Supplementary Materials

Exploring human pancreatic organoid modelling through single-cell RNA sequencing analysis

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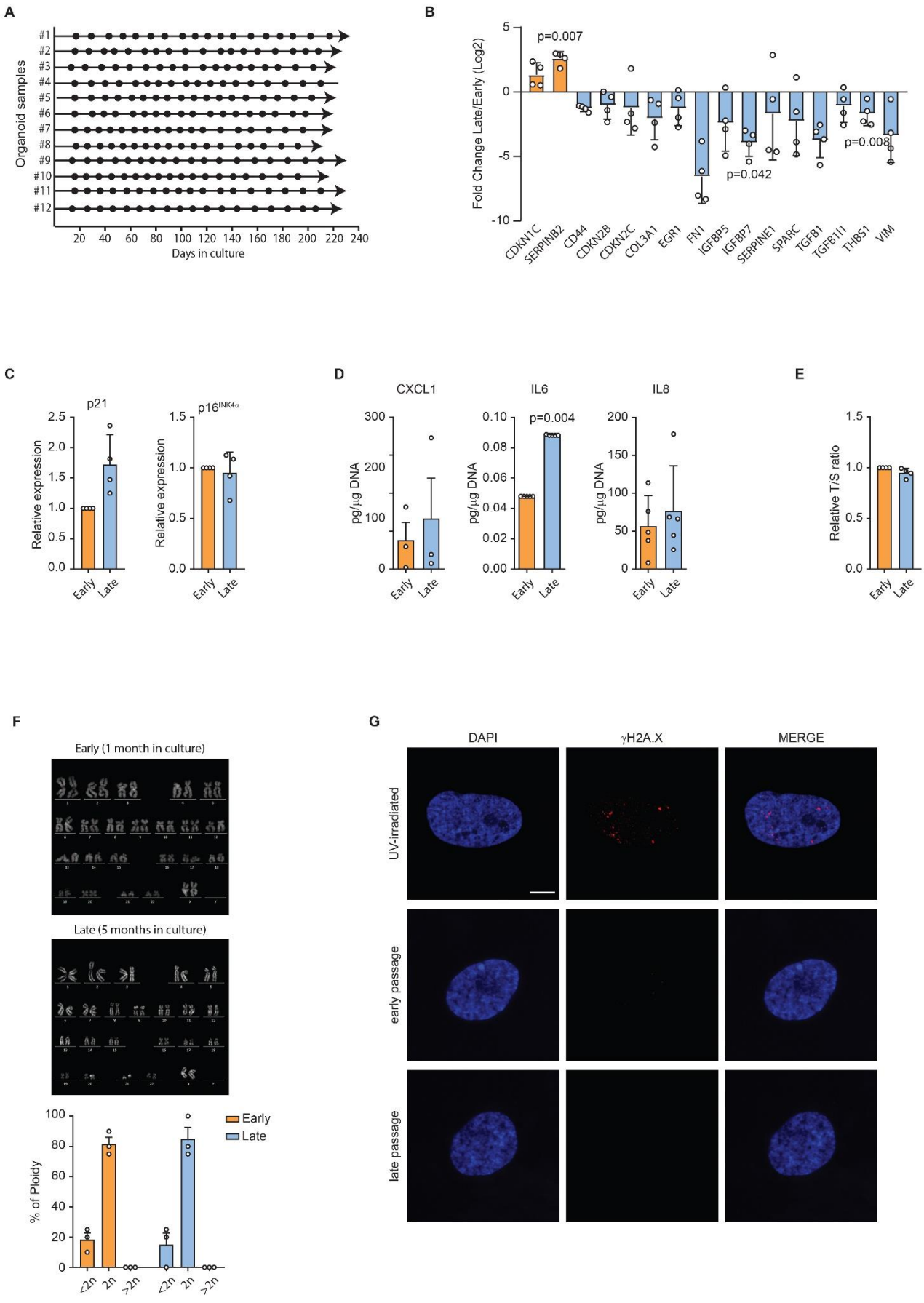
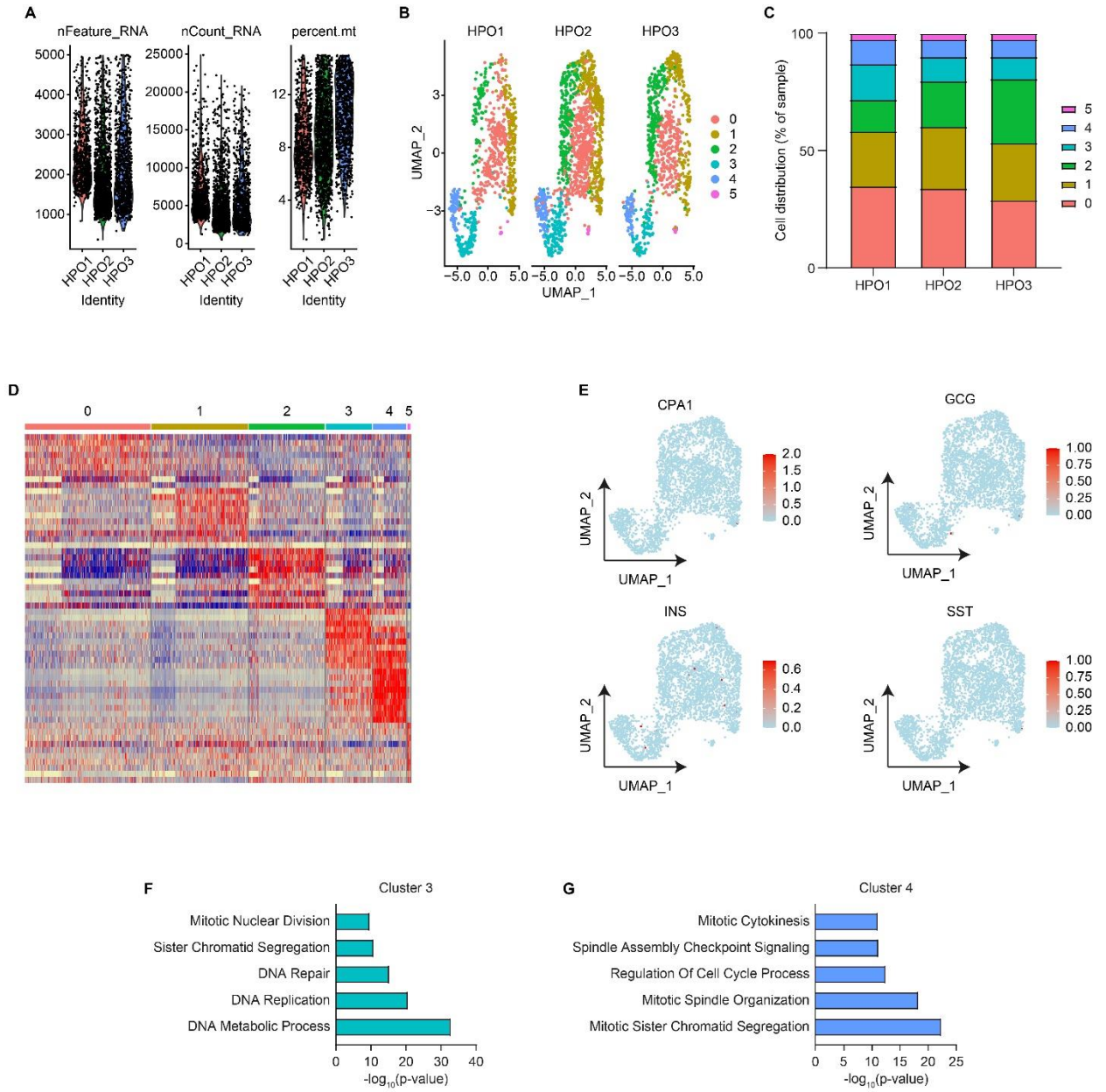
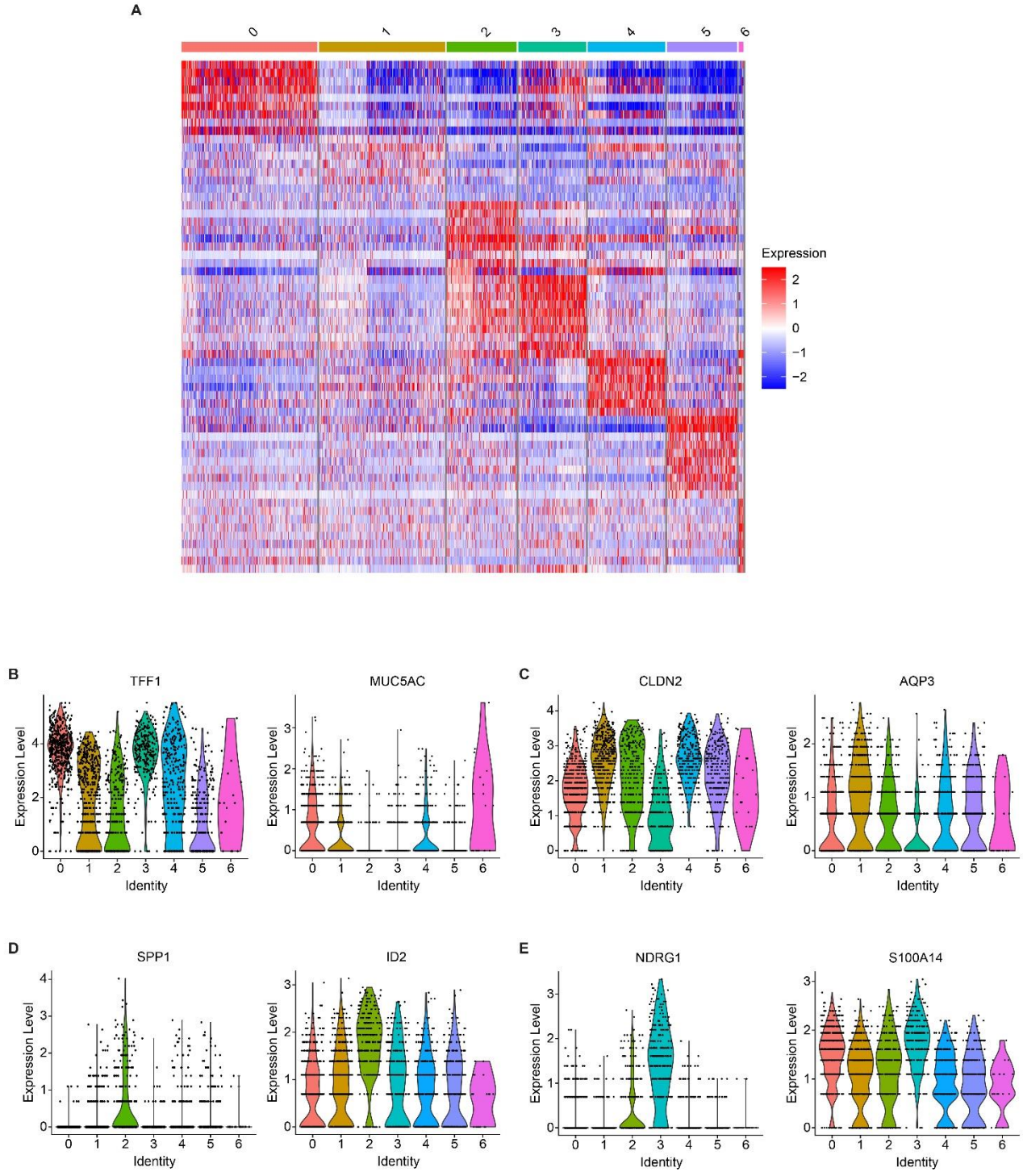

A
Organoid samples
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
20 40 60 80 100 120 140 160 180 200 220 240
Days in culture
B
Fold Change Late/Early (Log2)
5
0
-5
-10
p=0.007
p=0.042
p=0.008
CDKN1C
SERPINB2
CD44
CDKN2B
CDKN2C
COL3A1
EGR1
FN1
IGFBP5
IGFBP7
SERPINE1
SPARC
TGFB1
TGFB1I1
THBS1
VIM
C
p21
p16INK4a
Relative expression
Early
Late
D
CXCL1
IL6
IL8
pg/µg DNA
p=0.004
E
Relative T/S ratio
F
Early (1 month in culture)
Late (5 months in culture)
% of Ploidy
Early
Late
<2n
2n
>2n
G
DAPI
γH2A.X
MERGE
UV-irradiated
early passage
late passage

Supplementary Figure 1. Human pancreatic organoids can be expanded in long-term culture. (A) Schematic representation showing that human pancreatic organoids (hPOs) can be passaged over many months (n = 12 independent donors; circle = passage). (B) Fold change representation of selected genes by Fig. 2A. Data are means ± SD (n = 4 biologically independent samples). (C) The relative transcriptional level of p21 and $p16^{INK4a}$ genes were confirmed by qRT–PCR analysis on RNAs extracted from hPO early and late passages. Data are means ± SD (n = 4 biologically independent samples). (D) ELISA measurement of CXCL1, interleukin (IL)-6, and IL-8 secretion in medium from hPOs in early and late passages. Data are means ± SEMs (n = 3 biologically independent samples for CXCL1, and n = 5 biologically independent samples for IL6 and IL8). (E) Analysis of telomere length of human pancreatic organoids long-term cultured compared with early hPO. Data are means ± SD (n = 4 biologically independent samples). (F) Chromosome numbers were counted on a total of 20 metaphases. Data are means ± SD (n = 3 biologically independent samples). (G) Immunofluorescence for γH2A.X markers on hPO early and late passages. Scale bar, 10 μm.

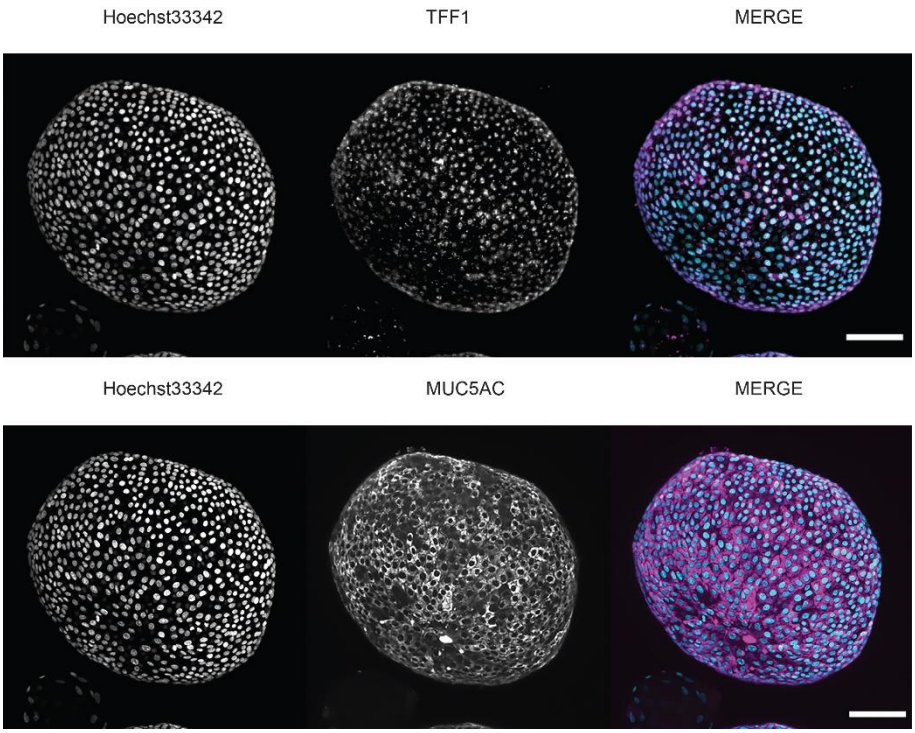
Supplementary Figure 2. Human pancreatic were genetically stable in culture. Array-CGH profiles of all chromosomes of two different lines of hPO at early (red dots) and late (blue dots) passages.



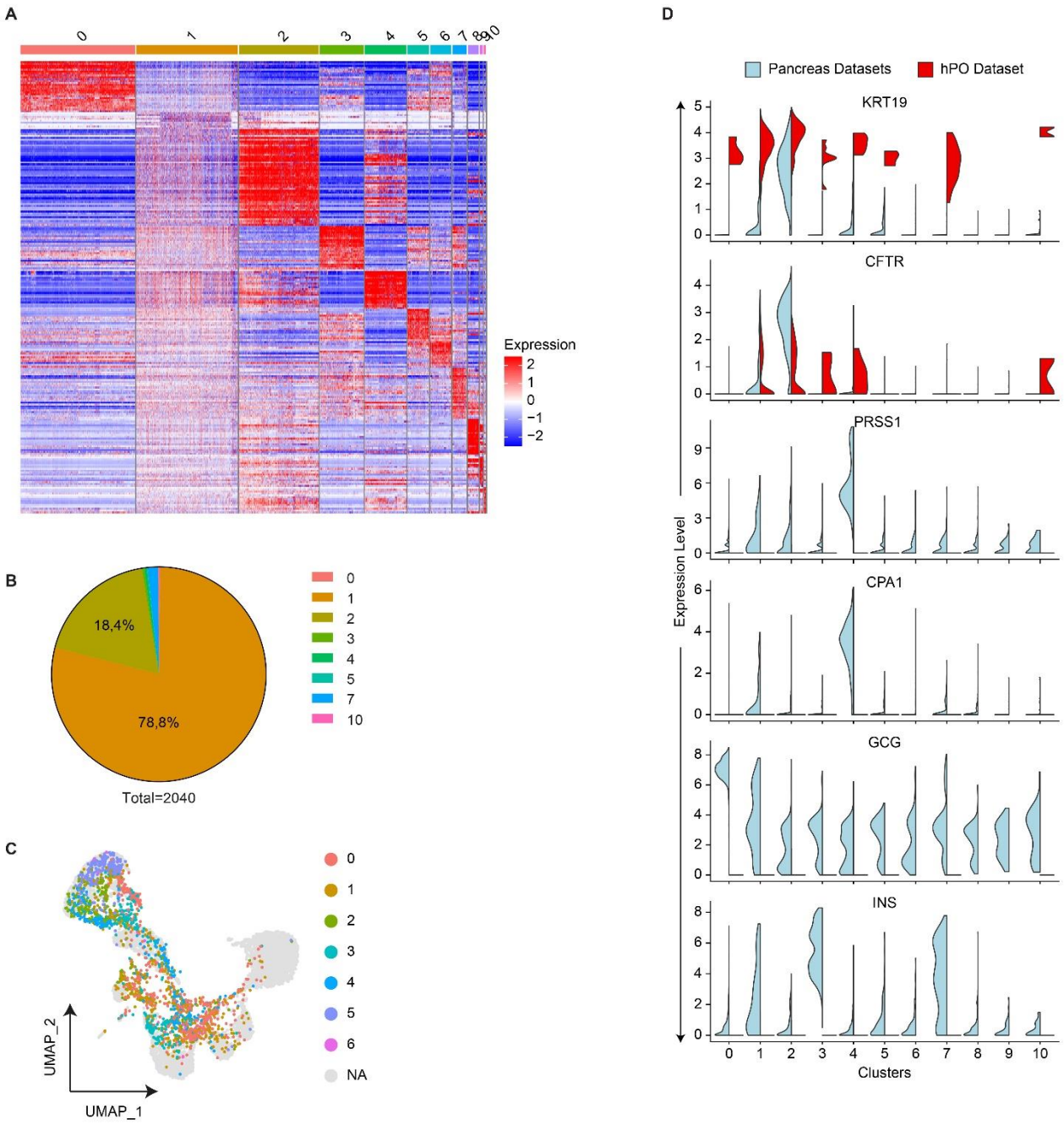
Supplementary Figure 3. Single-cell RNA sequencing analysis reveals a ductal cell identity of primary human pancreatic organoids. (A) Total number of expressed genes in each cell, total number of molecules detected within a cell and bar plot showing the expression of mitochondrial genes as a percentage of total mRNA in each cell. Each cell is represented by a single dot. (B) UMAP plot showing the contribution of each donor to the analyzed clusters. (C) Bar plot indicating the percentage distribution and contribution of various donors towards clusters. (D) Heatmap of the 10 most differentially expressed genes in each cluster from Fig. 2C. (E) UMAP plot showing the expression of archetypal acinar (CPA1) and endocrine (GCG, INS and SST) markers. (F and G) Five biological processes from Gene Ontology enriched in cluster 3 and 4.



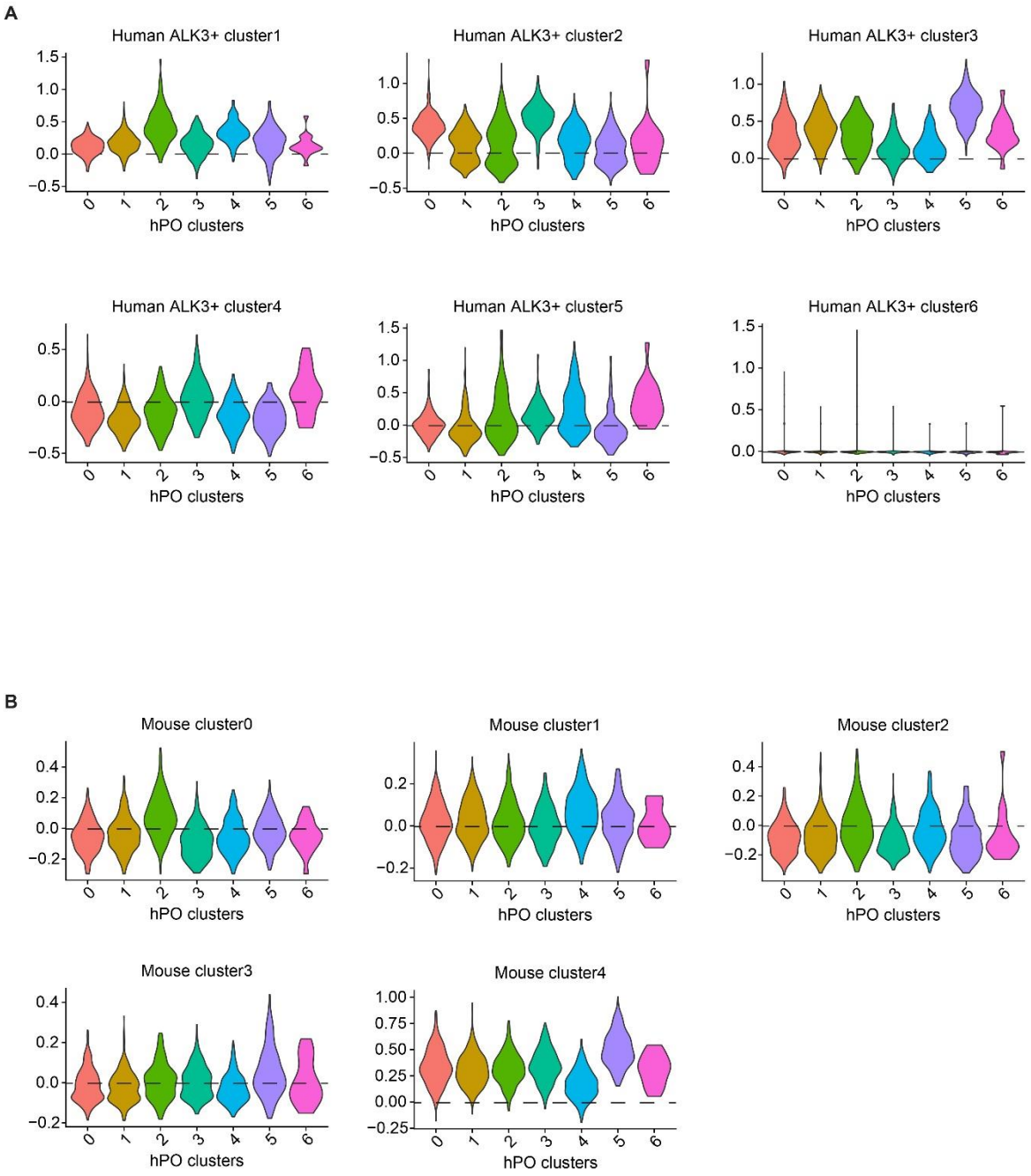
Supplementary Figure 4. Single-cell RNA sequencing analysis identify distinct ductal subtype populations in primary human pancreatic organoids. (A) Heatmap of the 10 most differentially expressed genes in each cluster from Fig. 3A. (B) Violin plots show differentially expressed genes (TFF1, MUC5AC, CLDN2 and AQP3) in cluster 0 and 1 (C) Violin plots show differentially expressed genes (SPP1, ID2, NDRG1 and S100A14) in cluster 2 and 3.



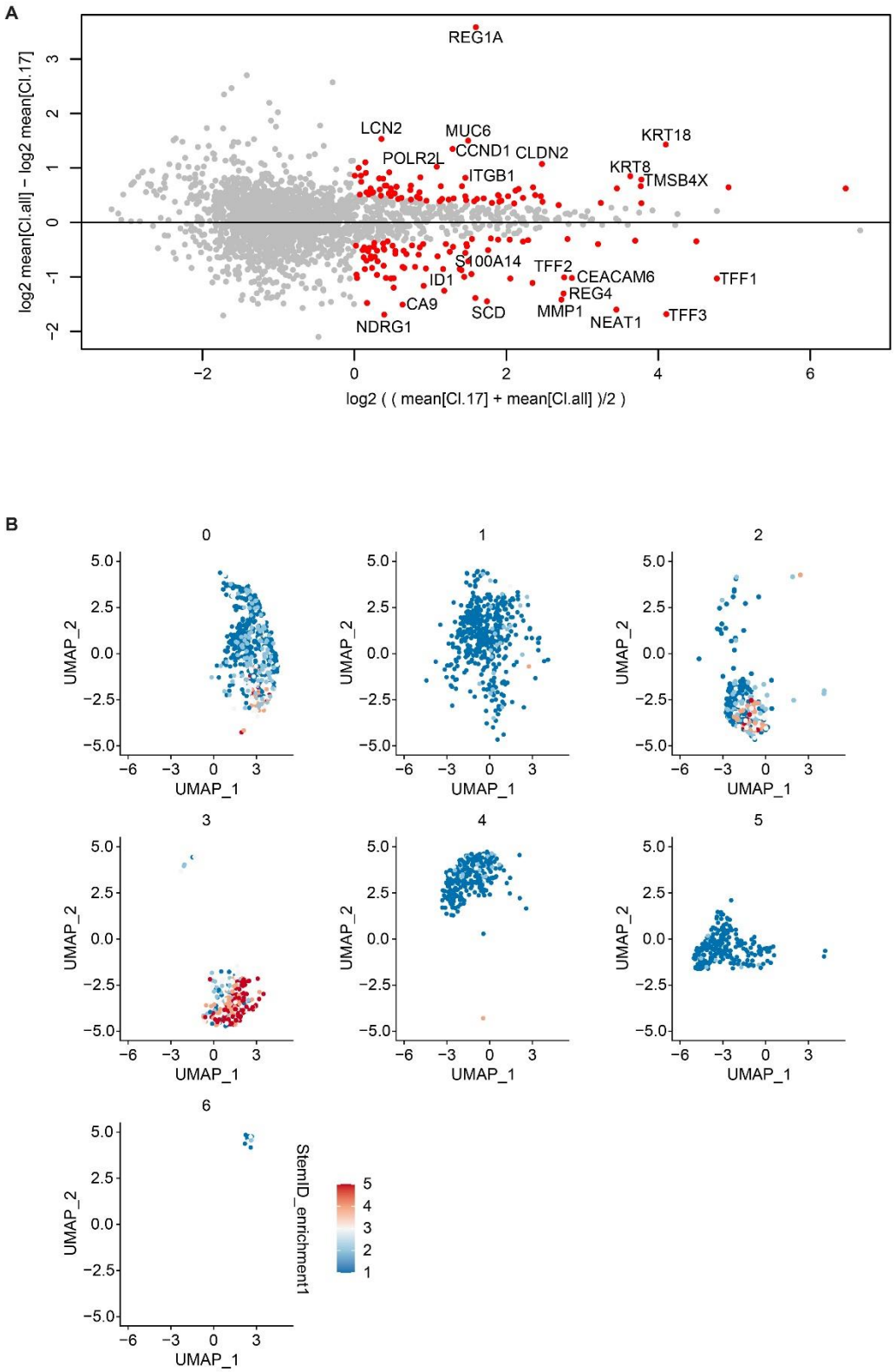
Supplementary Figure 5. Ductal intra-organoid heterogeneity. A) Confocal images of day-12 organoids immunostained for TFF1 and MUC5AC ductal subtype markers identified by scRNA-seq analysis, supporting the ductal heterogeneity of our hPOs. Scale bar, 100 μm.



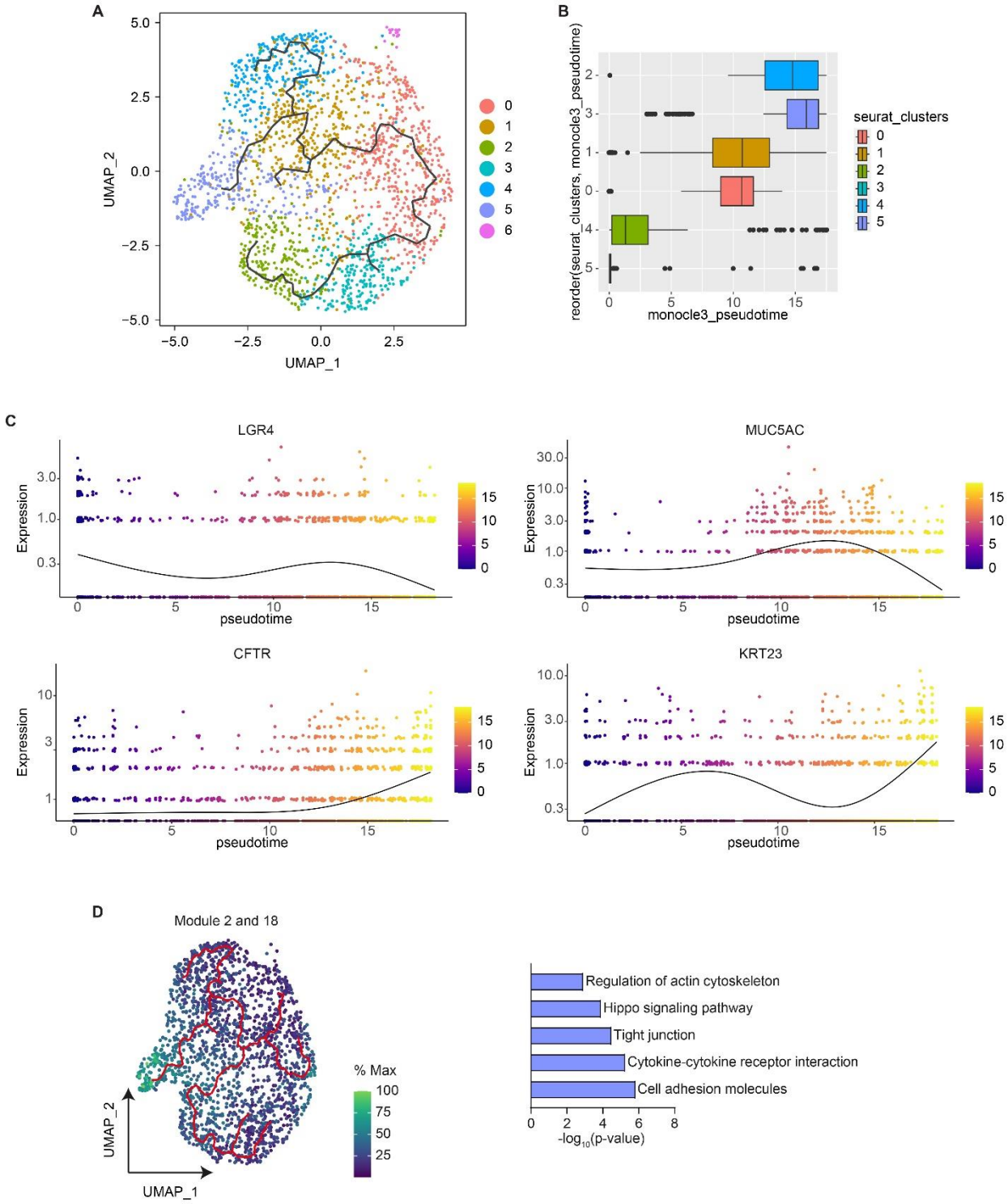
Supplementary Figure 6. Integration and comparison of primary human pancreatic organoids against human whole pancreas transcriptome. (A) Heatmap of the 10 most differentially expressed genes in each cluster from Fig. 4A. (B) Pie chart showing the contribution of the primary human pancreatic organoid datasets in each cluster from Fig. 4A. (C) UMAP plot illustrating the location of primary human pancreatic organoid cells in the integrated data sets. (D) Dual violin plots showing side by side gene expression distribution of cell clusters derived from whole pancreas datasets (light blue) and the primary human pancreatic organoids single-cell transcriptome (red).



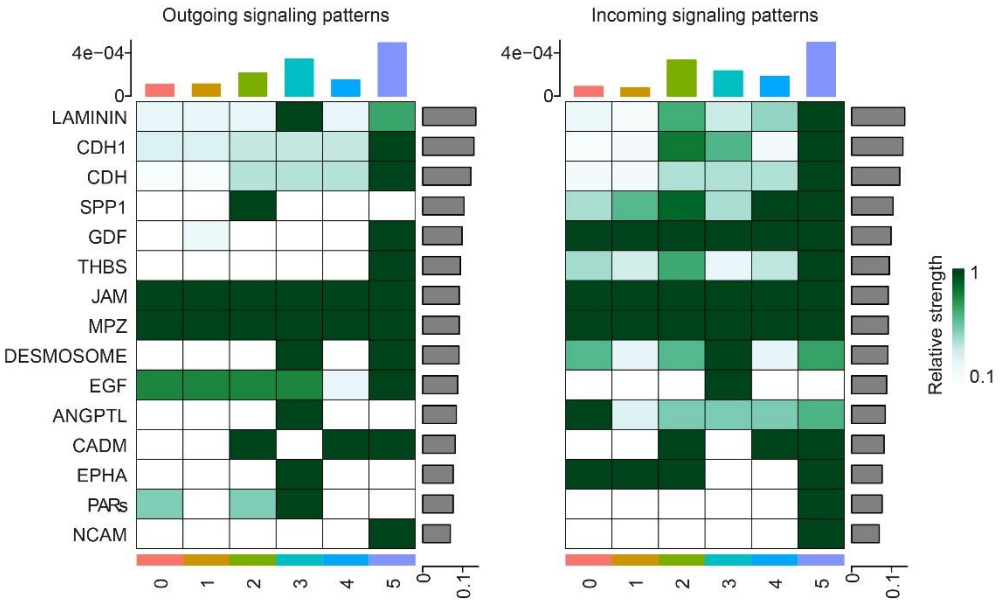
Supplementary Figure 7. Primary human pancreatic organoid ductal subpopulations are conserved in human and mouse. (A) Violin plots showing the score of homology between each of the human ALK3+ clusters {PMID: 32354994} to our primary human pancreatic organoid clusters. (B). Violin plots showing the score of homology between each of the human murine clusters {PMID: 34009124} to our primary human pancreatic organoid clusters.



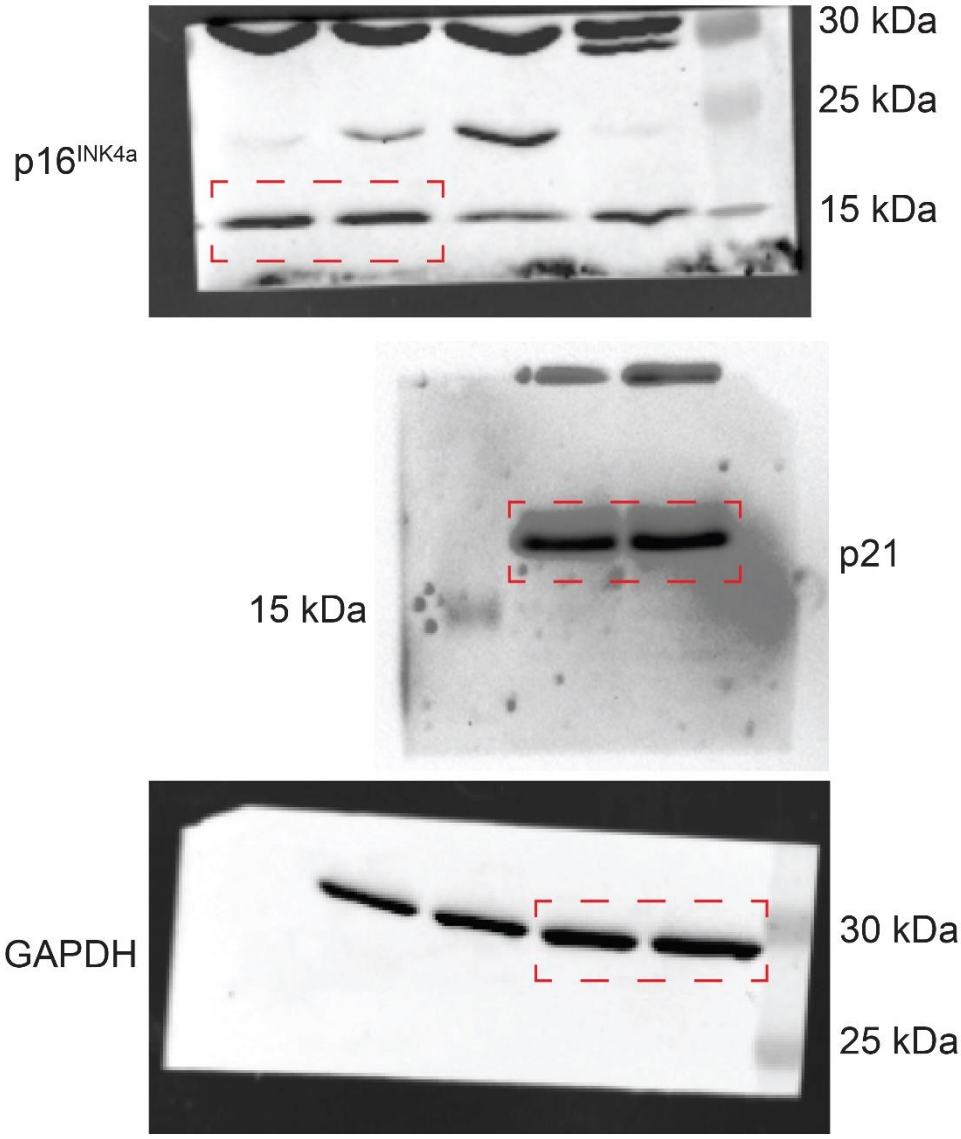
Supplementary Figure 8. RaceID3/StemID2 suggest primary human pancreatic organoids duct cluster 17 cells are the most progenitor-like. (A) Scatter plot showing the genes differentially expressed in RaceID3-derived cluster 17. (B) Enriched expression of the marker genes for the stem cells defined in RaceID3/StemID2 analysis in primary human pancreatic organoids clustering analysis.



Supplementary Figure 9. Pseudotemporal trajectory analysis of primary human pancreatic organoids. (A) Monocle 3 UMAP and trajectory are shown when primary human pancreatic organoid clusters are all included in the analysis. (B) Box plot showing the distribution of cells along the relative pseudotime axis, split by primary human pancreatic duct clusters. (C) Expression of an example gene from each duct subtype through the pseudotime. (D) Expression of modules 2 and 18 along trajectory and relative top 5 deregulated gene ontology biological processes terms.



Supplementary Figure 10. Cellchat analysis in primary human pancreatic organoids. Heatmap showing the summary of the signaling pathways that contribute to outgoing or incoming communication. The color bar represents the relative signaling strength of a signaling pathway across clusters. The bars indicate the sum of the signaling strength of each cluster or pathway.



Supplementary Figure 11. Uncropped and unedited western blot images used to prepare western blot shown in figure 1G. The western blot images are shown merged to molecular weight markers. The red rectangle indicates the portion presented in Figure 1G.

Supplementary Table 1. List of Antibodies for Immunostaining and Western Blot

Antibody name	Immunostaining	Western Blot	Catalog number	Manufacturer
P16-INK4a	-	1:1000	Cat# 551154; RRID: AB_394078	BD Biosciences
P21/WAF1	-	1:1000	Cat#05-345; RRID:AB_309684	Sigma-Aldrich
GAPDH (0411)	-	1:5000	Cat#sc-47724; RRID:AB_627678	Santa Cruz Biotechnology
E-Cadherin (24E10)	1:100	-	Cat#3195; RRID:AB_2291471	Cell Signalling Technologies
Ki67	1:100	-	Cat#ab16667; RRID:AB_302459	Abcam
Phalloidin-TRITC	1:5000	-	Cat# P1951, RRID:AB_2315148	Sigma-Aldrich
DAPI	1:10000	-	10236276001	Merck
Hoechst33342	1:1000	-	H1399	Molecular Probes
TFF1	1:100	-	Cat# ab76013, RRID:AB_1310784	Abcam
MUC5AC (45M1)	1:100	-	Cat# MA5-12178, RRID:AB_10978001	Thermo Fisher Scientific
SPP1	1:50	-	Cat# ab214050, RRID:AB_2894860	Abcam
CFTR (CF3)	1:200	-	Cat# MA1-935, RRID:AB_2081230	Thermo Fisher Scientific
Phospho-Histone H2A.X (Ser139) (20E3)	1:100	-	Cat# 9718; RRID: AB_2118009	Cell Signaling Technologies
KRT19	1:100	-	Cat# STJ24355, RRID: AB_3099474	St John's Laboratory
SOX9	1:50	-	Cat# AB5535, RRID:AB_2239761	Sigma-Aldrich

Supplementary Table 2. Primer Sequences for Gene Expression Analysis

Gene Name	Forward	Reverse
P21	CATGTGGACCTGTCACTGTCTTGTA	GAAGATCAGCCGGCGTTTG
P16/INK4a	TTCCCCCACTACCGTAAATG	CACTCCAGAAAACTCCAACACA
TEL	GGTTTTTGAGGGTGAGGGTGAGGGTG	TCCCGACTATCCCTATCCCTATCCCTA
36B4	CAGCAAGTGGGAAGGTGTAATCC	CCCATTCTATCATCAACGGGTACAA
ACTB	CACGATGGAGGGGAAGACGG	CGCCGCCAGCTCACCATG
TBP	GCCACGCCAGCTTCGGAGAG	CCGCAGCAAACCGTTGGGA