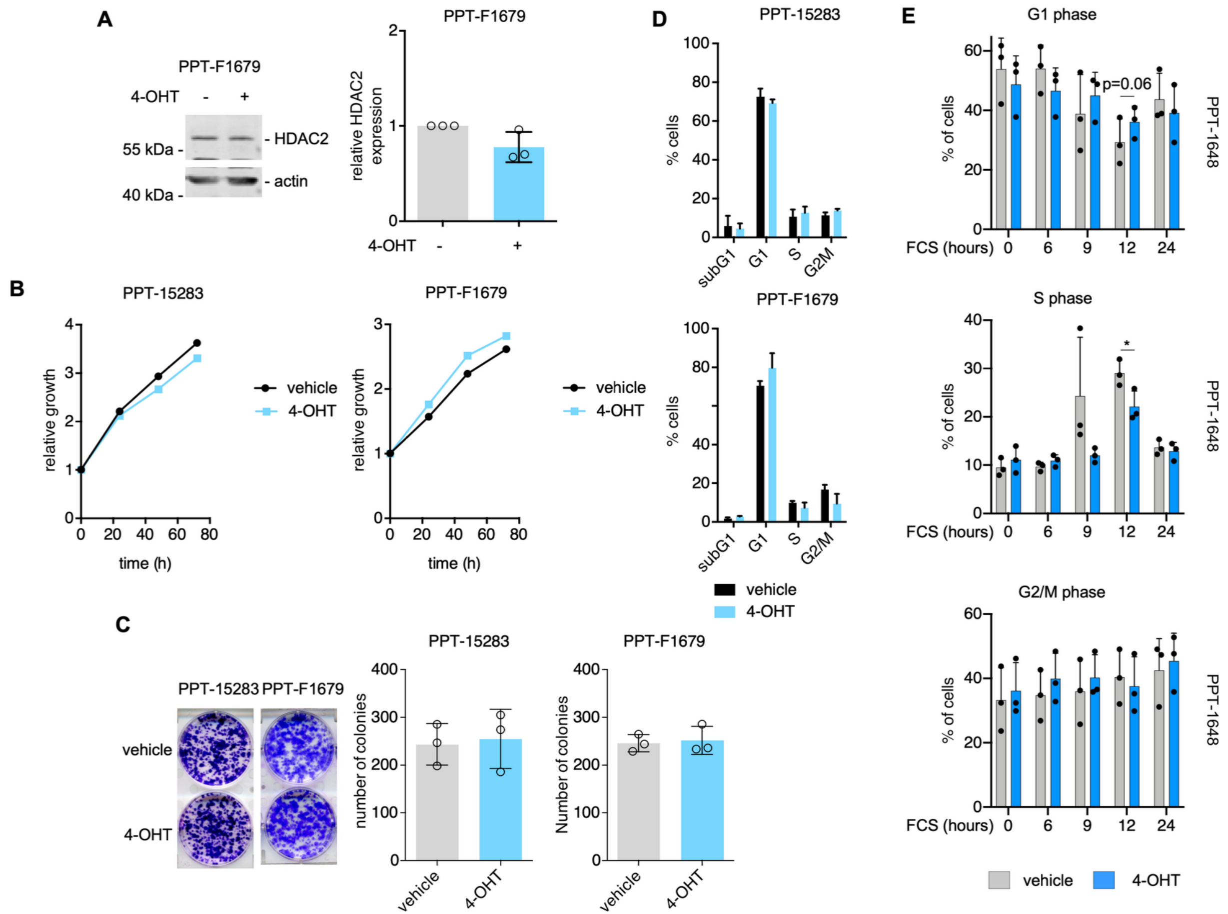
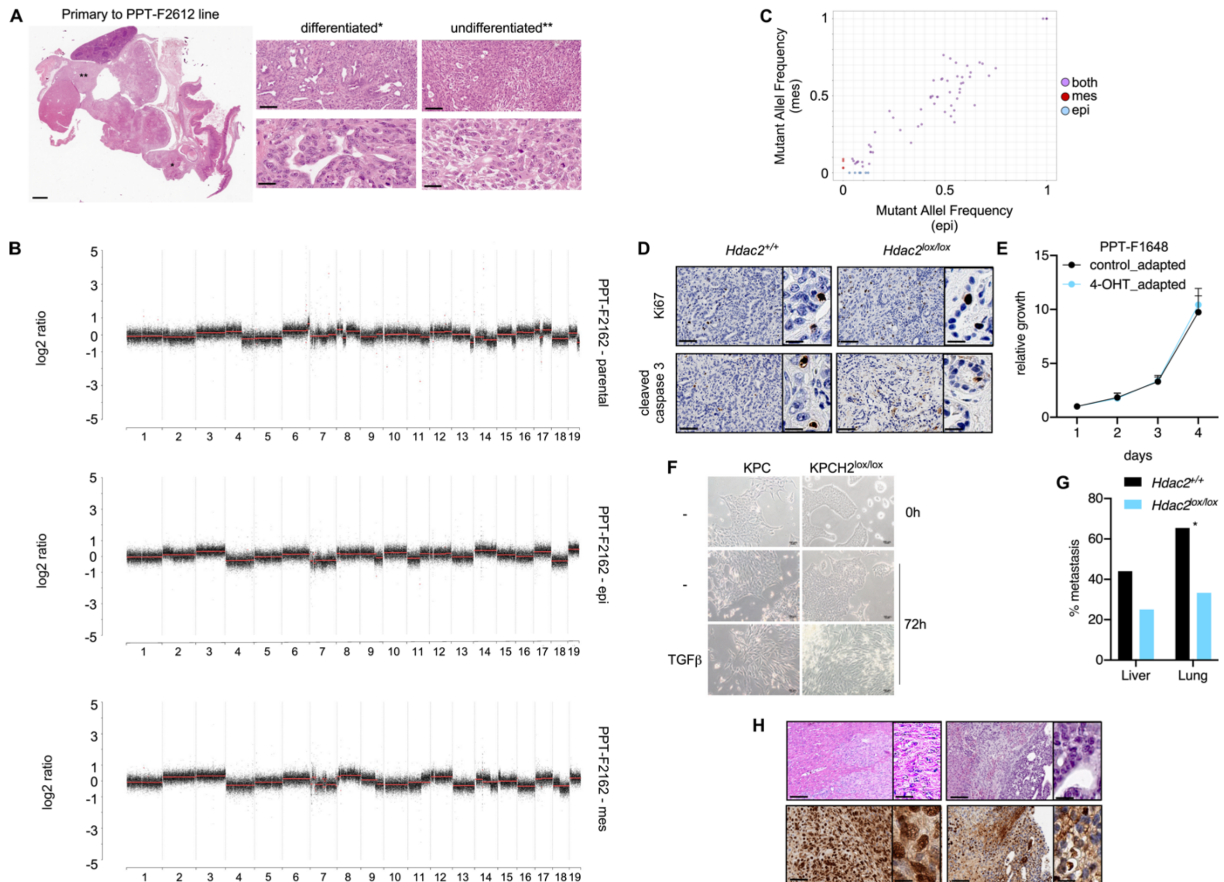
Supplemental Figure 1

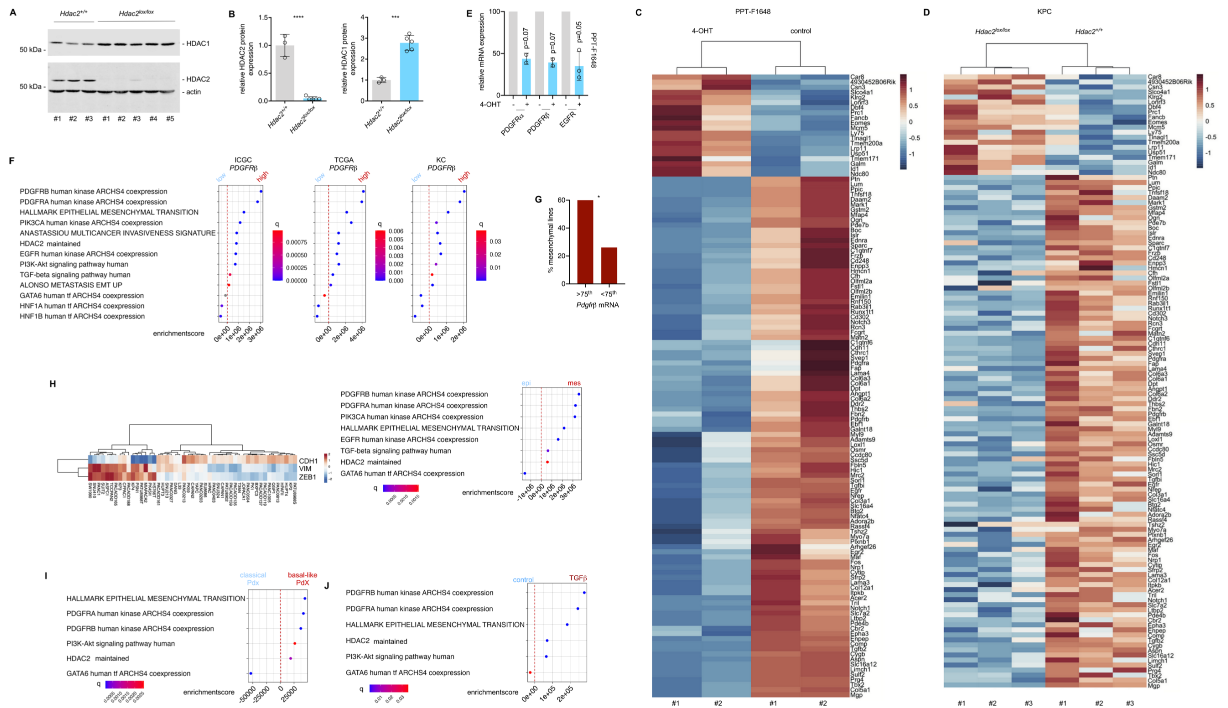


Supplemental Figure 1. Controlling potential Tamoxifen and Cre Toxicities.
A PPT-F1679 cells were treated for eight days with 600 nM 4-OHT or were left as vehicle treated controls. Left: Western blot of the expression of HDAC2. Actin was used as a loading control. Right: Quantification of HDAC2 protein expression from three independent experiments. **B** PPT-15283 (left) and PPT-F1679 (right) cells were treated for 8 days with 4-OHT (600 nM) or vehicle control. Afterwards, cells were plated in 96 wells and growth was determined by MTT assay over the indicated time points. Assay was performed in triplicate with three technical replicates. **C** PPT-15283 (left) and PPT-F1679 (right) cells were treated as in B. 2,000 cells were seeded in 24 well and clonogenic growth was analyzed after seven days. Left panel: representative clonogenic growth assay. Quantification (counting of Giemsa-stained colonies) of three independent experiments conducted as technical triplicates. **D** PPT-15283 (upper) and PPT-F1679 (lower) cells were treated as in B. Afterwards, propidium iodide cell cycle FACS analysis after eight days of treatment was performed. Three independent experiments were performed. **E** PPT-F1648 cells were treated for eight days with 600 nM 4-OHT or were left as vehicle treated controls. After serum starvation for cell cycle synchronization (24 hours), serum conatinig medium was added and propidium iodide cell cycle FACS analysis was performed in triplicates at the indicated time points. P value of a paired t-test is depicted or *<0.05.

Supplemental Figure 2

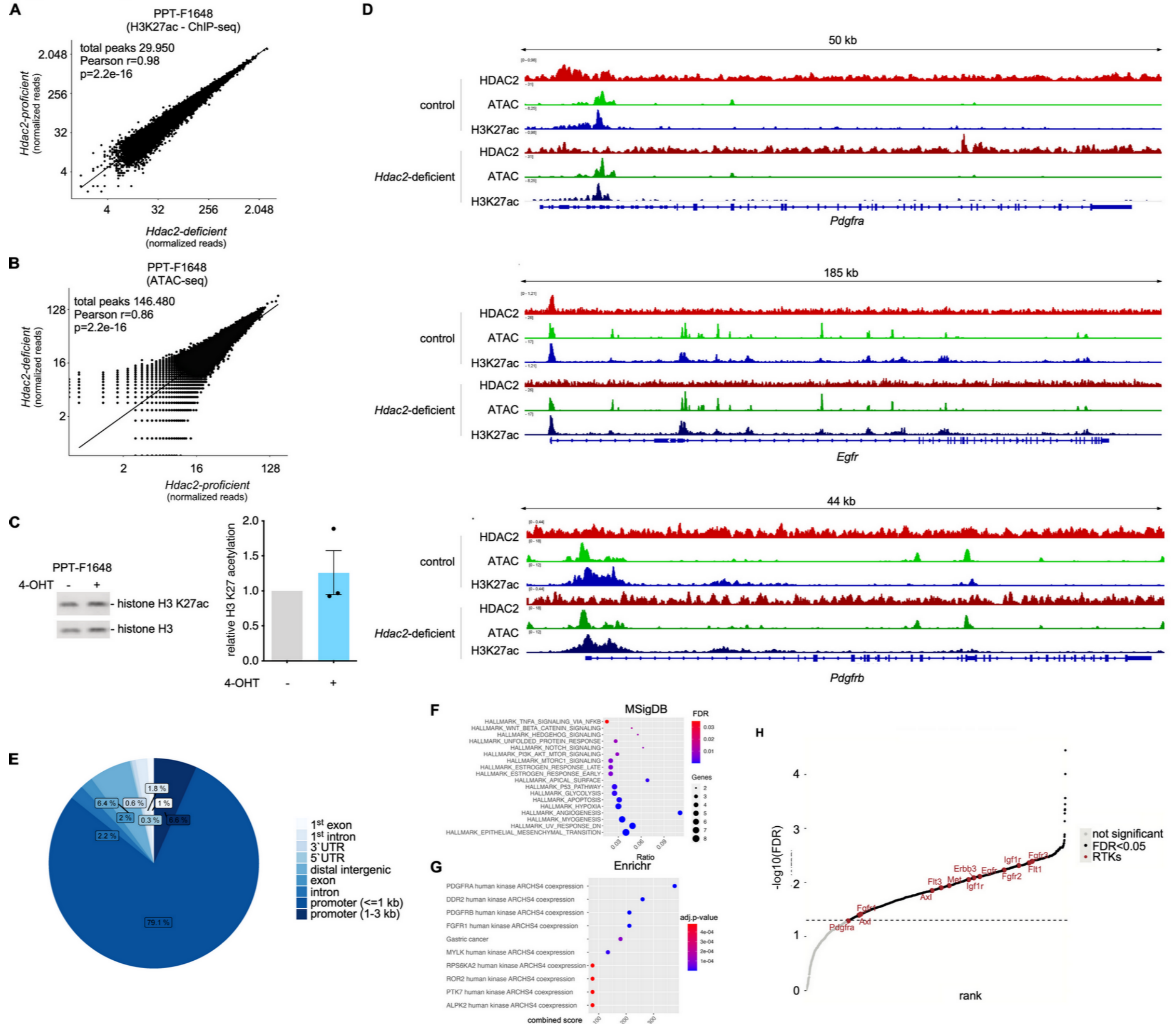


Supplemental Figure 2. TGFβ-induced EMT and organ-specific metastasis.
A H&E staining of the primary murine PDAC corresponding to the PPT-F2612 cell line. Left: outline, scale bar: 3mm Right: magnifications, scale bar: 100 μM and 20 μM. **B** Low-coverage-WGS based copy number profiles from parental PPT-F2612, epithelial (epi) PPT-F2612 and mesenchymal (mes) PPT-F2612 cells. **C** Allele frequencies of miss- and nonsense SNVs and indels of murine CancerGeneCensus (CGC) genes between epithelial (epi) and mesenchymal (mes) fractions (purple) exclusive to mes PPT-F2612 (red) or exclusive to epi PPT-F2612 (blue). **D** Representative IHC stainings of KI67 (upper panel) and cleaved caspase 3 (CC3) lower panel of murine PDACs of *KPC* and *KPCH2$^{lox/lox}$* mice. Scale bar: 100 μM and 20 μM. **E** Cell viability measurement of PPT-F1648 control and 4-OHT treated cells 9 passages after initial treatment. Cell viability was measured at indicated time points using MTT-Assay. **F** Epithelial *KPC* and *KPCH2$^{lox/lox}$* cell lines were treated with TGFβ (5 ng/ml) for the indicated time points. EMT was determined by morphology. Scale bar = 100 μM. **G** Fraction of metastasis into livers and lungs of *KPC* and *KPCH2$^{lox/lox}$* mice. * p value of a Fisher Exact test <0.05. Livers analyzed: *KPC*: n=25, *KPCH2$^{lox/lox}$*: n=24; Lungs analyzed: *KPC*: n=26; *KPCH2$^{lox/lox}$*: n=24. **H** Representative H&E and IHC HDAC2 staining of liver metastasis of *KPC* mice; scale bar: 50 μM and 10 μM.



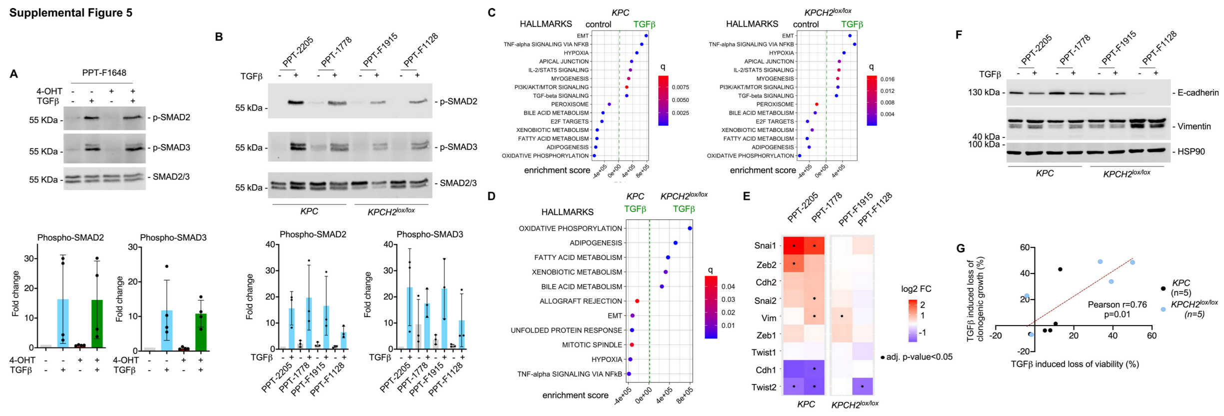
Supplemental Figure 3. HDAC2-maintained genes are connected to EMT, PDGFR-, EGFR-, PI3K-AKT-, and TGFβ-signaling.

A HDAC1 and HDAC2 western blot of HDAC2 in KPC (n=3) and KPCH2$^{lox/lox}$ (n=5) cell lines. Actin: loading control. **B** Quantification of A. P value of an unpaired t-test: ****<0.0001, ***<0.001. **C** and **D** Heatmap of genes in **C** vehicle compared to 4-OHT (600 nM) treated PPT-F1648 cells and **D** *KPC* (n=3) and *KPCH2$^{lox/lox}$* (n=3) cancer cells. Corresponding to the Venn diagram of Fig. 4A. **E** PPT-F1648 cells were treated for eight days with 600 nM 4-OHT or were left as vehicle treated controls. Quantitative PCR determines *Pdgfra* (n=2), *Pdgfrb* (n=2), and *Egfr* (n=3). P value of a paired t-test is depicted. **F** Two human PDAC datasets (curated TCGA, and curated ICGC) as well as one murine PDAC cell line dataset were analyzed. *PDGFRB* mRNA expression was separated into quartiles and cancers belonging to the highest quartile were compared to all others in a GSEA using the using GeneTrail3 web tool. The enrichment score (ES) is plotted and the q values were color coded. **G** *Pdgfrb* mRNA expression of a RNA-seq dataset of 38 murine PDAC cell lines was separated into quartiles and lines belonging to the highest quartile were compared to all others. The percentual fraction of cell lines with mesenchymal morphology was compared in both groups. * p of a Fisher exact test: <0.05. **H** Left panel: mRNA expression profiles of conventional human PDAC cell lines were separated by the expression of *CDH1* (epithelial marker), *VIM*, and *ZEB1* (mesenchymal markers). Epithelial lines (low *ZEB1* and *VIM* expression, high *CDH1* expression; right cluster) were compared to mesenchymal lines (high *ZEB1* and *VIM* expression, low *CDH1* expression; left cluster) using GSEA via the GeneTrail3 web tool. Right panel: Signatures enriched in both indicated groups. Enrichmentscores and color-coded q-values are depicted. **I** The E-MTAB-5039 corresponding dataset of differential expressed genes in basal-like and classical PdX models of PDAC was analyzed using GeneTrail3. Enrichmentscores were plotted and q-values are depicted. **J** Illumina beadchips expression arrays (GSE87472) of epithelial KPC cell treated with TGFβ or vehicle were analyzed by GEO2R. Differential expression with adj. p value<0.05 was considered. Probe set identifiers were collapsed to Max_probe using the GSEA4.0.3 software package. The log FC was used as a rank for a pre-ranked GSEA using GeneTrail3. Enrichmentscores were plotted and q-values are depicted.



Supplemental Figure 4. Chromatin alteration and HDAC2 binding to promoters.
A and **B** PPT-F1648 cells were treated for eight days with 4-OHT (600 nM) or were left as vehicle treated controls. Afterwards a H3K27ac ChIP-seq and ATAC-seq was performed. Dot plot showing **A** normalized H3K27ac ChIP-seq reads and **B** normalized ATAC-seq reads in *Hdac2*-proficient versus *Hdac2*-deficient cells. No significantly differences were identified by Perason correlation (H3K27ac ChIP-seq r=0.98, ATAC-seq r=0.86). **C** PPT-F1648 cells were treated as in A. Afterwards a western blot for histone H3 and histone H3 acetylated at K27 was performed. Left panel: representative Western blot; Right panel: Quantification of three independent experiments. **D** Genome browser view of HDAC2, H3K27ac and ATAC peaks in *Hdac2*-proficient and *Hdac2*-deficient PPT-F1648. The following gene regions are displayed: *Egfr*, *Pdgfra*, and *Pdgfrb*. **E** ChIPseeker annotations of significant HDAC2 peaks by (p adj. < 0.05). **F** and **G** Pathway Analysis of downregulated genes in *Hdac2*-deficient PPT-F1648 with significant HDAC2 peaks in the promoter region (TSS +/- 3000, p adj. < 0.05) were analyzed by **F** the MSigDB and **G** the Enrichr web tool and using the library KEGG 2021 and ARCH4 kinase co-expression. Adj. p value is color coded and Odds Ratio and the combined score is depicted. **H** Dotplot of HDAC2 peaks in PPT-F1648 cells. Peaks are ranked according to FDR. Significant peaks are shown in Black (p adj. < 0.05) and RTKs relevant in PDAC are shown in red.

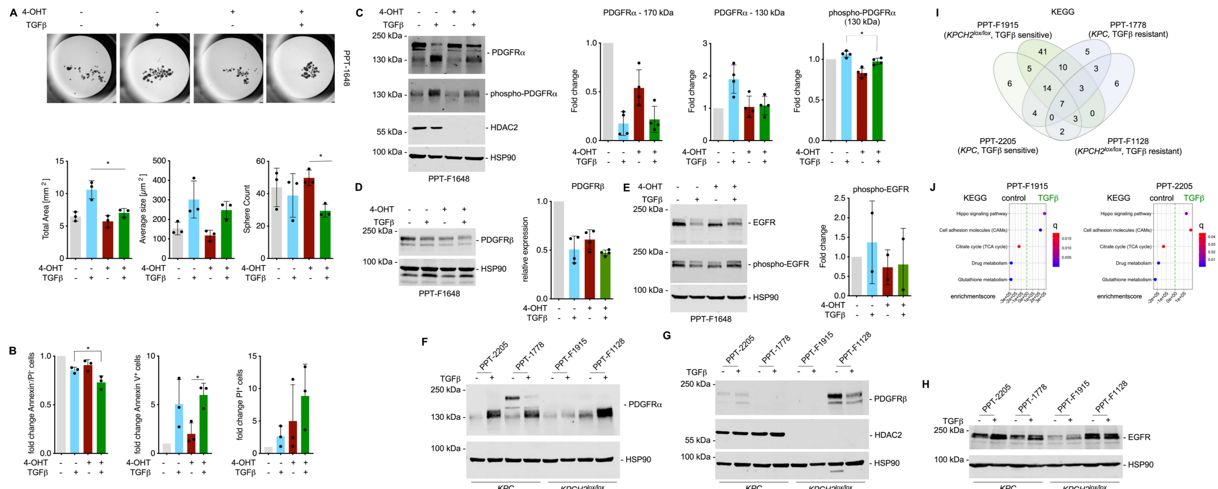
Supplemental Figure 5



Supplemental Figure 5. TGFβ-SMAD signaling in *Hdac2*-deficient PDAC cells.

A Western Blot of phospho-SMAD2 (Ser465/467), phospho-SMAD3 (Ser423/425), pan-SMAD2/3 of *Hdac2*-proficient and -deficient (4-OHT) PPT-F1648 cells treated with TGFβ (5 ng/ml) or control after 24 hours. One representative western blot out of four is depicted. Lower panel: Quantification of four independent experiments. **B** Western blot of phospho-SMAD2 (Ser465/467), phospho-SMAD3 (Ser423/425), pan-SMAD2/3 in two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) or left as a control for 24 hours. One representative western blot out of three is depicted. Lower panel: Quantification of three independent experiments. **C** RNA-seq of *Hdac2*-proficient (PPT-2205 and PPT1778) and -deficient (PPT-F1915 and PPT-F1128) treated for 24 hours with TGFβ (5 ng/ml) or were left as untreated controls were analyzed by GSEA using the GeneTrail3.0 platform. Treated and untreated samples of both lines were combined and the enrichmentscores and the color-coded q values are depicted. **D** Analysis of TGFβ (5 ng/ml) treated RNA-seq samples of *Hdac2*-proficient (PPT-2205 and PPT1778) and -deficient (PPT-F1915 and PPT-F1128) samples. Treated samples of both lines were combined and the enrichmentscores and the color-coded q values are depicted. **E** Differential expression of dedifferentiation marker genes in two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) after 24 hours in RNASeq. Log2Foldchange (log2 FC) is color coded and adjusted p value < 0.05 is indicated by (˙). **F** Western blot of E-cadherin and vimentin of two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. **G** Pearson correlation of TGFβ-induced loss of viability and clonogenic growth of five murine PDAC cell lines from *KPC* mice and five lines from *KPCH2*$^{lox/lox}$ mice treated with TGFβ (5 ng/ml) (corresponding to Fig. 5A-B).

Supplemental Figure 6



Supplemental Figure 6. TGFβ-induced cell fate decisions and RTK cross-signaling.
A Sphere formation assay of PPT-F1648 cells treated with TGFβ (5 ng/ml) or control after 72 hours. Pictures of spheres were analyzed by counts, total area and average size. *p<0.05. **B** FACS Apoptosis analysis of PPT-F1648 cells treated with TGFβ (5 ng/ml) or control for 72 hours. *p<0.05. **C** Western Blot of phospho-PDGF receptor α (Tyr1018), PDGF receptor α and HDAC2 of PPT-F1648 cells treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. One representative western blot out of four is depicted. Quantification of four independent experiments. *p<0.05. **D** Western Blot of PDGF receptor β of PPT-F1648 cells treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. One representative western blot out of four is depicted. Quantification of four independent experiments. **E** Western Blot of Phospho-EGF Receptor (Tyr1068) and EGF Receptor of PPT-F1648 cells treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. One representative western blot out of four is depicted. Quantification of four independent experiments. **F** Western blot of PDGF receptor α of two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. **G** Western Blot of PDGF receptor β and HDAC2 of two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. **H** Western Blot of EGF Receptor of two *Hdac2*-proficient and two *Hdac2*-deficient KPC cell lines treated with TGFβ (5 ng/ml) or control for 24 hours. HSP90: loading control. **I** KEGG signatures significantly enriched or depleted in TGFβ (5 ng/ml) treated models were analyzed by a Venn diagram. **J** Signatures defined in **I** regulated exclusively in sensitive lines as indicated in I.