Supplementary Materials and Methods

Immunoblot analysis

Cells and tissues were lysed in RIPA buffer (Pierce, Rockford, IL, USA) with Xpert Protease and Phosphatase Inhibitor Cocktail Solution (GenDepot, Katy, TX, USA). Total proteins were quantified using Bradford assay (Bio-Rad, Hercules, CA, USA). NuPAGE™ Sample Reducing Agent (Invitrogen, Carlsbad, CA, USA) was added to RIPA-lysed samples and denatured at 70℃ for 10 min. Samples were loaded on SDS-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 30 min at RT with 5% skim milk or 3% BSA in Tris-buffered saline (TBS) containing 0.1% Tween 20 and incubated with specific primary antibodies at 4℃ overnight. All listed primary antibodies were used at 1: 2000. Membranes were washed and incubated with HRP-conjugated secondary antibodies for 1 h at RT, developed immunoreactivity (Chemiluminescent HRP substrate, Millipore) and imaged on the ChemiDoc™ XRS+System (BioRad, Hercules, CA, USA).

Histology and immunofluorescence staining

Mouse tissues were harvested and fixed in 10% neutral buffered formalin solution. The formalin-fixed tissues were embedded in paraffin. 4 μm tissues sections were obtained on a microtome, deparaffinized, rehydrated, and stained with hematoxylin & eosin (H&E) or Masson's trichrome. For immunofluorescence staining, rehydrated sections were subjected to antigen retrieval in sodium citrate buffer (pH 6.0) and heated at 100℃ for 15-30min. Next, sections were incubated in blocking buffer (1% BSA in TBST) for 30 min at room temperature. Primary antibodies were diluted in blocking buffer and incubated with sections at 4℃ overnight. Fluorescent dye-conjugated secondary antibodies were diluted in blocking buffer and incubated with sections at room temperature for 1h. After staining, sections were mounted on fluorescence mounting medium (Dako). Images were acquired on a confocal microscope (Zeiss LSM 780).

For cryosection, pancreas tissue was harvested after perfusion with 10 ml PBS, followed by 10 ml 2% paraformaldehyde (PFA) in PBS. Tissues were fixed for 4 h in 4% PFA and then incubated in 15% sucrose/PBS for 4 h, and then in 30% sucrose/PBS overnight. Samples were

embedded in OCT compound and stored at -80°C. 10 μm frozen tissue sections were obtained on a cryomicrotome (Leica CM3050S).

Isolation and culture of pancreatic acinar cells

Procedures for isolation of primary acini from pancreas is described elsewhere. [1] Briefly, pancreas was removed, rinsed twice in Hank's Balanced Salt Solution (HBSS), sliced into 1-3 mm3 size with a razor blade and digested with collagenase P solution (10 mM HEPE, 10 mg/ml of collagenase P and 0.25 mg/ml of trypsin inhibitor in HBSS). The digestion was stopped by adding 10 ml of cold washing solution (5% Fetal Bovine Serum (FBS) and 10mM HEPES in HBSS). The dissociated pancreas pieces were washed twice with washing solution and centrifuged for 3 min at 450 x g at 4°C. The pellets were resuspended with culture media (2.5% FBS, 1% Penicillin-Streptomycin mixture (PS), 0.25 mg/ml of trypsin inhibitor, and 25 ng/ml of recombinant human Epidermal Growth Factor (EGF) in Waymouth's medium), then filtrated on a 100 μm cell strainer and seeded on a gelatin-coated culture plate or in a Matrigel dome.

Total RNA and Genomic DNA extraction from acinar cells

Total RNA was extracted from primary acinar cells using TRIzol™ (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Genomic DNA was obtained from primary acinar cells using Blood & Cell Culture DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. RNA and genomic DNA concentration were measured with a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

High-quality RNA extraction from pancreatic tissue

Pancreas was perfused by retrograde intraductal infusion of RNAlater™ (Invitrogen, Carlsbad, CA, USA) and kept overnight at 4°C (**Supplementary Figure 15**). The pancreas was chopped into ~ 1 mm^3 pieces with a razor blade and homogenized in TRIzol™ reagent using a FastPrep-24 bead homogenizer (MP Biomedicals, Santa Ana, CA, USA). Total RNA was extracted using TRIzol™ following the manufacturer's protocol.

Chromatin immunoprecipitation (ChIP) from pancreatic acinar cells

Chromatin was prepared for freshly isolated mouse primary acini. Briefly, acinar cells were crosslinked with 1% formaldehyde for 10 min at RT, followed by quenching with 125 mM glycine for 5min. Fixed cells were lysed and then sheared using the Sonics Vibra-Cell VCX130 Ultrasonic Processor for 2 min 30 sec of 1 sec ON/ 1 sec OFF at 50% amplitude. The fragmented chromatin was immunoprecipitated with anti-IgG, anti-ERRγ, and anti-H3K4me3. ChIP-qPCR primers are listed in **Supplementary Table 7**.

Quantitative real-time PCR

Total RNA was reverse transcribed to cDNA using HelixCript™ Easy cDNA Synthesis Kit (Nanohelix, Daejeon, South Korea). Quantitative real-time PCR was performed using Premier qPCR Mix [SYBR Green with ROX] (Nanohelix, Daejeon, South Korea) on a ViiA7 Real-Time PCR system (Applied Biosystems, Foster city, CA, USA). Relative quantification was based on the ΔΔCt method, and RPLP0 gene was used as a reference control. Primers were designed using NCBI Primer-BLAST and listed in **Supplementary Table 7**.

Transmission Electron Microscopy

Mice were heart perfused with 10 ml of sodium cacodylate buffer (pH 7.4) and subsequently with 20 ml fixative solution containing 2 % paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) and 2.5 % glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) in sodium cacodylate buffer. Pancreas tissue was dissected and diced into 1 mm^3 pieces under a stereo-microscope and post-fixed in the same fixative solution for 1 h. Tissue specimens were then post-fixed in 2% osmium tetroxide (OsO_4) containing 1.5% potassium ferrocyanide for 1 h at 4 °C. The fixed tissues were dehydrated using an ethanol series (50%, 60%, 70%, 80%, 90%, and 100%) for 20 min at each concentration and infiltrated with an embedding medium. After embedment, specimen was sectioned (60–70 nm) using an ultramicrotome (Leica Microsystems GmbH, Vienna, Austria). Then, they were double-stained with 2% uranyl acetate for 10 min and lead citrate for 5 min. The sections were then observed using a Tecnai G2 transmission electron microscope at 120 kV in (ThermoFisher, USA).

ERRα, ERRβ, ERRγ and ERα ligand binding assays

ERRs and ERα binding assays were conducted with Lanthascreen assay system (Life Technologies, Grand Island, NY, USA), which was based on TR-FRET, followed by manufacturer's instructions. Briefly, 4-hydroxytamoxifen was serially diluted 2-fold starting from 10 μM and 10 μL of each diluent was added to a 384 well plate. Then, GST-conjugated ERRα, ERRβ, ERRγ or ERα LBD (ligand-binding domain) was added to be 5 nM. Next, the mixture of fluorescein-conjugated coactivator PGC-1α (final concentration to be 500 nM) and Tb-a-GST antibody (final concentration to be 5 nM) was added. The reaction mixture was incubated at room temperature in dark state for 1 h. TR-FRET activity was measured at 340 nm excitation and 495 nm/520 nm dual emission using a microplate reader (Biotek, SynergyNeo, Winooski, VT, USA). The binding activity in the presence of 4-hydroxytamoxifen was represented as the percentage of control binding activity, and the IC50 value was calculated with non-linear regression fit with four parameters using the Prism 8.3 software. All IC50 values were statistically evaluated by R-squared value of goodness-of-fit, which was over 0.9.

BrdU Incorporation assay

Mice were intraperitoneally injected with bromodeoxyuridine (BrdU, 10 mg/ml) 3 d after the final tamoxifen injection. Pancreas tissues were harvested and fixed in 10 % neutral buffered formalin solution overnight. The formalin-fixed tissues were embedded in paraffin and cut at 4 μm. For DNA hydrolysis, paraffin sections were incubated in 1M HCl for 30 min to 1 h and washed thrice in PBS. Staining was performed following the aforementioned method.

RNA-Seq library generation

All transcriptome analyses described in this manuscript were performed with at least n=3 independent samples in indicated condition. Total RNA was isolated from cell pellets treated with RNAlater™ using the RNA mini kit (Qiagen) and treated with DNaseI (Qiagen) for 30 min at room temperature. Sequencing libraries were prepared from 100-500 ng total RNA using the TruSeq RNA Sample Preparation Kit v2 (Illumina) according to the manufacturer's protocol. Briefly, mRNA was purified, fragmented, and used for first-, then second-strand cDNA synthesis followed by adenylation of 3' ends. Samples were ligated to unique adapters

and subjected to PCR amplification. Libraries were then validated using the 2100 BioAnalyzer (Agilent), normalized, and pooled for sequencing. RNA-Seq libraries prepared from two biological replicates for each experimental condition were sequenced on the Illumina HiSeq 2500 using bar-coded multiplexing and a 100 bp read length.

High-throughput sequencing and analysis

Image analysis and base calling were performed with Illumina CASAVA-1.8.2. This yielded a median of 29.9M usable reads per sample. Short read sequences were mapped to a UCSC mm9 reference sequence using the RNA-seq aligner STAR [2]. Known splice junctions from mm9 were supplied to the aligner and de novo junction discovery was also permitted. Differential gene expression analysis, statistical testing and annotation were performed using Cuffdiff 2. Transcript expression was calculated as gene-level relative abundance in fragments per kilobase of exon model per million mapped fragments and employed correction for transcript abundance bias. RNA-Seq results for genes of interest were also explored visually using the UCSC Genome Browser.

Genomic data analysis

Genomic data analysis was performed by using on line free software such as DAVID. Heatmap and further analysis were performed by R Studio with gplot2 or Clusters and Java Treeview.

Glucose tolerance test and serum insulin measurement

For glucose tolerance test, over-night fasted mice were intraperitoneally injected with D-glucose (2 g of glucose/kg body mass). Glucose concentrations were measured at 0, 15, 30, 60 and 12 min using a Gluco Dr.TOP blood glucose meter (Allmedicus, Anyang, Republic of Korea). Serum insulin was analyzed via Mouse Ultrasensitive Insulin ELISA (ALPCO, USA).

Organic acid profiling analysis in pancreas

Pancreas samples from control and ERRγ cKO mice were prepared and subjected to organic acid profiling analysis as previously described.[3, 4] Deproteinization was performed by adding acetonitrile (100 μL) to pancreas tissue (1 mg) including $^{13}C_2$-succinic acid (0.5 μg) and 3,4-dimethoxybenzoic acid (0.1 μg) as internal standards. After centrifugation, the supernatants were added into deionized water (1 mL), an aliquot was adjusted to pH ≥ 12 using 5 M sodium hydroxide and then carbonyl group groups were converted to methoxime (MO) derivative using methoxyamine hydrochloride (1 mg) at 60℃ for 1 h. After the MO reaction, the aqueous phase was adjusted to pH ≤ 2 with 10 % H_2SO_4 and saturated with sodium chloride. Then extraction was performed by diethyl ether (3.0 mL) and ethyl acetate (2.0 mL). After addition of trimethylamine, the extracts were evaporated to dryness under a gentle stream of nitrogen (40℃). Prior to gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis, toluene (10 μL) and *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide with 1% *tert*-butyldimethylchlorosilane (20 μL) were added to the residue, and then heated at 60℃ for 60 min to form *tert*-butyldimethylsilyl (TBDMS) derivative.

Metabolic analysis

For monitoring metabolic rates, mice were housed individually in a metabolic chamber equipped with Oxymax/CLAMS instrumentation (Columbus Instruments, USA) and analysis was performed with mice fed ad libitum over 72 h. The respiratory exchange ratio (RER) is calculated as the ratio of VCO_2 to VO_2.

Extracellular acidification rate measurement

Extracellular acidification rate of primary pancreatic acinar cells was recorded in 96-well plates using a XF96 Extracellular Flux Analyzer and the XF Cell Mito Stress Test Kit (Seahorse Bioscience, North Billerica, MA) following the manufacturer's protocol. Briefly, primary acinar cells from C57BL/6J mice were seeded in a gelatin-coated 96-well cell culture plate and subsequently treated with GSK5182 or vehicle for 48 h. Cells were washed and pre-incubated in XF DMEM media (pH7.4) for 1 h prior to this assay. The Glycolysis Stress Tests were performed according to the manufacturer's protocol. Glucose (10 mM), Oligomycin (2 μM), and 2-deoxy-D-glucose (2-DG) (50 mM) were added as indicated.

Pancreatitis Human Cohort Analysis

To assess clinical relevance of ERRγ-dependent regulation, expression patterns of single or multiple genes were reanalyzed using human cohort samples from NCBI. Briefly, the CEL files were downloaded from the NCBI site (GSE143754 depository). Then, affy and oligo packages were utilized to load CEL files into R environment. The data frame of expression was established and normalized with the Robust Multiarray Averaging (RMA) method. To annotate Affymetrix data, the Human Transcriptome Array 2.0 (HTA-2.0) gene version was downloaded. The probe information was converted into gene symbol using the custom script. In addition, meta data including gender and disease status in each sample was extracted and merged into the R object. For further analysis, normal pancreas samples labelled with "_N" and chronic pancreatitis labelled with "_CP" were extracted. In order to compute significance between two groups, we conducted the Kruskal-Wallis test with the kruskal.test command. For the EMBL dataset, the E-EMBL-6 set was downloaded using the ArrayExpress package. The meta data including disease status was integrated into the expression object. Then, the data was normalized with the affyPLM package using the normalize.ExpressionSet.loess command. For pathway and enrichment analysis, the Enrichr tool was utilized. The gene sets including several gene lists were loaded into the Enrichr and significant pathways and cell types outputs were explored.

For single nucleus RNA-seq analysis, we established the dataset using the expression count tables from the EGAD00001006396, which were provided by the corresponding author of the study. In order to conduct snRNA-seq analysis, the Seurat package (v.4.0.4) was utilized. The expression count table and meta data were converted into the Seurat object. In order to minimize the batch effect, patient ID labelled with "TUM" was stratified. Moreover, expression restoration was conducted using the RunALRA command. After merging samples, normalization and RunHarnomy commands were used. Cell type or cluster was examined and defined based on the previous study. To visualize expression with conditions, the gene expression was extracted with the FetchData command and vlnplot was utilized for individual gene expression examination.

Human genetic study of the *ESRRG* locus

Subject selection. The study cohort was selected from the UK Biobank using the methods previously described.[5] Briefly, possible pancreatitis diagnosis consisted of all subjects with CP or AP diagnosis from the following data fields: ICD9, ICD10, death certificate, self-reported, age at first reported K85 diagnosis, and age at first reported K86 diagnosis. CP subjects were defined as a confirmed diagnosis of CP from either ICD9, ICD10, or death certificate data fields. AP subjects were defined as a confirmed diagnosis of AP from either ICD9, ICD10, or death certificate data fields excluding those with an additional CP diagnosis in any of those same data fields. This resulted in 6625 possible pancreatitis, 1027 confirmed CP, and 3329 confirmed AP with no CP. Finally, 24000 control subjects were selected at random from the rest of the UK Biobank who had no possible pancreatitis diagnosis.

NAPS2 subjects were ascertained, phenotyped and genotyped as previously described.[6-9] Standardized questionnaires were used for data collection and SNV arrays (Illumina HumanOmniExpress BeadChip and HumanCoreExome) were used for genotyping[7], with supplemental, targeted genotyping as previously described. [9, 10]

Genomic and covariate data. We used the previously published imputed genotype data (Bulk Data Category 100319) from the UK Biobank filtered at a minor allele frequency of 1%, Hardy Weinberg Equilibrium of $1x10^{-10}$, and a genotyping call rate of 10%. Original genotype data was collected on a custom Axiom array and imputed using a combination of the Haplotype Reference Consortium and UK10K Haplotype resource. Genetic principal components used in this analysis were generated and published by the UK Biobank as data field f22009. Covariate data was pulled either directly for sex (DF: f31) and BMI (DF: f21001) or computed for smoking status [current=1, Former/Never=0](DF: 20116) and age as of January 2021 assuming survival [01/01/2021 – 01/(Birth Month)/(Birth Year)](DFs: f52 & f34).

NAPS2 data were genotyped using the Illumina HumanOmniExpress BeadChip and imputed using the Sanger imputation service against the 1000 genomes phase 3 haplotype resource. Imputed data were filtered at a minor allele frequency of 1%, Hardy Weinberg Equilibrium of $1x10^{-10}$, and a genotyping call rate of 10% prior to analysis.

Genetic association analysis. We conducted a GWAS of AP and CP using logistic regression and accounting for age, sex, body mass index, smoking status, and the first six principal components of ancestry as covariates in PLINK2. After accounting for missing data this left cohorts of 23996 participants for CP (960 cases, 23036 controls) and 26116 participants for AP

(3080 cases, 23036 controls).

The NAPS2 GWAS of CP subjects only was conducted using logistic regression and accounting for age, sex, body mass index (BMI), smoking status, and the first six principal components of ancestry as covariates in PLINK2.

Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM) or standard deviation (SD). Statistical comparisons were analyzed using Student's t-test or two-way ANOVA. Statistically significant differences are represented as *p <0.05, ** p < 0.01, *** p < 0.005. Data analysis was performed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, CA, USA).

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