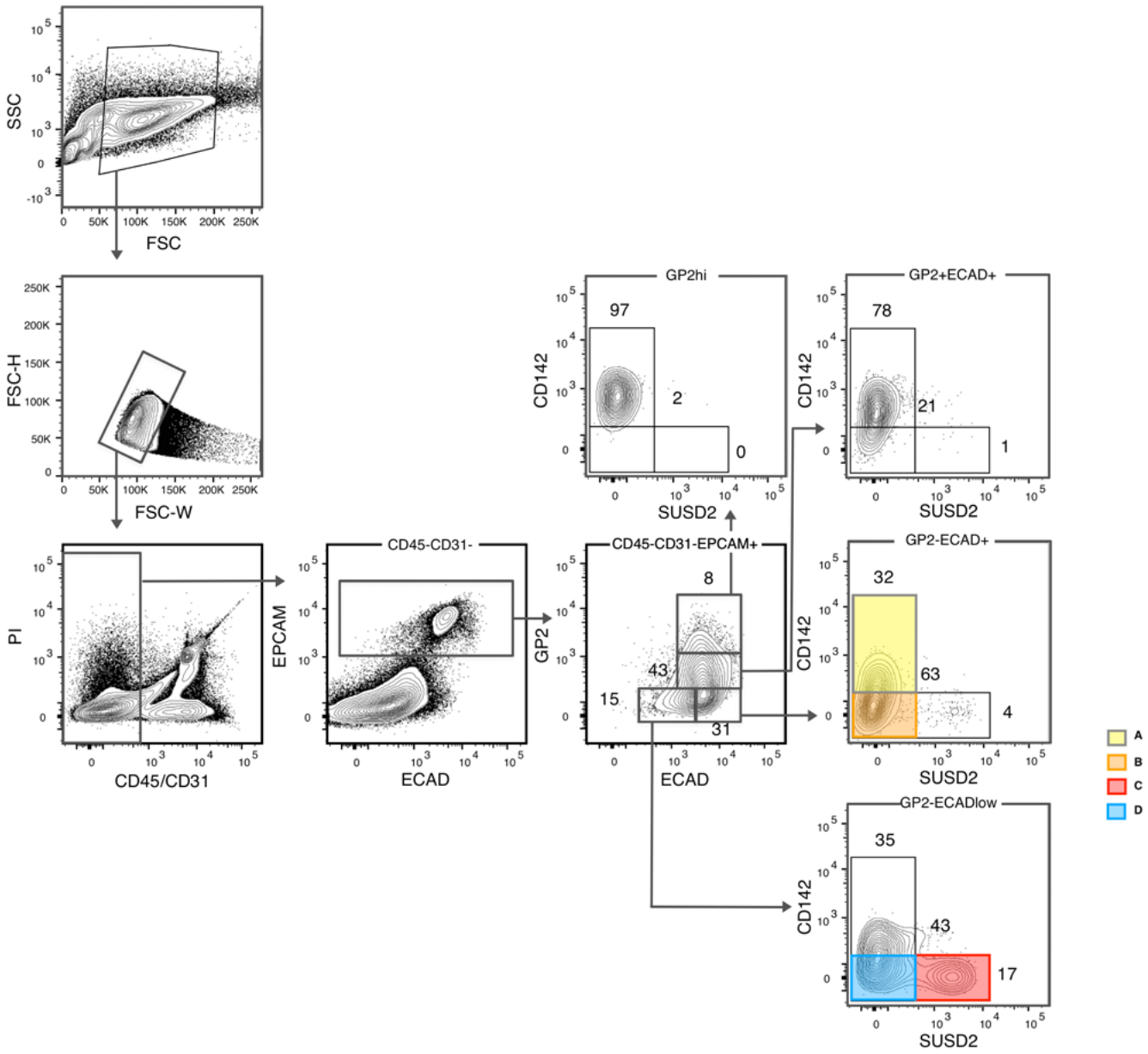
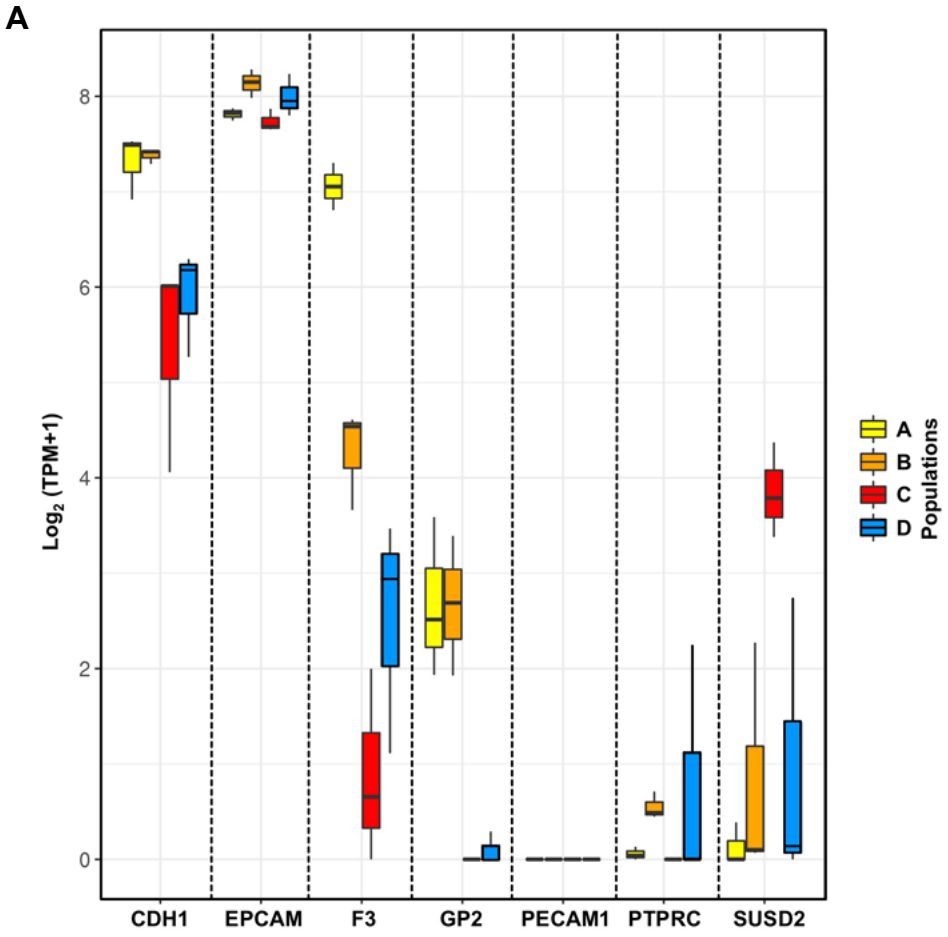
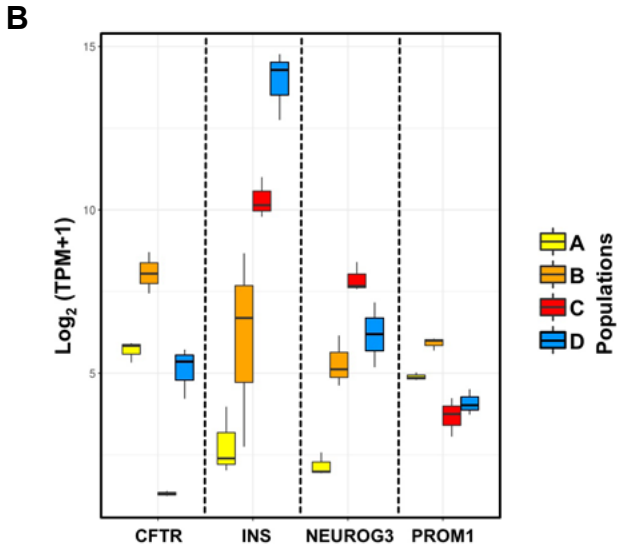
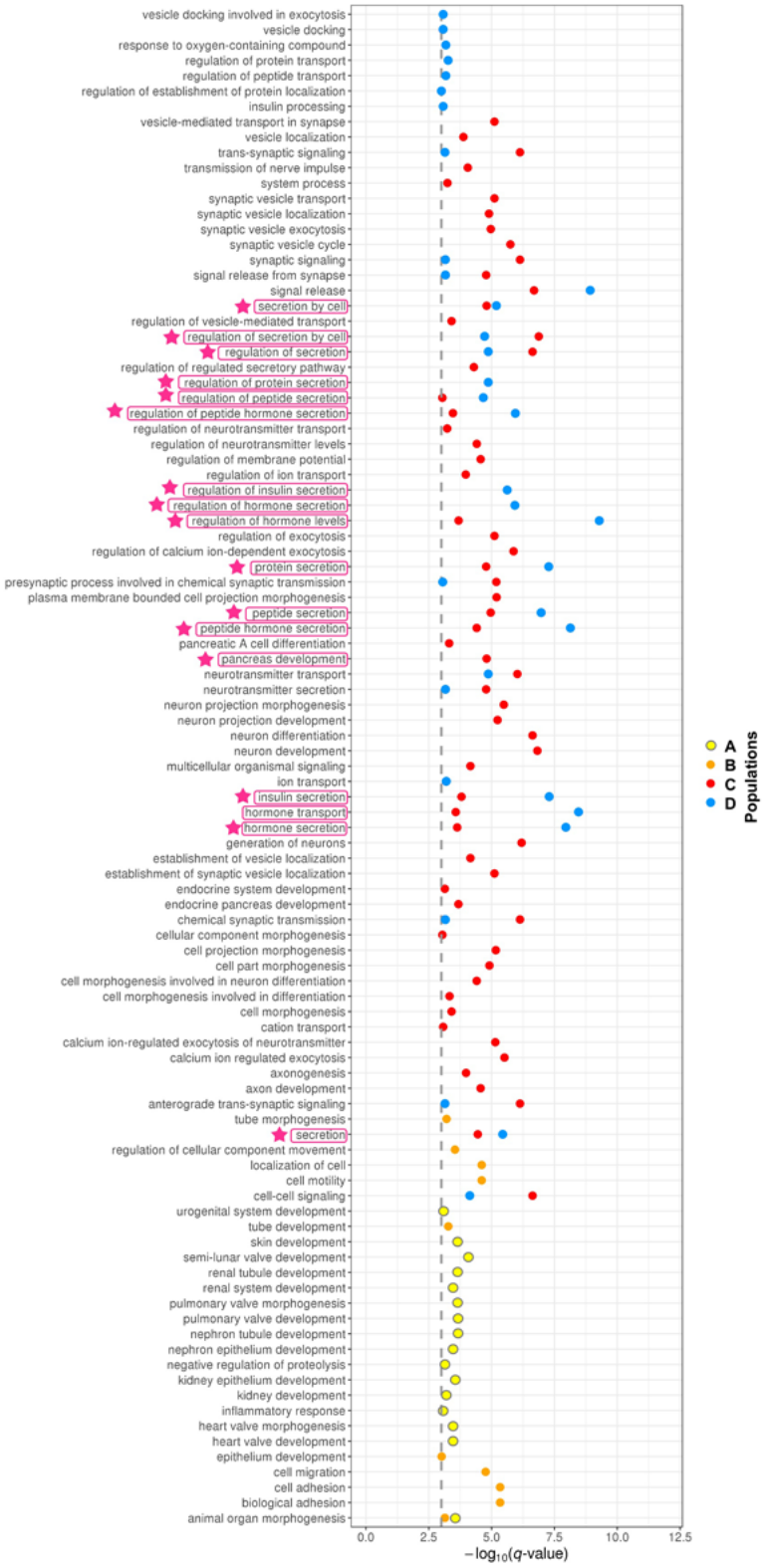


Supplementary Figure 1: Gating strategy for the expression of GP2, ECAD, CD142 and SUSD2 Human fetal pancreata at 9WD were stained for CD45, CD31, EPCAM, ECAD, GP2, CD142 and SUSD2. Doublet cells were excluded from the analysis with FSC-H and FSC-W (middle top plot). Propidium iodide (PI) was used to exclude dead cells as shown in the right top plot in the diagonal. GP2 and ECAD expression was analyzed in the CD45-CD31-EPCAM+ population. CD45-CD31-EPCAM- population was used as negative control to set up the GP2-ECAD+ and GP2+ECAD+ gates. GP2+ECAD+ population was used to set up the gate for ECAD levels. CD142 and SUSD2 expression was analyzed in GP2hiECAD+, GP2+ECAD+ and GP2-ECADlow. GP2hiECAD+ population was used as a positive control for the expression of CD142 and as a negative control for the expression of SUSD2. This gating strategy was applied to each pancreatic stage.

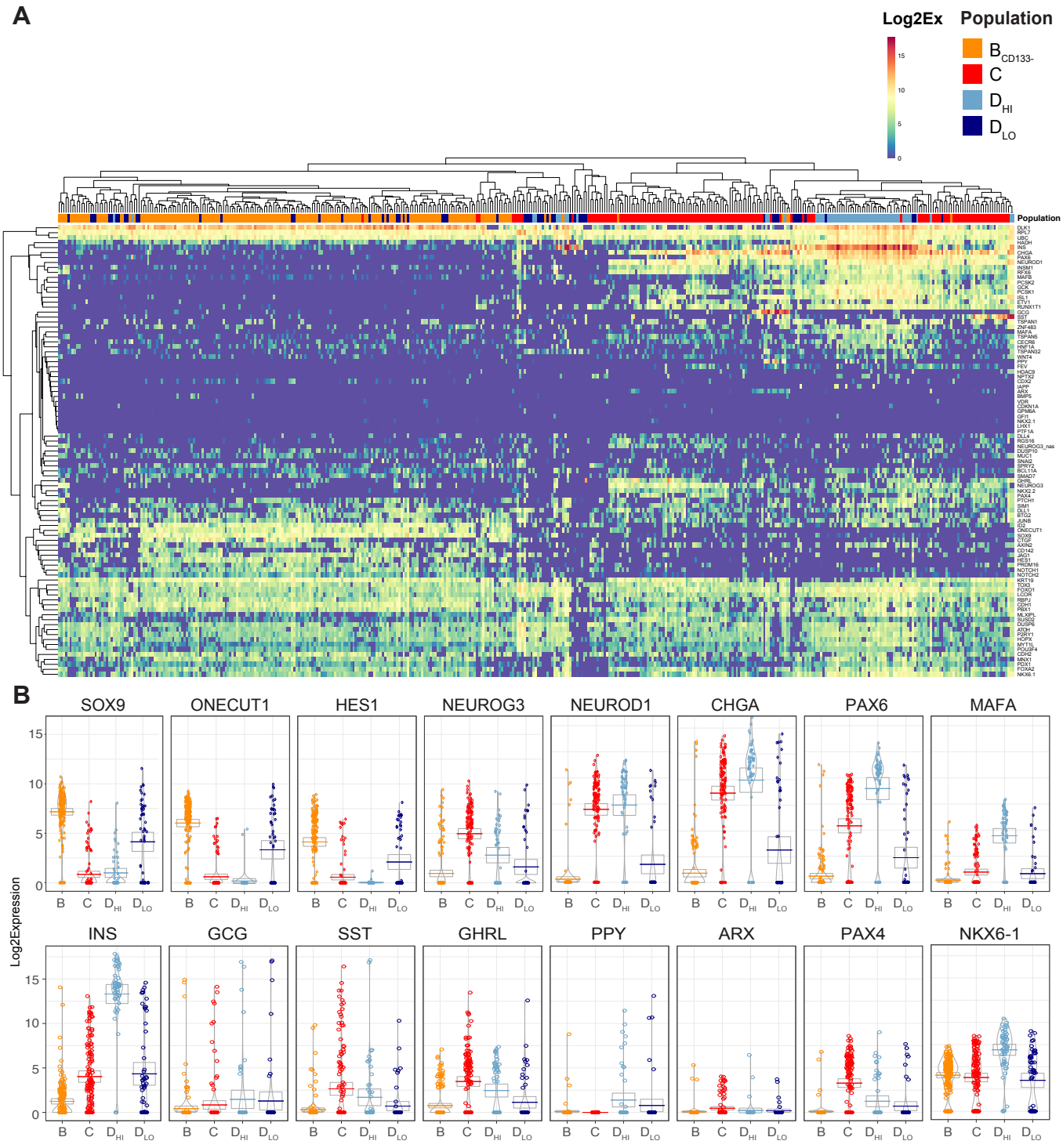




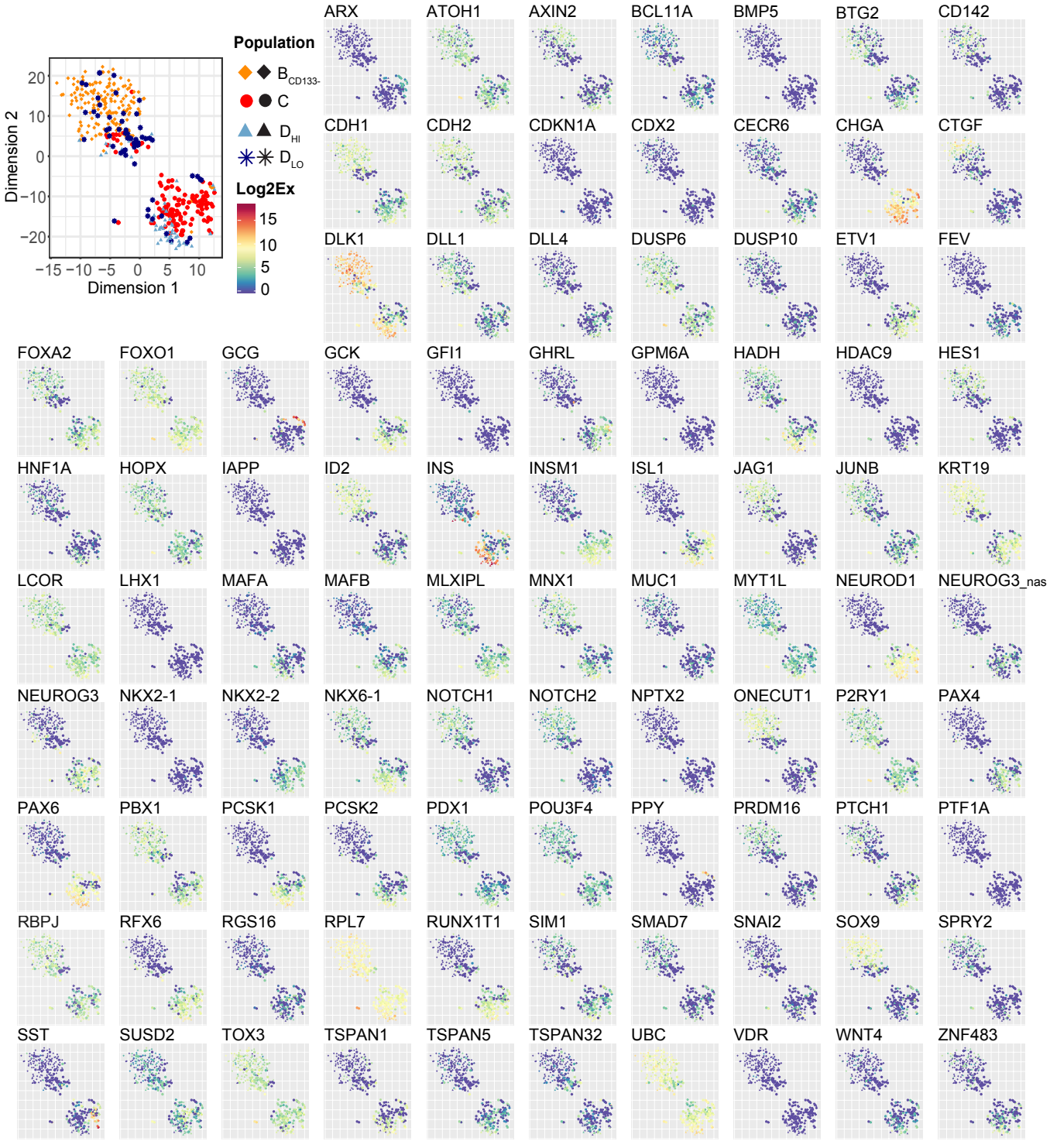
Supplementary Figure 2: Expression of membrane protein genes, ductal and endocrines genes in population A-D at 9WD
(A) Expression of genes encoding membrane proteins (CDH1, EPCAM, F3, GP2, PECAM1, PTPRC and SUSD2) used for the cell sorting of population A, B, C and D. CDH1 codes for ECAD, F3 for CD142, PECAM1 for CD31, PTPRC for CD45. **(B)** Expression of CFTR, NEUROG3 and PROM1 in populations A, B, C and D



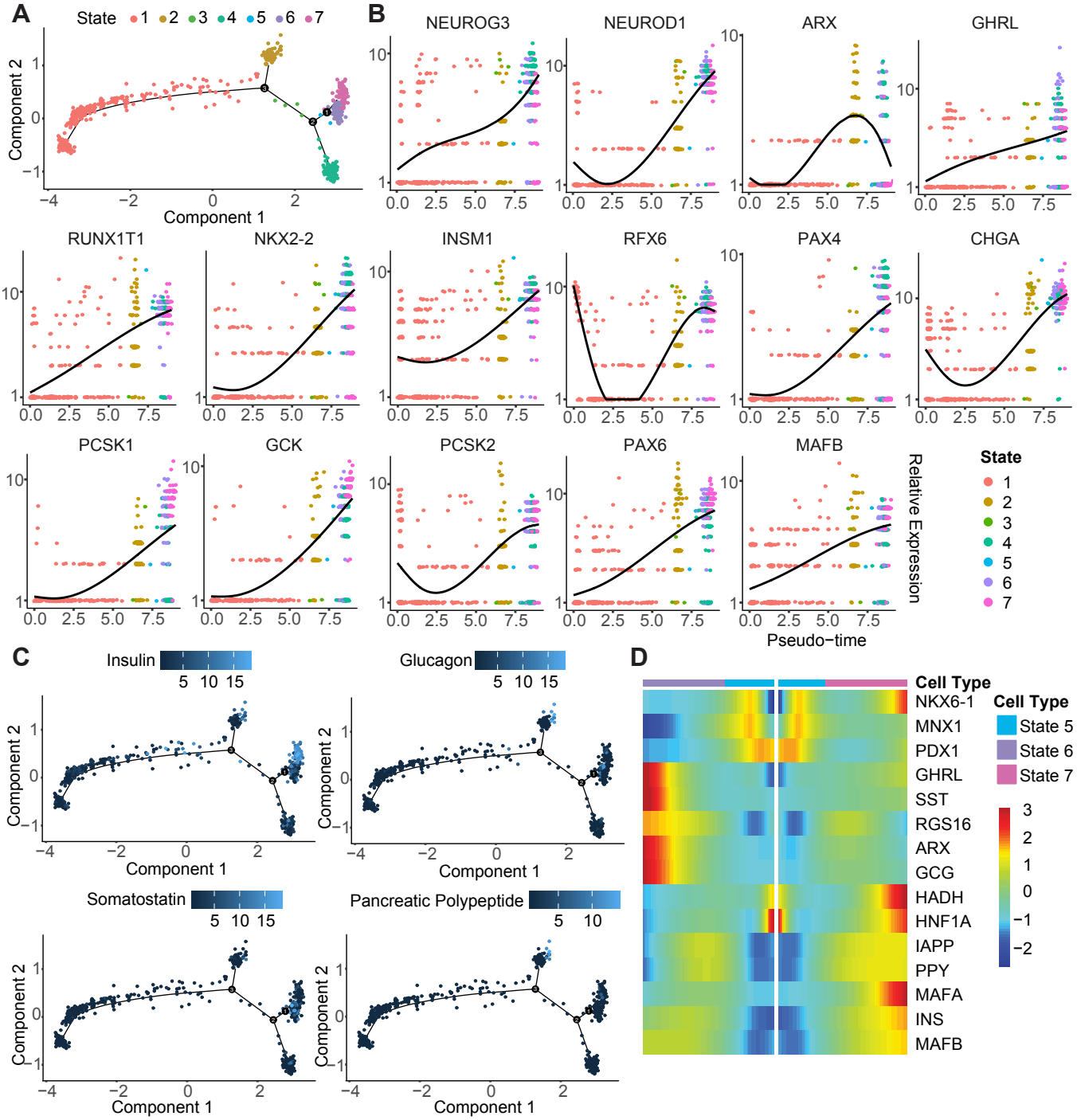
Supplementary Figure 3: Gene set enrichment analysis on population A, B, C and D GSEA analysis on populations A, B, C and D at 9WD using Gene ontology database (FDR <1%).



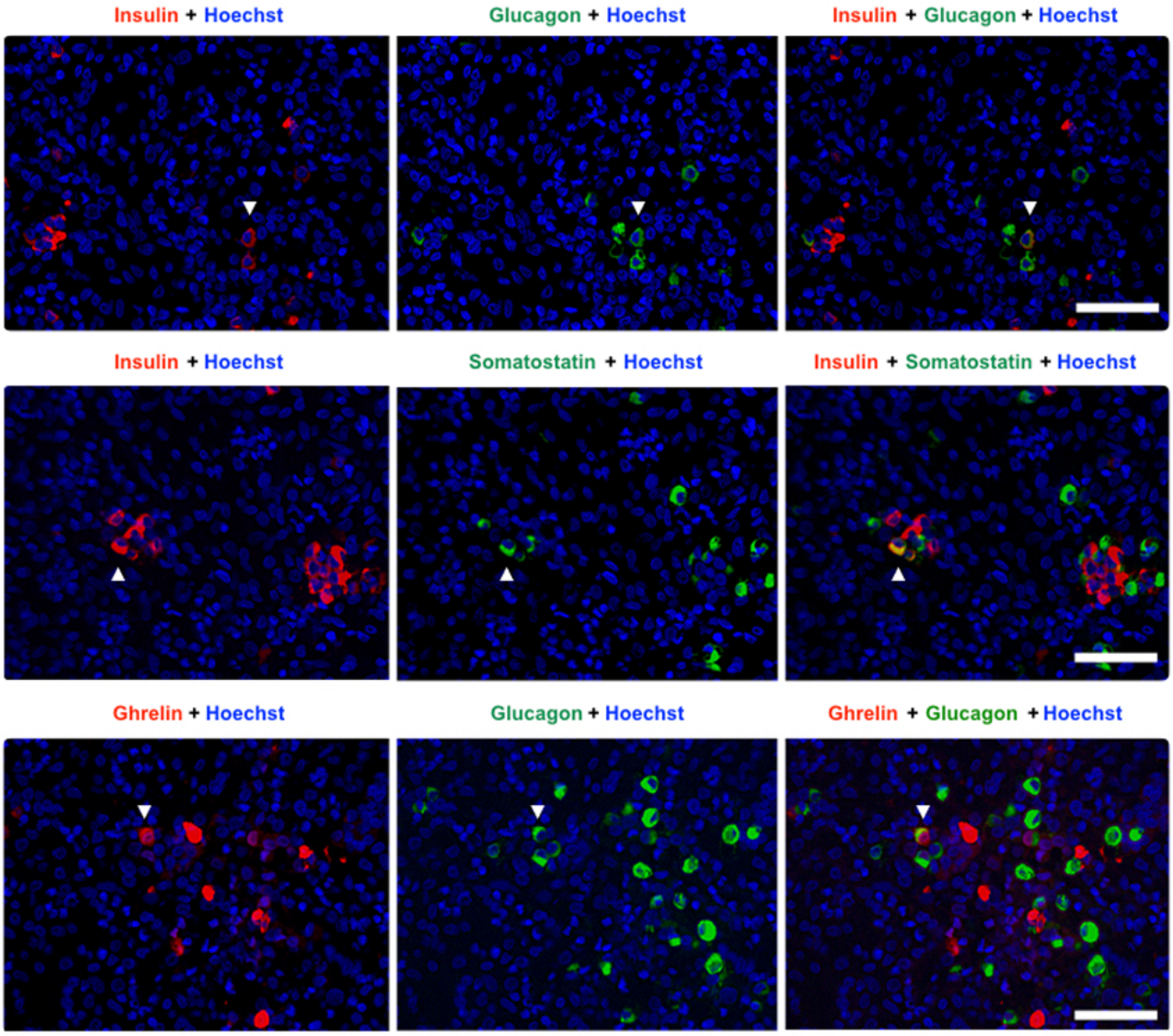
Supplementary Figure 4: Unsupervised hierarchical clustering of single-cell qPCR data from sorted human fetal pancreatic cells
(A) Unsupervised hierarchical clustering of sorted human fetal pancreatic cells at 9WD, showing all genes for populations B_{CD133-}, C, D_{HI} and D_{LO}. **(B)** Pirate plots showing the expression levels of specific genes in the four sorted populations. Each plot is a combination of box plots (25%-75%), means, violin plots and individual data points.



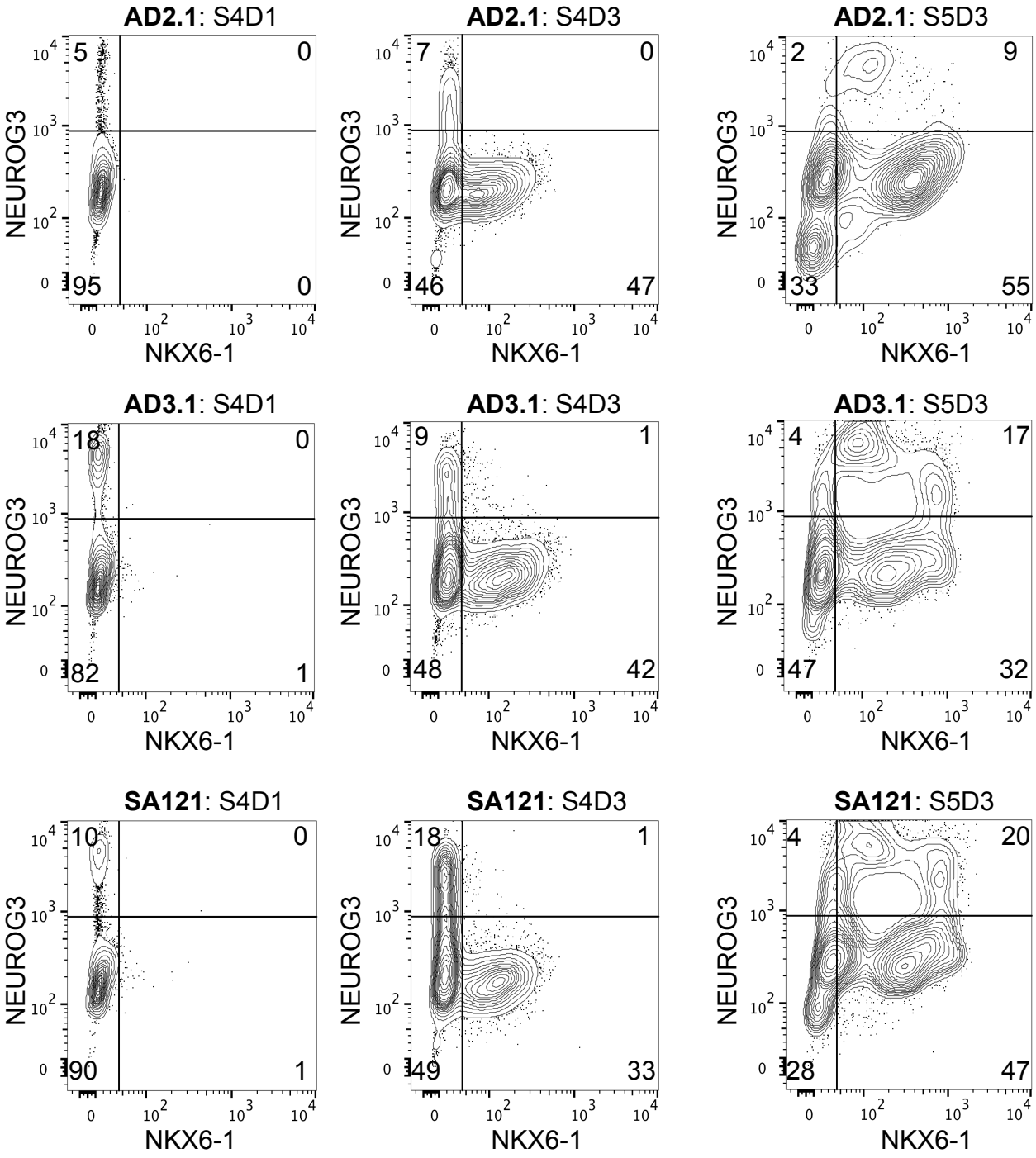
Supplementary Figure 5: Extended gene expression profiling of individual human fetal pancreas cells. t-SNE plots (corresponding to Fig. 5B) colored according to gene expression level of the indicated genes.



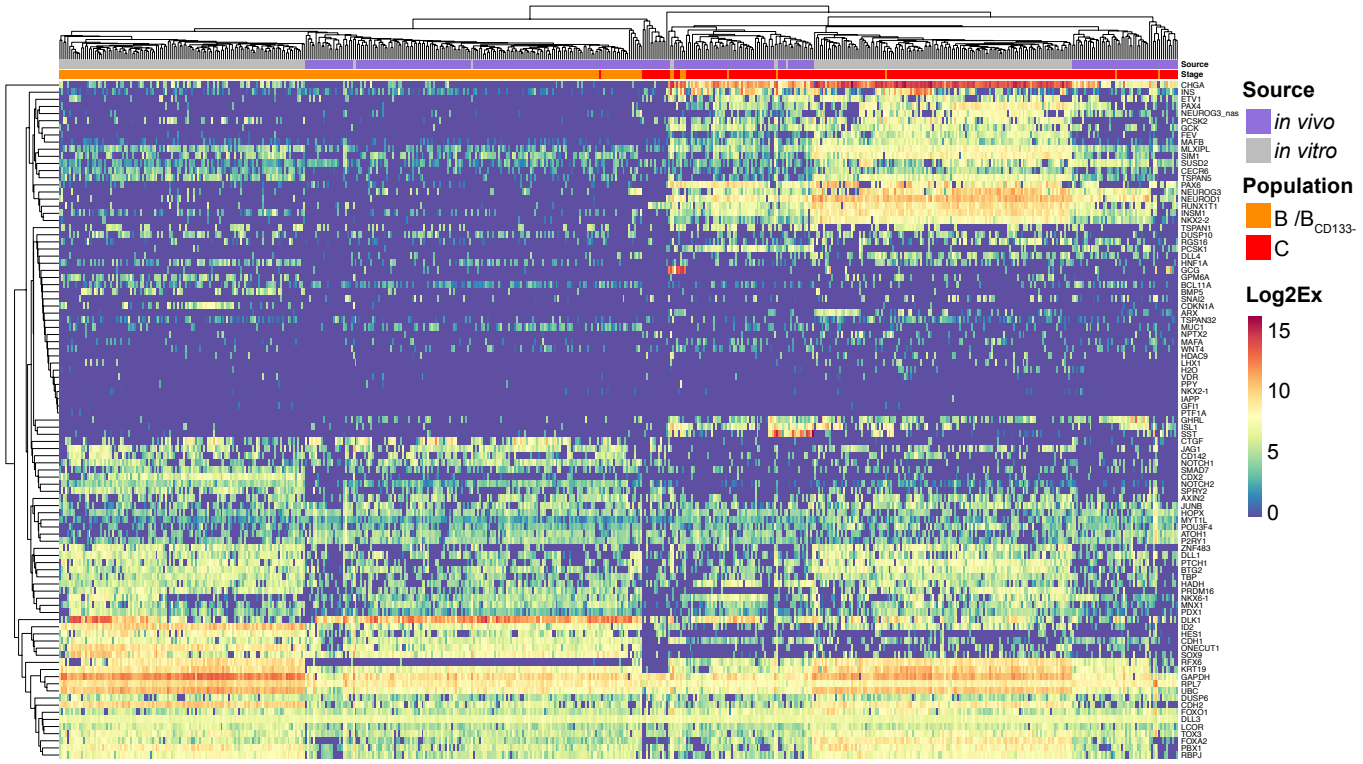
Supplementary Figure 6: Pseudotemporal ordering of single-cell gene expression data from human fetal pancreas. (A) Developmental trajectory of human fetal pancreatic cells (corresponding to Fig. 5C) colored by states. **(B)** Gene expression plots showing the pseudotemporal development of key genes involved in pancreas development. Gene expression level is shown on the y-axis; pseudotime on the x-axis. Each data point represents a single cell and is colored according to state on the trajectory shown in A. **(C)** Developmental trajectory of human fetal pancreatic cells (corresponding to Fig. 5C) colored by gene expression levels of selected hormonal genes. **(D)** Heat map showing pseudotemporal development of gene expression for the two cell fates derived from branching point 1 on the developmental trajectory shown in A. Cells at this branching point differentiates towards either State 6 or State 7.



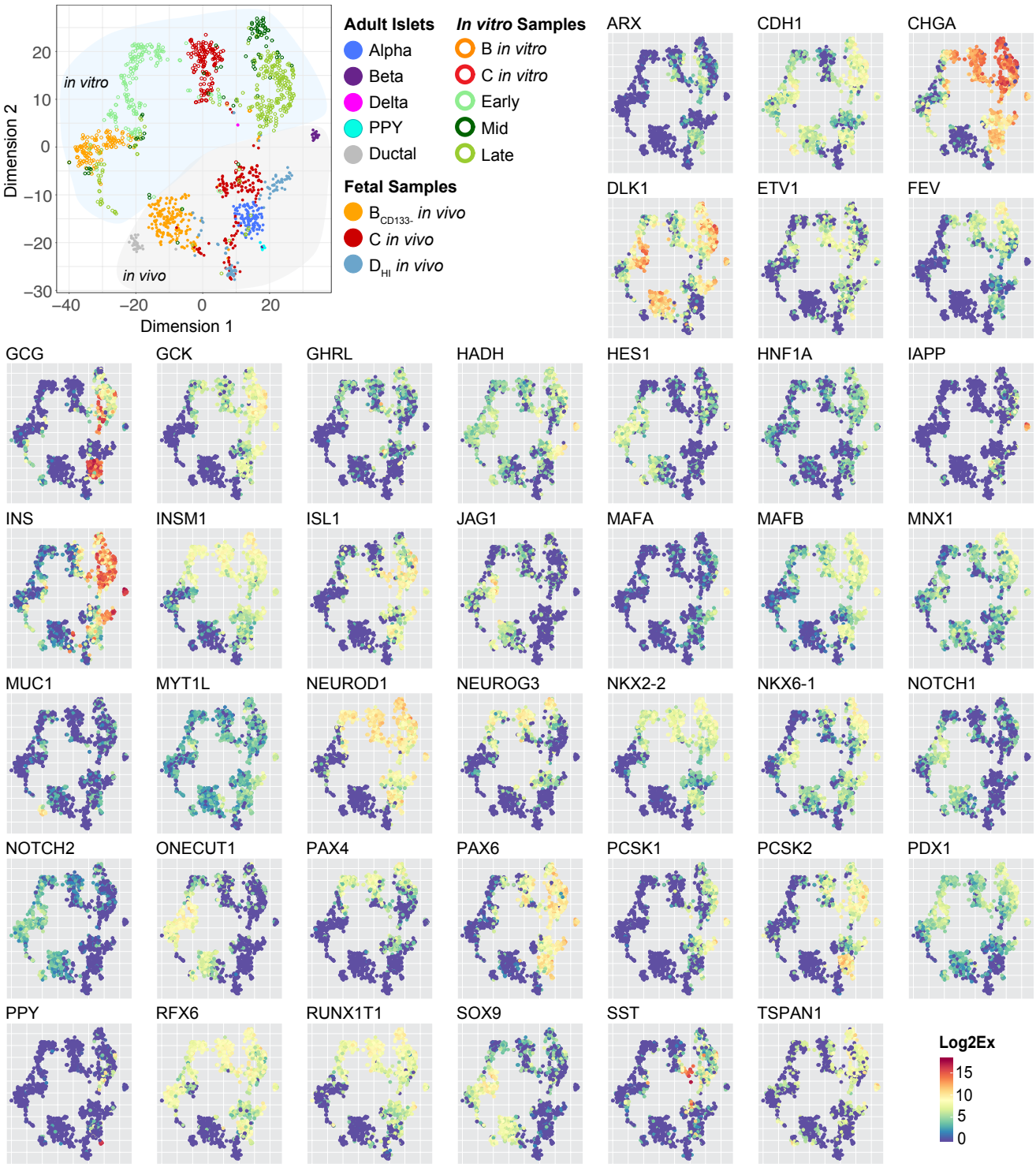
Supplementary Figure 7: Co-expression of pancreatic hormones in human fetal pancreas. Immunofluoresence staining for insulin, glucagon, somatostatin and ghrelin on pancreatic section at 10WD. Scale bar: 50µm. Arrowheads indicate double hormone positive cells.



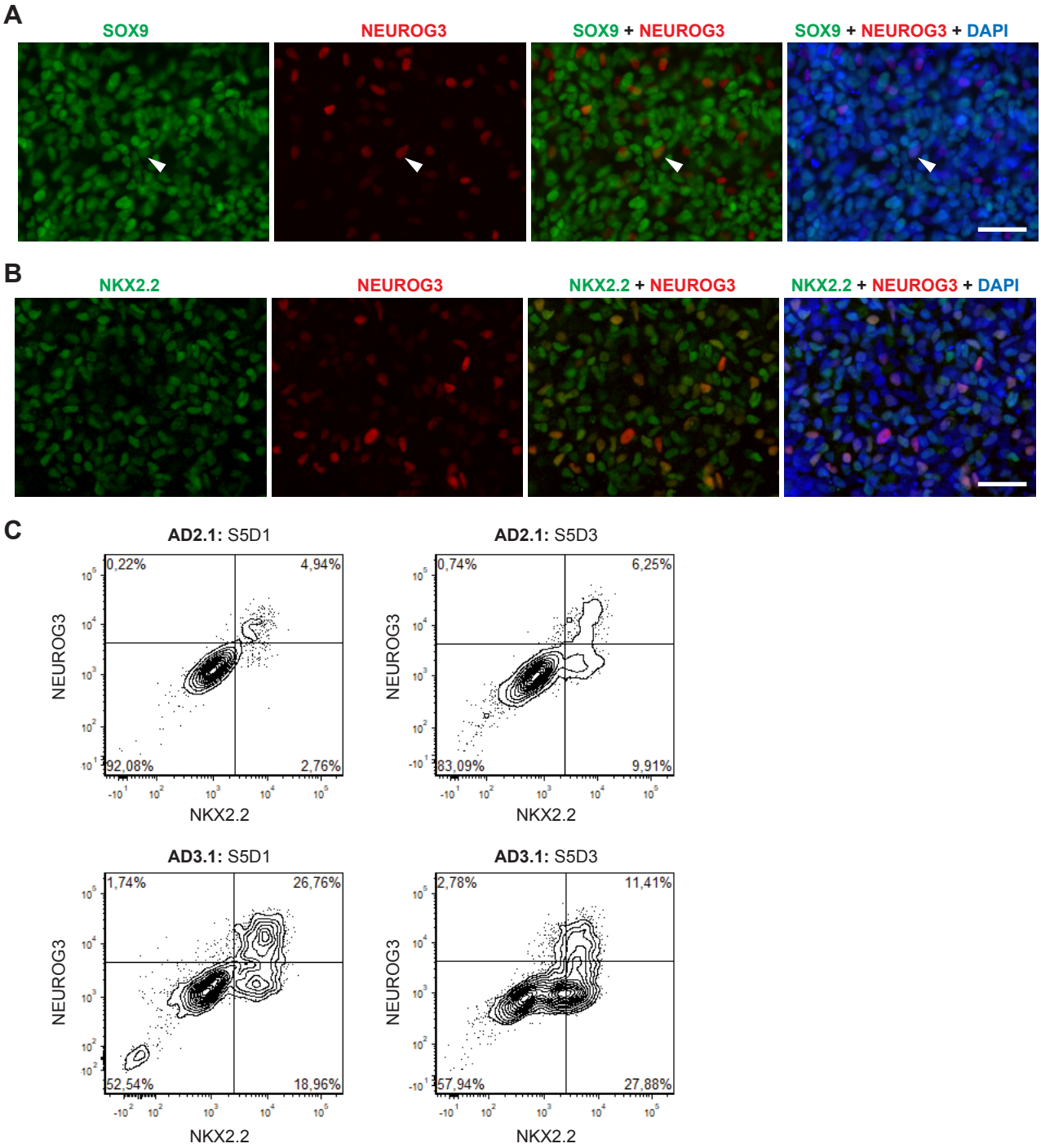
Supplementary Figure 8: Generation of endocrine progenitors from hPSCs. Representative flow cytometry plots showing the percentage of cells expressing NEUROG3 and NKX6-1 protein at S4D1, S4D3 and S5D3 of the differentiation protocol for each of the hPSC-lines SA121, AD2.1 and AD3.1 used for sorting based on cell-surface markers and subsequent single-cell qPCR analysis.



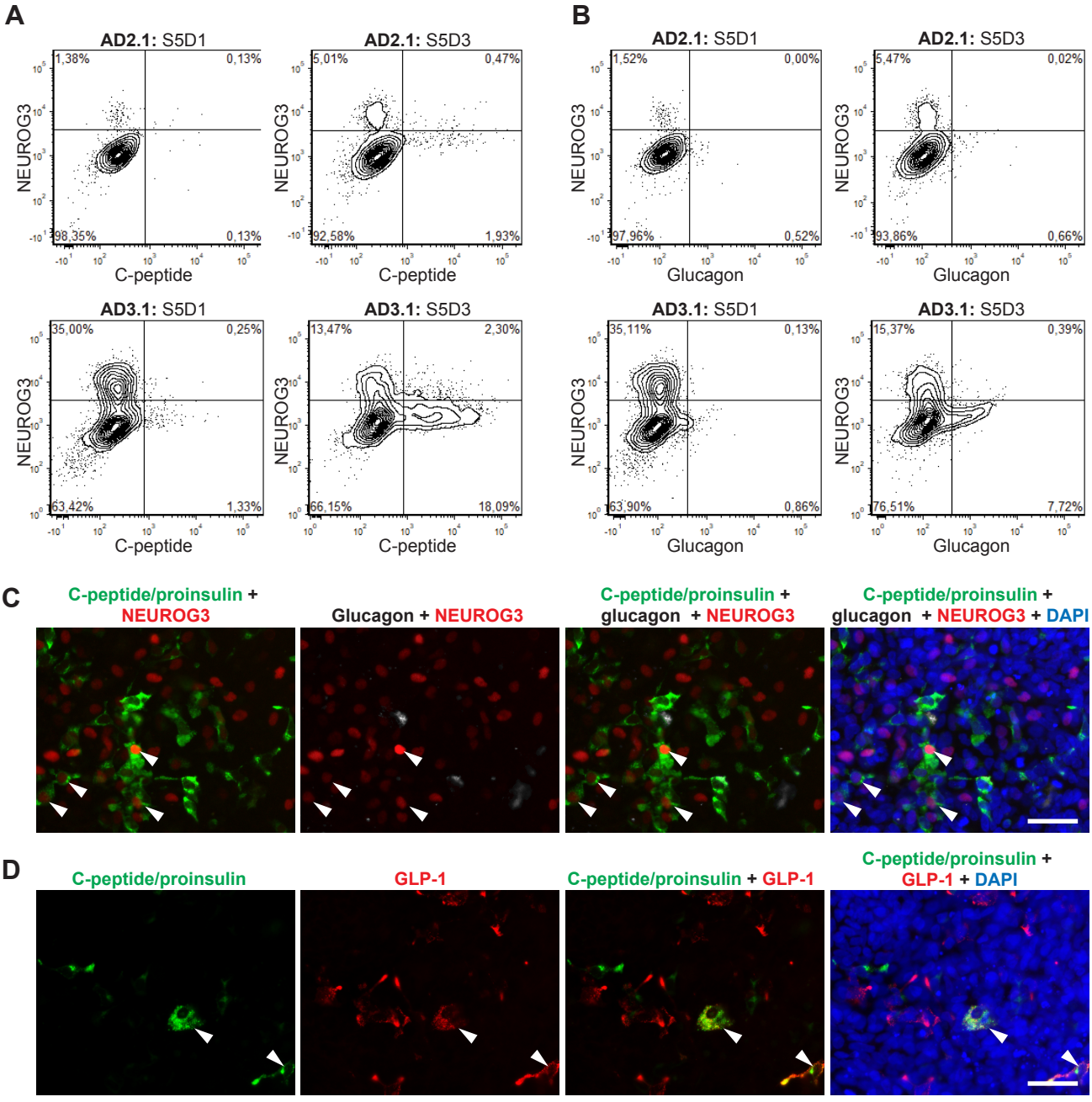
Supplementary Figure 9: Extended comparison of the single-cell expression profile of pancreatic cells generated *in vitro* to *in vivo* fetal and adult pancreas Hierarchical clustering of single-cell qPCR data showing the expression of all genes for populations B and C sorted from human fetal pancreas ("*in vivo*") or hPSC-derived pancreatic cells ("*in vitro*"). Refers to Fig. 7A and B.



Supplementary Figure 10: Gene expression in single pancreatic cells from human fetal pancreas, adult human islets or generated *in vitro* from hPSCs. t-SNE plots corresponding to Fig. 7D colored according to gene expression level of the indicated genes.



Supplementary Figure 11: Characterization of SOX9 and NKX2-2 protein co-expression in hPSC-derived endocrine progenitor subpopulations. (A) Representative image of co-staining for SOX9 (green) and NEUROG3 (red) at S5D1 of in vitro differentiated cultures (hiPSC line AD2.1). Arrowhead indicates a SOX9+/NEUROG3+ cell. Nuclei are counterstained with DAPI. Scale bar: 50 µm. **(B)** Representative image of co-staining for NKX2-2 (green) and NEUROG3 (red) at S5D1 of in vitro differentiated cultures (hiPSC line AD3.1). Nuclei are counterstained with DAPI. Scale bar: 50 µm. **(C)** Flow cytometry analysis for co-expression of NKX2-2 and NEUROG3 at S5D1 and S5D3 of in vitro differentiated cultures (hiPSC lines AD2.1 is shown in the top panel and AD3.1 in the bottom panel).



Supplementary Figure 12: Characterization of hormone expression at the protein level in hPSC-derived endocrine progenitor subpopulations. (A) Flow cytometry analysis for co-expression of NEUROG3 and C-peptide or **(B)** NEUROG3 and glucagon, at S5D1 and S5D3 of in vitro differentiated cultures (hiPSC lines AD2.1 is shown in the top panel and AD3.1 in the bottom panel). **(C)** Representative image of co-staining for C-peptide/proinsulin (green), NEUROG3 (red) and glucagon (white) at S5D1 of in vitro differentiated cultures (hiPSC line AD2.1). Arrowheads indicate co-expression of C-peptide and NEUROG3. Scale bar: 50 µm. **(D)** Representative image of co-staining for C-peptide/proinsulin (green) and GLP-1 (red) at S5D1 of in vitro differentiated cultures (hiPSC line AD3.1). Arrowhead indicates co-expression of C-peptide/proinsulin and GLP1. Scale bar: 50 µm.

Table S1: List of the 1000 genes differentially expressed in populations A, B, C and D

Click here to Download Table S1

Table S2: Gene ontology functional classification

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Table S3: Single cell qPCR processed data (Log2 expression values), primer sequences and information on number of cells analyzed

Click here to Download Table S3