Supplemental Materials and Methods

Animals. The mice were handled in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. All experiments were performed in mice of mixed background and in age-controlled fashion. Both genders were used. Tamoxifen (Sigma) was given at 0.17mg/g via oral gavage for three consecutive days to mice that were 3.5 to 4.5 weeks old.

The transgenic mouse lines used in this study were as follows and have been previously described: *Ptf1a-Cre,* [1] *Arid1a*^{floxed}*,* [2] *Kras*^{G12D}*,* [3] *Trp53*^{floxed}, [4] *Sox9-CreER,* [5], *Ptf1a*^{CreER}, [6]*, Rosa-LSL-tdTomato* [7]. Please see Supplementary Table 2 for genotyping primer.

Duct size was measured by identifying the largest duct on each section and then measuring the maximum diameter.

Cell culture. Immortalized human pancreatic ductal epithelial (HPDE) cells (ATCC) [8] were maintained in keratinocyte-SFM media supplemented with bovine pituitary extract and epidermal growth factor per manufacturer instruction (Thermo Fisher Scientific, 17005042).

siRNA. The following Silencer Select siRNA (Ambion) were used: Negative Control (4390843), *ARID1A* (s15785 and s15786), *MYC* (s9129 and s9130). Forward transfection was performed with Lipofectamine RNAiMAX per manufacturer's instruction (Thermo Fisher Scientific).

Generation of HPDE *ARID1A* null cells

Mouse *Arid1a* gRNA (GCTGCTGCTGATACGAAGGTTGG) was cloned into LentiGuide-puro plasmid (Addgene #53963). Active lentivirus was prepared in 293T cells in 10-cm dish. The day after seeding cells, each dish was transfected with pVsvg, pLenti-gag pol, *Arid1a* gRNA or

LentiCas9-Blast plasmid (Addgene #53962) using Lipofectamine 3000 (Life Technologies). Virus containing medium was collected at 60h after transfection. For creating the CAS9 stable expressing HPDE cell line, we infected cells with lentivirus carrying CAS9, followed by blasticidin selection (10 ug/ml) for 4 days. We then infected CAS9 expressing HPDE cells with *Arid1a* gRNA lentivirus. Three days after the infection, we selected cells for 3 days with puromycin at 2ug/ml. Then, we plated 50 to 200 cells in 15 cm dishes for single clone selection. We picked single clones when they grew big enough and verified their genotype and *ARID1A* expression by PCR and Western blot. Clones that retained ARID1A on Western blot were used as wild-type control and ones that did not express ARID1A were used as experimental lines.

Immunohistochemistry and Immunofluorescence. Tissues were fixed overnight in 4% paraformaldehyde, embedded in paraffin, and dehydrated. For immunohistochemistry, antigen retrieval was performed with sodium citrate buffer. Incubation with primary antibody was overnight at 4ºC. Detection was performed with ABC kit (Vector Laboratories) and DAB kit (Vector Laboratories) or MOM kit (Vector Laboratories). Primary antibodies used were anti-ARID1A, 1:500 dilution (Cell Signaling, 12354s); anti-KRT19, 1:50 (DSHB, TROMA-III-S); anti-HNF-4α, 1:200 (Santa Cruz, sc-8987); anti-SOX9, 1:2000 (Millipore, AB5535); anti-PDX1, 1:2000 (EMD Millipore, 07-696); anti-CD45, 1:200 (eBioscience, 13-0451); anti-ER, 1:10000 (Millipore, 06-935); anti-PR, 1:100 (Thermo Scientific; 9102-S0); anti-MUC1, 1:100 (Thermo Scientific, RB-9222-P0); anti-MUC5ac, 1:200 (MS 145-P0); anti-MUC2, 1:100 (Santa Cruz, 15334), anti-RFP, 1:2000 (Rockland, 600-401-379), anti-Claudin 18, 1:1000 (Thermo 700178), anti-CPA-1, 1:1000 (R&D AF2765), anti-MYC 1:500 (Abcam, ab32072), and anti-CDX2 1:1000 (Cell Signaling, 12306). Alcian blue (AMTS, KTABP1) and Sirius red staining (NovaUltra, IW-3012) were performed according to manufacturers' instructions.

For immunofluorescence, primary antibody used were anti-RFP 1:200 (Rockland, 600-401-379), anti-tdTomato (Biorbyt, orb182397), anti-KRT19 1:2 to 1:10 (DSHB, TROMA-III-S), anti-insulin, 1:150 (Cell Signaling 4590), anti-glucagon, 1:200 (Cell Signaling, 2760), anti-amylase, 1:200 (Sigma A8273). DAPI was used as nuclear stain (Vector Laboratories).

Cell Counting. To determine proliferative rates of duct cells in *Sox9-CreER* mice, all duct cells from one section of each mouse were counted. The number of Ki-67 positive/CK-19 positive double stained cells were divided by the total number of CK-19 positive cells. To determine efficiency of the *Sox9-CreER* recombinase, the entire section from each mouse was examined. The number of tomato positive/CK-19 positive double stained cells were divided by the total number of CK-19 positive cells. To determine the total PanIN area, the total claudin-18 positive area was divided by total pancreas area. One section was quantified per mouse.

Western Blot. Mouse pancreata were lysed in Tissue Protein Extraction Reagent (Thermo Scientific Pierce). Primary antibodies used were anti-ARID1A, 1:2000 (Sigma Aldrich, HPA005456); anti-MYC, 1:2500 (Abcam ab32072); anti-ß-ACTIN, 1:15000 (Cell Signaling, 4970); anti-ß-TUBULIN, 1:5000 (Cell Signaling, 2128); anti-phospho-mTOR 1:1000 (Cell Signaling, 2974); anti-mTOR 1:1000 (Cell Signaling, 2983); phospho-S6 ribosomal protein 1:1000 (Cell Signaling, 5364); S6 ribosomal protein1:1000 (Cell Signaling, 2217).

RNA Extraction and RT-qPCR. Total RNA was isolated using TRIzol (Invitrogen). cDNA synthesis was performed with 1 mg of total RNA using miScript II RT Kit (QIAGEN). RT-qPCR expression was measured using the standard delta-delta Ct method.

RNA sequencing.

Pancreata from six to eight-week old mice were utilized. Total RNA was isolated as above, and the libraries were prepared with the Ovation RNA-Seq Systems 1-16 based on manufacturer instructions (Nugen). Indexed libraries were multiplexed in a single flow cell and underwent 75 base pair single-end sequencing on an Illumina NextSeq500 using the High Output kit v2 (75 cycles) at the UTSW Children's Research Institute Sequencing Facility. Adaptors and low-quality sequences were trimmed from raw sequencing reads (Phred score < 20) using trim galore package (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The reads were aligned to the GRCm38/mm10 using HiSAT2, [9] and duplicates removed by SAMtools. [10] Read counts were then generated for annotated genes based GENCODE V20 with featureCounts. [11,12] edgeR was used to perform differential gene analysis with FDR < 0.05 as cutoff. [13] and GSEA analysis was performed with a pre-ranked gene list by log fold change. [14]

Chromatin immunoprecipitation and sequencing

Cells were fixed with fresh 1% formaldehyde for 10 min at room temperature and 125 mM glycine was added to inactivate the fixation. Cells were washed by cold PBS 3 times and collected. Cross-linked chromatin was sonicated into fragments of a size range between 250 and 500 nucleotides (Covaris). H3K27Ac antibody (Abcam, ab4729) was incubated with the solubilized DNA fragments at 4°C overnight. Antibody–chromatin complexes were captured by protein A/G Agarose beads (Pierce #20423) and eluted with 1% SDS after extensive washing. The cross-link between DNA and chromatin proteins was reversed by incubation overnight at 65°C. DNA was purified by QIAquick PCR Purification Kit (Qiagen 28104) and dissolved into 30 μL TE buffer per immunoprecipitation. Libraries were prepared per manufacturer instructions (Kapa LTP library preparation kit, KK8230) and sequenced by Illumina Nextseq 500 at the UTSW Children's Research Institute Sequencing Facility.

For analysis, adapters and low quality sequences (quality score < 25) were removed and then reads were mapped onto GRCh38 reference genome using BWA. [15] We performed filtering by removing alignments with mapping quality less than 30 (MAPQ < 30) and removing duplicate reads identified by SAMBAMBA 0.6.6. [16] The mapped reads were compiled into whole genome WIG profile using MACS 2.1.0 with the fragment size set to the 200 bp and peak calling threshold set at p-value = 0.01. [17] We defined the H3K27ac peaks, which were normalized by the corresponding input, as enrichment. Finally, using deepTools 2.3.5, [18] we calculated mean enrichment scores for the genome regions of genes in the Hallmark MYC Targets V2 GSEA signature, the Reactome Peptide Chain Elongation GSEA signature, and all known genes, and visualized the enrichments through profile plots and heatmaps, whose x-axis represents the distance to closest transcription start site (TSS).

Protein synthesis measurements. O-propargyl-puromycin (OPP) was used to measure protein synthesis rates [19,20]. The assay was performed using an Alexa Fluor 594 dye, based on manufacturer instruction (Thermo Fisher Scientific, C10457). Briefly, the cells were incubated with OPP for 30 minutes, fixed with 1% PFA for 15 minutes at 4ºC, permeabilized with saponin (0.05% in PBS) for 5 minutes at room temperature, and the Click-iT reaction performed. Fluorescence levels were measured using a FACS Aria flow cytometer (BD Biosciences). Data were analyzed using FlowJo (Tree Star). Cycloheximide (Sigma, 100ug/mL) was added for 30 minutes before fixation.

EZH2 inhibitor treatment. Four-week old KCA mice were utilized and randomized to either vehicle alone or compound treatment. The groups were balanced for gender and initial cyst volume. EPZ011989 (provided by Epizyme, Inc.) was given at 250 mg/kg twice a day at a dosing volume of 10ml/kg via oral gavage for four weeks. [21] The vehicle was 0.5% sodium carboxymethyl cellulose and 0.1% Tween-80.

Magnetic resonance imaging (MRI). MRI scanning was conducted using a 7T small animal MRI scanner (Agilent (Varian), Inc,) equipped with a 40 mm Millipede RF coil (ExtendMR LLC) under inhalational anesthesia. The mice were placed supine head first with the border of liver and kidneys centered with respect to the center of the RF coil. MRI acquisitions were gated using the respiratory triggering. Two-dimensional scout images were obtained in the axial, coronal, and sagittal planes to ensure the positioning. The kidneys and spinal cord were used as the visual margins to plan the coronal sections. High-resolution T2 weighted fast spin-echo images with fat suppression were acquired on these coronal sections. Fifteen coronal images per animal were imported into ImageJ and the T2 hyperintense area denoting the cysts were measured using the freehand selection function. The areas were summed to generate the volume of the cysts per animal.

Statistical Analysis. The data is presented as mean ± SEM. The Mann-Whitney u test was used for continuous variables, the Fisher's exact test for categorical variables, and two-way analysis of variance for repeated measures. Statistical significance in all figures is displayed as *P < 0.05; **P < 0.01; ** P < 0.001.

Data Sharing. RNA-seq and ChIP-seq data can be found at XXXX

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