of IF staining for Insulin (Ins; green) or Glucagon (Gcg; green) in combination with tdTomato (red) and DAPI (blue) in KF and KYYF pancreata after ~1.5 months of TAM treatment. Scale bar indicates 100 μm. $n = 5$.

(F) Heatmap of relative mRNA levels of indicated pancreatic lineage markers in KYYF cells at indicated times after CRE treatment. $n = 3$.

(G) Relative *Sox2* mRNA levels in KYYF cells at indicated time points after Ad-CRE infection. $n = 3$.

(H) Relative mRNA levels of indicated genes *Yap*+ and *Yap*- KYYF cells at 3 days after infection with lentiviruses carrying vector control or Sox2 shRNA #2. $n = 3$.

$*P < 0.05$. $**P < 0.005$. $***P < 0.0005$. ns: not significant. Error bars indicate s.d.



Murakami *et al*. Figure S7

Figure S7. Related to Figure 7; Effects of Yap ablation on p53 mutant pancreatic tumor cells in vitro and in vivo.

(A) Percent of DNA methylation within the CpG islands of the indicated gene promoters in WT, KF, and KYYF pancreata. $n = 3$.

(B) Percent of global DNA methylation in KYYF cells at 3 days after treatment of DMSO or 5 μM of 5-Aza. $n = 3$.

(C) Growth curve of KYYF cells untreated or treated with SAM (50 μM) and SAH (1 μM) after 4 days of infection with Ad-GFP or Ad-CRE. $n = 3$.

(D, E) Relative mRNA levels of indicated genes in p53 mutant primary human PDAC (D) and Panc-1 (E) cells expressing vector control or shYap. $n = 3$.

(F) Representative IHC images of Amylase, tdTomato and Yap in the well-differentiated and poorly-differentiated PDAC regions within the same KPYYF pancreas after ~1 month of TAM treatment. Scale bar indicates 300 μm.

$*P < 0.05$. $**P < 0.005$. $***P < 0.0005$. Error bars indicate s.d.