Supplemental Material and Methods

Compounds. TGFβ was purchased from PeproTech (Rocky Hill, NJ, USA), 4-Hydroxytamoxifen (4-OHT) and α-Tocopherol (TOCO) was purchased from Sigma (Munich, Germany). N-Acetylcysteine (NAC) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Mouse lines. All animals were bred on a mixed *C57Bl/6;129S6/SvEv* (RRID:MGI:5657570) genetic background. The following mouse lines were used to engineer genetically defined pancreatic cancer models: *Pdx1-Cre* and *LSL-Kras*^{G12D} (1); *Ptf1a*^{Cre} (2); *LSL-p53*^{R172H} (3); *Hdac2*^{lox} (4); *Pdx1-Flp*, *FSF-Kras*^{G12D}, and *R26CAG-FSF-Cre*^{ERT2} (5); *FSF-p53*^{frt} (6); *LSL-R26-Tva* (7) Genotyping of the mouse lines was described (2,5,7–9). Animal studies were conducted in compliance with European guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committees of the Technische Universität München and Regierung von Oberbayern.

Cell lines. Generation of murine PDAC cell lines has been described (10). Cells were cultured in DMEM high glucose medium (Thermo Fisher Scientific/Life technologies, Waltham, MA, USA) supplemented with 10% (v/v) fetal calf serum (FCS) (FCS superior, Merck Millipore/Biochrom, Berlin, Germany) and 1% (v/v) penicillin/streptomycin (Thermo Fisher Scientific/Life technologies, Waltham, MA, USA). All cell lines were isolated and established in the Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universität München and cultivated for less than 30 passages after isolation. For the experiments, the cells were cultivated for a maximum of 12 passages before returning to another vial of the same stock. The identity of the murine PDAC cell lines was verified using genotyping PCR and the cell lines were tested for Mycoplasma contamination by a PCR-based methodology using a multiplex PCR strategy once per month. To test for mycoplasma contamination, 2 ml of supernatant from confluent cell cultures, cultured in antibiotic-free medium for two weeks, were taken and centrifuged at 250 g for 2 min. The supernatant was transferred in a new epi and centrifuged at 20,000 g for 10 min. Afterwards, the pellet was resuspended in 50 µl PBS, heat inactivated at 95°C for 3 min and used as DNA template for subsequent PCR analysis.

Mycoplasma and genotyping primers are depicted in STable1. Genotypes of the used cell lines can be found in STable1. To activate Cre^{ERT2}, PDAC cells were treated with vehicle (ethanol) or 600nM 4-hydroxytamoxifen (4-OHT) for 8 days. Afterwards, cells were left for 3 days to recover before being used for individual assays.

Differential trypsinization. Separating epithelial and mesenchymal fractions of the cell lines was performed as described previously (11). In brief, the parental cell line was trypsinized shortly (ca. 60 sec) to detach the mesenchymal fractions of the cell line and this suspension was further cultivated in a separate flask. The parental cell line was trypsinized again for a longer time (ca. 5 min) to remove all remaining mesenchymal cells and to keep only the fully epithelial fraction in the flask. The fully epithelial fraction was cultivated further as described above. Differential trypsinization was performed until a clear separation of the morphology was observed (at least 5 times).

Viability assay and clonogenic assay. Viability was measured using MTT assay. 2,000 dispersed cells were plated in 96-well plates (#353072, Corning, Falcon, Corning, NY, USA), or low-attachment 96-well plates (#3474, Corning, Costar, Corning, NY, USA), left over-night, followed by TGFβ or inhibitor treatment as depicted. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (10 mg/ml) was purchased from Sigma-Aldrich (Munich, Germany). 10 µL of the MTT dye was added per well followed by incubation for 4 hours at 37°C. Media was removed and the formazan crystals dissolved in 200 µL DMSO:EtOH (1:1) (v/v). Cell viability was determined by measuring the absorption at 595 nm in a Multiskan FC microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA). For clonogenic assay, 2,000 dispersed cells were plated in 24-well plates (#353047, Corning, Falcon, Corning, NY, USA), attached overnight, followed by TGFβ treatment as depicted. Subsequently, cells were left to grow for 7 days in growth medium containing 10% (v/v) FCS supplemented with 1% (v/v) penicillin–streptomycin. At the endpoint, cells were washed with PBS, fixed with ice-cold methanol (100%), stained with Giemsa (Merck, Darmstadt, Germany) and scanned for visualization. As TGFβ-treated cells do not form distinct colonies, at the endpoint, cells were washed with PBS, stained with crystal violet (Sigma-Aldrich, Munich, Germany) and scanned

for visualization. For quantification, colonies were counted or crystal violet was resolved in 1% SDS solution (Serva Electrophoresis GmbH, Heidelberg, Germany) and absorbance was measured at 595nm in a microplate reader.

Caspase activity. Caspase activity was measured using Caspase-Glo® 3/7 Assay (#G8091, Promega, Walldorf, Germany) and normalized to CellTiter-Glo® Luminescent Cell Viability Assay (#G7570, Promega, Walldorf, Germany). 2,000 dispersed cells were plated in 96-well plates (Corning, Costar, Corning, NY, USA), left overnight, followed by TGFβ or control treatment as depicted. For CellTiter-Glo® Luminescent Cell Viability Assay, 25 µL of the CellTiter-Glo® reagent was added per well followed by incubation for 30 min at 37°C. Simultaneously, 50 µL of Media was removed from each and 50 µL of Caspase-Glo® 3/7 reagent was added to each well followed by incubation for 1 h at 37°C. Cell viability and caspase 3/7 activity were determined by measuring the emission at 560 nm in a FLUOstar OPTIMA microplate reader (BMG LABTECH, Ortenberg, Germany).

Sphere forming assay. Pancreatic cancer spheres were generated by culturing pancreatic cancer cells (100,000 cells/200 µl) in low-attachment 96-well plates (#3474, Corning, Costar, Corning, NY, USA). After 96 hours, pictures were taken with the stereo microscope Leica M205 FA (Leica Microsystems, Wetzlar, Germany). For subsequent analysis, Image J (ImageJ, RRID:SCR_003070) was used. Spheres were analyzed by counts, total area and average size.

Cell-cycle, FITC Annexin V Apoptosis, ROS and Repopulation FACS. For cell-cycle FACS analysis, cells were seeded in 10 cm dishes (#353003, Corning, Falcon, Corning, NY, USA), left over-night and harvested the next day or for cell cycle synchronization experiments, starved for 24 h by addition of serum free media and then released into cell cycle by media change. At indicated time points, cells were harvested by trypsinization and washed once in PBS followed by fixation in 1 ml cold 70% (v/v) EtOH. After 24 h, EtOH was removed and cells were washed in PBS. RNA was digested by adding RNAse (Sigma-Aldrich, Munich, Germany) (final concentration 0.05 mg/ml) for 1 h at 37°C. Cells were stained by adding 25 µl propidium iodide (PI) (25 mg/ml) (Sigma-Aldrich, Munich, Germany) and measured using flow cytometry on a Gallios™ Flow Cytometer (Beckman Coulter, Krefeld, Germany). For dead cell staining

and ROS measurements, cells were seeded in 10 cm dishes (#353003, Corning, Falcon, Corning, NY, USA) and left overnight. Cells were treated with TGFβ or vehicle. After 72 h, cells were harvested by trypsinization and prepared for FITC Annexin V Apoptosis Detection Kit I (RRID:AB_2869082) (#556547, BD Biosciences, Heidelberg, Germany) or CellROX™ Deep Red Flow Cytometry Assay Kit (#C10491, Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. All samples were measured by flow cytometry on a Gallios™ Flow Cytometer (Beckman Coulter, Brea, CA, USA) and data were analyzed with the FlowJo software (FlowJo, LLC, Ashland, OR, USA, RRID:SCR_008520). For Repopulation Assay, GFP positive cells (*Hdac2* proficient) were mixed with GFP negative cells (*Hdac2* deficient) in a 50:50 ratio and vice versa and 8,000 cells per well were then seeded in 6-well plates. After 24 hours cells were treated with TGFβ or vehicle for 96 hours. Afterwards cells were washed in PBS and trypsinated and detatched using PBS (+2% FCS). Technical triplicates were pooled and singularized. Then samples were measured using flow cytometry on a Gallios™ Flow Cytometer (Beckman Coulter, Brea, CA, USA) and data were analyzed with the FlowJo software (FlowJo, LLC, Ashland, OR, USA). Additionally, non mixed cells were seeded and treated in the same manner in order to normalize GFP positive and GFP negative cells vs debris.

Vectors and Transduction. Gateway cloning: EGFP template was amplified with overhangs for Restricition Enzyme digestion (DraI, XbaI). EGFP template was ligated into Entry Vector pENTR1A no ccDB (w48-1) (Addgene, #17398, RRID:Addgene_17398) after Restriction Enzymatic digestion of Vector using Enzymes DraI and XbaI. Annealing was performed by adding 1 µl T4 DNA Ligase, 1 µl of 10x T4 DNA Ligase Buffer (New England Biolabs, #B0202S) and 7 ul H_2O together with 100 ng of total DNA (1:3 Vector:Template molar Ratio) in a 1.5 ml Eppendorf Tube. The solution was heated for 5 minutes at 95°C on a heating block, then removed and slowly cooled down for 30 minutes at room temperature. A 1:50 dilution was used for subsequent cloning. Resulting Vectors Resulting Vectors were transformed in *E.coli Stbl3* bacteria and selected on LB-Agar (ROTH, #X969.2) plate with 100 µg/ml Kanamycin. Single colonies were amplified in LB-Medium supplemented with 100 µg/ml of Kanamycin. The

plasmid was isolated with the “NucleoSpin Plasmid, Mini Kit for Plasmid DNA” (Machery Nagel, #740588.250) according to the manufactures protocol. All plasmids were Sanger sequenced to test proper insertion. Next, Gateway Cloning was performed using the Gateway LR Clonase Enzyme Mix (Invitrogen, 11791043) into Destination Vecor pLEX_305-N-dTAG (Addgene #91797, RRID:Addgene_91797) according to manufacturers protocol. Resulting Vectors were transformed in *E.coli Stbl3* bacteria and selected on LB-Agar (ROTH, #X969.2) plate with 100 µg/ml Ampicilin. Single colonies were amplified in LB-Medium supplemented with 100 µg/ml of Ampicilin. The plasmid was isolated with the “NucleoSpin Plasmid, Mini Kit for Plasmid DNA” (Machery Nagel, #740588.250) according to the manufactures protocol. All plasmids were Sanger sequenced to test proper insertion. For lentiviral production, $1x10^6$ HEK293FT cells (RRID:CVCL_6911) were seeded in a 10 cm dish in DMEM + 10% FBS. The next day, the medium was aspirated, and fresh medium was added. In addition, the transfection mix (18 µl Lipofectamin, 280 µl Optimem and 10 µl of a Plasmid mix (1.25µg psPAX2 (RRID:Addgene_12260) (psPAX2 was a gift from Didier Trono), 0.75 µg pMD2 (RRID:Addgene_12259) (pMD2 was a gift from Didier Trono), 2 µg of the different lenti-vectors filled to 10 µl with H_2O) was mixed and incubated for 5 minutes at room temperature added dropwise to the medium. On the following day, the medium was aspirated and new DMEM + 30% FBS was added. The same was done on the next day, but the aspirated medium was saved in a 15ml Falcon tube. On the last day, the medium was again aspirated and pooled with the medium of the previous day, filtered, and the lentivirus stored at -80°C.

Lentiviral Cell transduction: 100,000 – 150,000 PPT-F1648 cells were seeded in two wells of a 6-well plate for 24 hours. Afterwards, 1 ml of the lentivirus containing medium was added together with 8 µg/ml Polybrene. After 8 hours, 1 ml of Medium with 10% FBS was added and cultured overnight. Medium was refreshed and after additional 24 hours and cells were selected for pLEX_305-N-dTAGEGFP with 8 µg/ml Puromycin (Invivogen, #ant-pr-1) for 48 hours.

Cell lysis and western blot. To prepare whole-cell extracts (WCEs), cell lysis buffer (#9803S, Cell Signaling Technology, Leiden) supplemented with protease and phosphatase inhibitors

(cOmplete, EDTA-free Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany) and Phosphatase-Inhibitor-Mix I (Serva, Heidelberg, Germany)) was used. Whole-cell extracts were normalized for protein, heated at 95°C for 5 min in protein loading buffer (6 mM Tris-HCl (pH 6.8), 70mM SDS, 10% (v/v) glycerol, 1% (v/v) β-mercaptoethanol, 0.015mM bromophenol blue), loaded onto 10% SDS-polyacrylamide gels and proteins were transferred to nitrocellulose membranes (GE Healthcare Life Sciences, Freiburg). Afterward, membranes were incubated in blocking buffer (5% (w/v) skim milk, 0.1% (v/v) Tween in PBS) and incubated with primary and DyLightTM-conjugated secondary antibodies. Western blots were visualized with the Odyssey Infrared Imaging System (Licor, Bad Homburg, Germany) and western blots were quantified using Odyssey software. Antibodies used are depicted in TableS1.

Quantitative reverse-transcriptase PCR. Total RNA was isolated from PDAC cell lines using the Maxwell® 16 LEV simply RNA Purification Kit (Promega, Walldorf, Germany) following the manufacturer's instructions. Quantitative mRNA analysis was performed using a real-time PCR analysis system (TaqMan, PE StepOnePlus, Real-Time PCR System; Applied Biosystems Inc., Carlsbad, CA, USA) (RRID:SCR_015805) with Promega GoTaq qPCR Master Mix as fluorescent DNA binding dye. Data analysis was carried out with StepOne software (RRID:SCR_014281, Applied Biosystem; Thermo Fisher Scientific, Waltham, MA, USA) according to the ΔΔCt method. qPCR primers are depicted in TableS1.

Histochemistry, immunohistochemistry, and analysis. For histopathological analysis, murine tissues were fixed in 4% formaldehyde (Carl Roth, Karlsruhe, Germany), embedded in paraffin, and sectioned (thickness: 2.5 µm). Tissues were stained with hematoxylin and eosin as described (12)**.** Immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections was performed on a BonRxm system (Leica, Wetzlar, Germany). Briefly, the tissue was dewaxed with deparaffinization solution and heat mediated antigen retrieval was performed for 20 min with epitope retrieval solution 1 (Leica, Wetzlar, Germany). Sections were incubated with the following primary antibodies: Hdac2 (ab12169; 1:400; Abcam, RRID:AB_2118547), Ki67 (RRID:AB_302459), ab16667; 1:50; Abcam) and cleaved Caspase 3 (9664; 1:150; Cell Signaling, RRID:AB_2070042). For detection, a Polymer Refine Detection Kit (Leica, Wetzlar,

Germany) was used either with (Hdac2) or without (Ki67 and cleaved Caspase 3) post-primary antibody. 3,3′-diaminobenzidine tetrahydrochloride is used as chromogen for visualization in this kit. Slides were counterstained with hematoxylin, dehydrated and coverslipped and scanned with slide scanner Leica AT2 and images were captured by Aperio ImageScope (RRID:SCR_020993) (Leica Biosystem, Nußloch, Germany). Tumor grading of the murine PDACs was performed by an experienced comparative pathologist (KS) with respect to the consensus classification of pancreatic neoplasms in genetically engineered mice (13) taking the human WHO classification (*WHO Classification of Tumours*, 5th Edition, Vol. 1) also into account. Grading is always performed with respect to the worst grade occurring in a significant amount of a neoplasm (>10%).

Metastasis Frequency. Lungs and livers were investigated macroscopically for metastases at necropsy. For microscopical detection of metastasis, liver and lung tissues of *Hdac2*-proficient and -deficient *KPC* mice, were fixed in 4% formaldehyde (Carl Roth, Karlsruhe, Germany) and embedded in paraffin. The tissues were cut into serial sections (10 sections á 2.5µm (= series 1)) separated by 100 µm between each series. Per organ and mouse at least 10 series were cut and analysed by an experienced comparative pathologist (KS) to differentiate metastases from background lesions. The first sections of each series were H&E stained and screened for metastasis. Local invasion from the primary tumor to the liver was excluded and not counted.

RNA-Seq and Microarray analysis, Venn-diagrams and heatmap, gene set enrichment analysis, and datasets. mRNA was extracted from control and 4-OHT treated PPT-F1648 cell as described above and quality was controlled by densitometry using agarose gel electrophoresis and quality control measures of the genomics and proteomics core facility of the DKFZ Heidelberg. RNA-sequencing (RNA-Seq) was carried out by the genomics and proteomics core facility of the DKFZ (Heidelberg, Germany; B25M reads/sample (single-end reads); Illumina HiSeq 2000 (RRID:SCR_020132), San Diego, CA, USA). Next-generation sequencing data were analyzed using the Galaxy platform (RRID:SCR_006281)(28). TrimGalore! (Galaxy Version 0.4.2) was used to remove adapters from FASTQ files. Reads

were mapped to the mouse reference genome mm10 using bowtie2 (RRID:SCR_016368) (14). Aligned reads which overlap to features in the GTF annotation file, obtained from the UCSC genome browser (RRID:SCR_005780), were calculated using htseq-count 0.6.1galaxy3 (RRID:SCR_011867) (15). The resulting countmatrix was imported into R v4.0.5. Technical replicates were summarized and lowly expressed genes were filtered according to the following criteria: For each gene the read count has to be 5 or more in at least 80% of the samples. Prior differential expression analysis with DESeq2 v1.30.1 (RRID:SCR_015687) (16). Dispersion of the data was estimated with a parametric fit using the genotype as explanatory variable. The Wald test was used for determining differentially regulated genes between genotypes. Shrunken log2 fold changes were calculated afterwards. A gene was determined to be differentially regulated if the p-value was below 0.05. Rlog transformation of the data was performed for visualization and further downstream analysis. For RNA-seq of TGFβ treated *Hdac2*-proficient and -deficient KPC cell lines, mRNA was extracted as described above. For this dataset, RNA sequencing was performed at the Sequencing Core Unit at the TranslaTUM, Technical University Munich (TUM). Library preparation for bulk-sequencing of poly(A)-RNA was done as described previously (17). Briefly, barcoded cDNA of each sample was generated with a Maxima RT polymerase (Thermo Fisher) using oligo-dT primer containing barcodes, unique molecular identifiers (UMIs) and an adaptor. 5`-ends of the cDNAs were extended by a template switch oligo (TSO) and full-length cDNA was amplified with primers binding to the TSO-site and the adaptor. NEB UltraII FS kit was used to fragment cDNA. After end repair and A-tailing a TruSeq adapter was ligated and 3'-end-fragments were finally amplified using primers with Illumina P5 and P7 overhangs. In comparison to Parekh et al. (17), the P5 and P7 sites were exchanged to allow sequencing of the cDNA in read1 and barcodes and UMIs in read2 to achieve a better cluster recognition. The library was sequenced on a NextSeq 500 (Illumina) (RRID:SCR_014983) with 67 cycles for the cDNA in read1 and 16 cycles for the barcodes and UMIs in read2. Data was processed using the published Drop-seq pipeline (v1.0) to generate sample- and gene-wise UMI tables (18). Reference genome (GRCm38 or GRCh38) was used for alignment. Transcript and gene definitions were used

according to the GENCODE Version M25. RNAseq analysis was performed with R-Studio (RRID:SCR_000432) (R version v4.1.) and DEseq2 v 1.32.0. Genes with sum (read counts) < n (number of Samples) were removed and remaining counts were normalized and transformed using regularized log transformation (rlog) implemented in the DEseq2 package. The rlog normalized matrix was used to perform a GSEA using the GeneTrail 3.0 (SCR_006250) web tool with default setting.Total RNA of murine *Hdac2*-deficient (n=3) and -proficient (n=3) KPC cell lines were prepared, processed according to Affymetrix standard protocols, and hybridized onto the GeneChip Affymetrix Mouse Gene ST 1.0 (Affymetrix, Santa Clara, CA, USA). Microarray raw data was imported into R v4.0.5. Data was RMA normalized with the rma function provided from the Bioconductor (RRID:SCR_006442) oligo (RRID:SCR_015729) (19) package and probes were summarized to probesets. For differential expression analysis data was then modeled with Limma (RRID:SCR_010943) v3.46.0 (20) using the genotype as explanatory variable. Most recent probeset annotations were downloaded from ENSEMBL (RRID:SCR_002344) (GRCm38p6 v102) and used to assign probeset to genes. Probesets, where no gene was annotated to, were discarded from analysis. A gene was determined to be differentially regulated between *Hdac2* knockout and wt condition if the adjusted p-value was below 0.05. For Microarray and RNAseq data differentially regulated genes were stratified according to the direction of regulation and the intersection was determined. The TCGA PAAD dataset RNA-seq V2 was downloaded via the cBioPortal (RRID:SCR_014555) platform (http://www.cbioportal.org/) (12/2018). The dataset was curated according to (21), excluding normal tissue, cancers with low cellularity, and non-PDAC samples (n=146). Normalized human PDAC RNA-seq data and associated clinical data obtained from Bailey and colleagues (22). All acinar cell carcinomas and all intraductal papillary mucinous neoplasms were excluded in this dataset (n=81). RNA-seq of murine PDAC cell lines (n=38) was described recently (11). The E-MTAB-5039 corresponding dataset of differential expressed genes in basal-like and classical PDAC cancer was directly retrieved via (23) from the supplemental table (1-s2.0-S2211124717316066-mmc4). Illumina bead chips (GSE87472) (24) of epithelial *KPC* cells treated with TGFβ or vehicle were analyzed by GEO2R (RRID:SCR_016569) and

differential expression with adj. p value<0.05 was considered. Association of regulated genes was conducted with the following pipelines: Molecular Signature Database (MSigDb) (https://www.gsea-msigdb.org/gsea/msigdb/annotate.jsp) and gene set enrichment analysis (GSEA) tool (25); Enrichr(https://amp.pharm.mssm.edu/Enrichr/) (RRID:SCR_001575) (26,27); Gene Trail3 (28). Signatures not available in the Gene Trail3 web service were downloaded via MSigDb or Enrichr. Thresholds used in the different analyses are described in the figure legends. Venn`s diagrams were generated with https://bioinfogp.cnb.csic.es/tools/venny/ (RRID:SCR_016561). Differential expressed genes were visualized with heatmapper (RRID:SCR_016974) (29) or ClustVis (RRID:SCR_017133) (30). Microarray mRNA expression data of *Hdac2*-deficient and -proficient murine PDAC cells can be accessed on GEO: GSE144798; RNA-Seq data of 4-OHT and vehicle-treated PPT-F1648 cells can be accessed on ENA: PRJEB35204. RNA-seq data of TGFβ-treated *Hdac2*-deficient and -proficient murine PDAC lines can by accessed on ENA: PRJEB47385.

ATAC-Seq. ATAC-seq was done as previously described (31)**.** In brief, 50,000 cells were pelleted at 500 g for 5 min at 4˚C, resuspended in 50 µl of lysis buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% NP40, 0.1% Tween-20, 0.01% digitonin) and spun at 500 g for 10 min at 4˚C to collect nuclei. Nuclei were subsequently resuspended in 50 µl Transposase mixture containing 25 µl 2 × tagmentation buffer, 16.5 µl PBS, 0.25 µl 2% digitonin, 0.5 µl 10% Tween-20, 5.25 µl H2O, 2.5 µl Tn5 Transposase (Illumina Nextera DNA Library Preparation Kit). Reactions were incubated for 30 min at 37˚C in a thermomixer shaking at 1000 rpm and DNA purified using PCR clean-up MinElute kit (Qiagen, Hilden, Germany). The transposed DNA was subsequently amplified in 50 µl reactions with custom primers as described (32). Amplified libraries were purified with the PCR clean-up MinElute kit (Qiagen, Hilden, Germany) and size selected for fragments <600 bp using the Agencourt AMPure XP beads (Beckman Coulter, Pasadena, CA, USA). Libraries were quality controlled by Qubit and Agilent DNA Bioanalyzer analysis. High throughput sequencing was performed on a HiSeq 1500 system (RRID:SCR_018006) according to the standard Illumina protocol for 50 bp single-end.

ChIP-Seq. ChIPseq was done as previously described (33). Briefly, For H3k27ac 1-2 million cross-linked cells (1% formaldehyde, 10min RT) were lysed in 100 µl Buffer-B (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 1%SDS, 1× protease inhibitors (Roche Diagnostics, Mannheim, Germany) and sonicated in a microtube (Covaris; 520045) using a Covaris S220 device to shear the chromatin to an average size of 100–500 base pairs long (settings: temperature 4˚C, duty cycle 2%, peak incident power 105 W, cycles per burst 200). Successively, lysates were diluted with 900 ul of Buffer-A (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.5 mM EGTA,1% Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate, 140 mM NaCl, 1× protease inhibitors-Roche) and centrifuged 10 min, 4˚C, 12000g. 150 ul of sonicated chromatin was then incubated for 4h at 4˚C on a rotating wheel with 3 µg of antibody conjugated to 10 µl of Protein G Dynabeads (Thermo Fisher Scientific, Waltham, MA, USA). The antibody used was H3K27ac (Diagenode, Denville, NJ, USA; Pab-174-050, RRID:AB_2716835). Beads were washed four times with Buffer-A (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.5 mM EGTA,1% Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate, 140 mM NaCl, 1× protease inhibitors-Roche) and once with Buffer-C (10 mM Tris–HCl, pH 8.0, 10 mM EDTA). Beads were resuspended in 100 µl elution buffer (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 1% SDS) and incubated 20 min at 65˚C. Supernatant was transferred to a new tube. Crosslink reversal of immunoprecipitated DNA was carried out overnight at 65˚C. Then 100 µl TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA) was added, RNA was degraded by 4 µl RNase A (10 mg/ml) for 1 h at 37˚C and proteins were digested with 4 µl Proteinase K (10 mg/ml) at 55˚C for 2h. Finally, DNA was isolated by phenol:chloroform:isoamyl alcohol purification followed by ethanol precipitation. Purified DNA was used as input for library preparation with MicroPlex Library Preparation Kit v2 (Diagenode, cat. C05010012) and processed according to the manufacturer´s instructions. Libraries were quality controlled by Qubit and Agilent DNA Bioanalyzer analysis. High throughput sequencing was performed on a HiSeq 1500 system according to the standard Illumina protocol for 50bp single-end reads using v3 reagents.

For HDAC2 ChIPseq, 10 million cross-linked cells per sample (1% formaldehyde, 10min RT then quenched with 0.125 Glycine, 5min RT) were lysed in 5 Aliquots in 200 µl Lysis Buffer I

(50 mM HEPES–KOH, pH 8.0 1 mM EDTA, 0.25% Triton X 100, 140mM NaCl, 0.5% NP-40,10% Glycerol, 1× protease inhibitors (Roche Diagnostics, Mannheim, Germany) on ice for 10 min, spun down and supernatant was removed. Next, pellet was resuspended 200 µl Lysis Buffer II (10 mM Tris–HCl pH 8.0, 1 mM EDTA, 200 mM NaCl, 0.5 mM EGTA, 1× protease inhibitors (Roche Diagnostics, Mannheim, Germany) on ice for 10 min, spun down and supernatant was removed. Then, the pellet was resupended in 300 µl Shearing (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl, 0.1% SDS, 0.1% Na-deoxycholate, 0.5% N-Lauroylsarcosine, 1× protease inhibitors (Roche Diagnostics, Mannheim, Germany) and incubated on ice for 10 min and sonicated in a 1.5 ml Bioruptor Plus TPX microtubes using a Bioruptor device to shear the chromatin to an average size of 100–500 base pairs long (settings: temperature 4˚C, 30s on 30s off, Intensity: High, 3x 10 min + 1x5 min Cycles, spin down between each 10 min cycle). After successful size check, sonicated chromatin was precleared with 100 µl blocked Dynabeads by incubating for 1h at 4°C on a rotating wheel (35 rpm) and 5% of precleared sample was used for Input (Beads were blocked using IP buffer (10mM Tris pH 8.0, 140mM NaCl 0.5M EGTA, 1mM EDTA,0.1% Na-deoxycholate, 0.5% N-Lauroylsarcosine), +0.5% BSA, 1h at 4°C). Then 1.25 ml of sonicated chromatin was incubated overnight at 4˚C on a rotating wheel with a 1:50 Dilution of antibody HDAC2. The antibody used was anti-HDAC2 (HDAC2, D6S5P, cell signaling, mAb #57156, RRID:AB_2756828). The next day, 150 µl blocked beads were added to antibody-bound chromation and incubated at 4°C for 2 hours (rotating, 35 rpm). Next, beads were washed twice with 1ml Low Salt Wash Buffer (10mM Tris-HCl, pH 8.0, 1 mM EDTA pH 8.0, 140mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% Nadeoxycholate) (5 min, 20 rmp, 4°C). Low-binding Eppendorf tubes were changed after first wash. Next, beads were washed with 1ml High Salt Wash Buffer (10 mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0, 500 mM NaCl, 1%Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate)(5 min, 20 rmp, 4°C). Next, beads were washed with 1ml LiCl Wash Buffer (10 mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate)(5 min, 20 rmp, 4°C). Then, 1x TE-buffer was added and Beads were placed on a magnet and supernatant was aspirated completely. For crosslink reversal of immunoprecipitated DNA

beads were resuspended in 250 µl ChIP Elution Buffer (10 mM Tris-HCl pH 8.0 0.5% SDS, 300mM NaCl, 5mM EDTA pH 8.0). Input sampes were filled up to 70 µl Chip Elution Buffer. Next 2 µl RNAse A (10mg/ml, PureLink RNAse A, Invitrogen) was added and Input Chromatin were incubated at 37°C for 30 min (900 rpm). Afterwards, 2 µl of Proteinase K (20mg/ml, Applichem A4392,0010) was added and beads or Input Chromatin were incubated at 55°C for 1h (600rpm) and then incubated at 65°C for 8h for decrosslinking. Afterwards beads were separated from supernatant using a magnet and supernatant was collected in a new tube. 60 µl elution buffer was added to the beads and incubated for one minute and then elution buffer was combined with supernatant. 1 µl Proteinase K was added to eluates and incubated at 55°C for 1 h (600 rpm). For DNA Purification, 500 µl Ampure Beads (A63881, Agencourt AMPure XP, Beckman Coulter) were added the decrosslinked Chromation samples. Entire Volume was then mixed for at least 10 times and beads and DNA was incubated at room temperature for 10 min. Afterwards tubes were placed on a magnet and supernatant was removed. Next, Beads were washed with 80% Ethanol twice. Beads were then dried for 5 minutes before adding 20 µl Elution buffer (10 mM Tris pH-8.0). Beads were incubated for 5 minutes and 20 µl Eluate was collected in a new tube. Eluate concentrations were determined using Qubid HS DNA Assay. Major parts of the HDAC2-ChIP protocol were developed in the lab of Roland Rad by Christine Klement as well as Thorsten Kaltenbacher by combining the previous protocol with the protocols of Blecher-Gonen et al. (34) and Imbeault et al. (35). Purified DNA was used as input for library preparation with NEBNext Ultra II FS DNA Library Prep Kit for Illumina and processed according to the manufacturer´s instructions. Libraries were quality controlled by Qubit and Agilent DNA Bioanalyzer analysis. The library was sequenced on a NextSeq 500 (Illumina) with 75 cycles for the cDNA in read1 and 16 cycles for the barcodes and UMIs in read2.

Bioinformatic analyses of ATAC- and ChIP-seq. For H3K27ac ChIP-seq and ATAC-seq analyses, reads were mapped to the mouse genome version mm10 using Bowtie (RRID:SCR_005476) (36) (version 1.1.2) with standard parameters. Duplicate reads were removed using Picard (http://broadinstitute.github.io/picard) (RRID:SCR_006525). H3K27ac

peaks were identified with DFilter (version 1.6) (37) and peak coverage was calculated with Homer annotatePeaks (38). Pearson correlation analysis for H3K27ac peak coverage was performed with R (http://www.R-project.org/) using the rcorr function of the package Hmisc package (https://CRAN.R-project.org/package=Hmisc). Genome browser screenshots were taken from IGV (Integrative Genomics Viewer, RRID:SCR_011793) (39). Data were deposited in GEO: GSE155753. For HDAC2 ChIP-seq, data was analyzed with the nf-core chipseq pipeline v1.2.2 (40) with the following parameter settings: --macs_pvalue 0.005 and --broad_cutoff 0.005. Input Samples were merged prior to analysis. Bam and peak files were then imported into R v4.1.0 and analyzed with DiffBind (RRID:SCR_012918) v 3.2.4 (Parameters: normalize=DBA_NORM_LIB, summits = 350, method=DBA_DESEQ2) (41,42). Peaks were annotated with ChIPSeeker (RRID:SCR_021322) (Parameter: annoDb="org.Mm.eg.db") (43). For downstream analysis peaks located within 3 kb around the TSS of a gene and a FDR below or equal to 0.05 were selected. These genes were intersected with significant genes from the RNAseq analysis and resulting gene lists were used as input for pathway analysis with EnrichR within the Hallmark gene set collection. Motif Enrichment analysis of ChIP-peaks was performed using Homer (RRID:SCR_010881) (38) with the following parameters: findMotifsGenome.pl <peak/BED file> mm10 <output directory> -size 50. Data were deposited in GEO: GSE183823

Low-Coverage-WGS. Purified DNA was used as input for library preparation with NEBNext Ultra II FS DNA Library Prep Kit for Illumina and processed according to the manufacturer´s instructions. Libraries were quality controlled by Qubit and Agilent DNA Bioanalyzer analysis. The library was sequenced on a NextSeq 500 (Illumina) with 75 cycles for the cDNA in read1. Afterwards CNVs were analyzed according to the https://github.com/roland-rad-lab/ Pipeline (44). Data was deposited to ENA: PRJEB47385

WES Sequencing libraries were generated using Agilent SureSelectXT Mouse Exon Kit (Agilent Technologies, CA, USA) following manufacturer's recommendations. Briefly, fragmentation was carried out by hydrodynamic shearing system (Covaris, Massachusetts, USA) to generate 180-280bp fragments. Remaining overhangs were converted into blunt ends

via exonuclease/polymerase activities and enzymes were removed. After adenylation of 3' ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. Captured libraries were enriched in a PCR reaction to add index tags to prepare for hybridization. Products were purified using AMPure XP system (Beckman Coulter, Beverly, USA) and quantified using the Agilent Bioanalyzer 2100 system (RRID:SCR_019715). 300bp paired end sequencing was performed on an Illumina NovaSeq instrument (RRID:SCR_016387). Afterwards sequenced samples were analyzed according to the MoCaSeq Pipeline (44). Before mapping, raw sequencing reads were trimmed using Trimmomatic (RRID:SCR_011848) version 0.39. Leading and trailing bases with Phred scores below 25 and reads shorter than 50 nucleotides were removed. In addition, the average base quality within a sliding window of 10 nucleotides should be above 25 to keep the read for further downstream analysis. Reads were aligned to the GRCm38.p6 reference genome using BWA-MEM 0.7.17 (RRID:SCR_010910) with default settings. PCR duplicates were marked with samblaster (RRID:SCR_000468) version 0.1.26 together with Picard tools version 2.20.0 and realignment around indels was performed with GATK (RRID:SCR_001876) toolkit version 4.2.0.0. Mutect2 was used for calling somatic mutations with default settings. Potential somatic events were filtered for SNPs by excluding Single Nucleotide Variants (SNVs) which were listed in version 5 of the Mouse Genome Project SNP database. Somatic point mutations were included in the final list, if the read coverage for each position was at least 5 in both control and tumor, variant frequency was at least 5% and read count supporting the variant nucleotide was at least 2 in the tumor sample and equal to 0 or 1 in the control. Further, SNVs marked as strand or PCR bias artefacts by 'LearnReadOrientationModel' were excluded from further analysis. Annotation of somatic events was conducted with SNPeff (RRID:SCR_005191) version 4.3 . Data was deposited to ENA: PRJEB47385.

Statistical methods. Analysis of variance or two-sided Student's t-test was used to investigate statistical significance, as indicated. Kaplan–Meier curve was analyzed by the Log-rank test. P-values were calculated with GraphPad Prism6/8 (GraphPad Software, San Diego, CA, USA,

RRID:SCR_002798), and unless otherwise illustrated, all data were determined from at least three independent experiments and presented as mean and standard deviation (S.D.). Multiple testing was corrected according to Bonferroni.

References Supplemental Material and Methods

1. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 2003;4:437–50.

2. Eser S, Reiff N, Messer M, Seidler B, Gottschalk K, Dobler M, et al. Selective Requirement of PI3K/PDK1 Signaling for Kras Oncogene-Driven Pancreatic Cell Plasticity and Cancer. Cancer Cell. 2013;23:406–20.

3. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004;119:847–60.

4. Montgomery RL, Davis CA, Potthoff MJ, Haberland M, Fielitz J, Qi X, et al. Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. Gene Dev. 2007;21:1790–802.

5. Schönhuber N, Seidler B, Schuck K, Veltkamp C, Schachtler C, Zukowska M, et al. A next-generation dual-recombinase system for time- and host-specific targeting of pancreatic cancer. Nat Med. 2014;20:1340–7.

6. Lee C-L, Moding EJ, Huang X, Li Y, Woodlief LZ, Rodrigues RC, et al. Generation of primary tumors with Flp recombinase in FRT-flanked p53 mice. Dis Model Mech. 2011;5:397–402.

7. Seidler B, Schmidt A, Mayr U, Nakhai H, Schmid RM, Schneider G, et al. A Cre-loxP-based mouse model for conditional somatic gene expression and knockdown in vivo by using avian retroviral vectors. Proc National Acad Sci. 2008;105:10137–42.

8. Diersch S, Wirth M, Schneeweis C, Jörs S, Geisler F, Siveke JT, et al. KrasG12D induces EGFR-MYC cross signaling in murine primary pancreatic ductal epithelial cells. Oncogene. 2015;35:3880–6.

9. Stojanovic N, Hassan Z, Wirth M, Wenzel P, Beyer M, Schäfer C, et al. HDAC1 and HDAC2 integrate the expression of p53 mutants in pancreatic cancer. Oncogene. 2016;36:1804–15.

10. Burstin J von, Eser S, Paul MC, Seidler B, Brandl M, Messer M, et al. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex. Gastroenterology. 2009;137:361–71, 371.e1-5.

11. Mueller S, Engleitner T, Maresch R, Zukowska M, Lange S, Kaltenbacher T, et al. Evolutionary routes and KRAS dosage define pancreatic cancer phenotypes. Nature. 2018;554:62–8.

12. Diersch S, Wenzel P, Szameitat M, Eser P, Paul MC, Seidler B, et al. Efemp1 and p27(Kip1) modulate responsiveness of pancreatic cancer cells towards a dual PI3K/mTOR inhibitor in preclinical models. Oncotarget. 2013;4:277–88.

13. Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, et al. Pathology of Genetically Engineered Mouse Models of Pancreatic Exocrine Cancer: Consensus Report and Recommendations. Cancer Res. 2006;66:95–106.

14. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.

15. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics. 2014;31:166–9.

16. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

17. Parekh S, Ziegenhain C, Vieth B, Enard W, Hellmann I. The impact of amplification on differential expression analyses by RNA-seq. Sci Rep-uk. 2016;6:25533.

18. Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell. 2015;161:1202–14.

19. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics. 2010;26:2363–7.

20. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47–e47.

21. Peran I, Madhavan S, Byers SW, McCoy MD. Curation of the pancreatic ductal adenocarcinoma subset of The Cancer Genome Atlas is essential for accurate conclusions about survival related molecular mechanisms. Clin Cancer Res. 2018;24:clincanres.0290.2018.

22. Initiative APCG, Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016;531:47–52.

23. Nicolle R, Blum Y, Marisa L, Loncle C, Gayet O, Moutardier V, et al. Pancreatic Adenocarcinoma Therapeutic Targets Revealed by Tumor-Stroma Cross-Talk Analyses in Patient-Derived Xenografts. Cell Reports. 2017;21:2458–70.

24. Krebs AM, Mitschke J, Losada ML, Schmalhofer O, Boerries M, Busch H, et al. The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat Cell Biol. 2017;19:518–29.

25. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. P Natl Acad Sci Usa. 2005;102:15545–50.

26. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44:W90-7.

27. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles G, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. Bmc Bioinformatics. 2013;14:128.

28. Stöckel D, Kehl T, Trampert P, Schneider L, Backes C, Ludwig N, et al. Multi-omics enrichment analysis using the GeneTrail2 web service. Bioinformatics. 2016;32:1502–8.

29. Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, Maciejewski A, et al. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res. 2016;44:W147-53.

30. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 2015;43:W566–70.

31. Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods. 2017;14:959–62.

32. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods. 2013;10:1213–8.

33. Cernilogar FM, Hasenöder S, Wang Z, Scheibner K, Burtscher I, Sterr M, et al. Pre-marked chromatin and transcription factor co-binding shape the pioneering activity of Foxa2. Nucleic Acids Res. 2019;47:9069–86.

34. Blecher-Gonen R, Barnett-Itzhaki Z, Jaitin D, Amann-Zalcenstein D, Lara-Astiaso D, Amit I. High-throughput chromatin immunoprecipitation for genome-wide mapping of in vivo protein-DNA interactions and epigenomic states. Nat Protoc. 2013;8:539–54.

35. Imbeault M, Helleboid P-Y, Trono D. KRAB zinc-finger proteins contribute to the evolution of gene regulatory networks. Nature. 2017;543:550–4.

36. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009;10:R25.

37. Kumar V, Muratani M, Rayan NA, Kraus P, Lufkin T, Ng HH, et al. Uniform, optimal signal processing of mapped deep-sequencing data. Nat Biotechnol. 2013;31:615–22.

38. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol Cell. 2010;38:576–89.

39. Robinson JT, Thorvaldsdóttir H, Wenger AM, Zehir A, Mesirov JP. Variant Review with the Integrative Genomics Viewer. Cancer Res. 2017;77:e31–4.

40. Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, et al. The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol. 2020;38:276–8.

41. Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature. 2012;481:389–93.

42. Brown RS and G. DiffBind: Differential binding analysis of ChIPSeq peak data. http://bioconductor.org/ [Internet]. Available from: http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf

43. Yu G, Wang L-G, He Q-Y. ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. Bioinformatics. 2015;31:2382–3.

44. Lange S, Engleitner T, Mueller S, Maresch R, Zwiebel M, González-Silva L, et al. Analysis pipelines for cancer genome sequencing in mice. Nat Protoc. 2020;15:266–315.