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Supplemental Information

Heterogeneity of SOX9 and HNF1β in Pancreatic Ducts Is Dynamic

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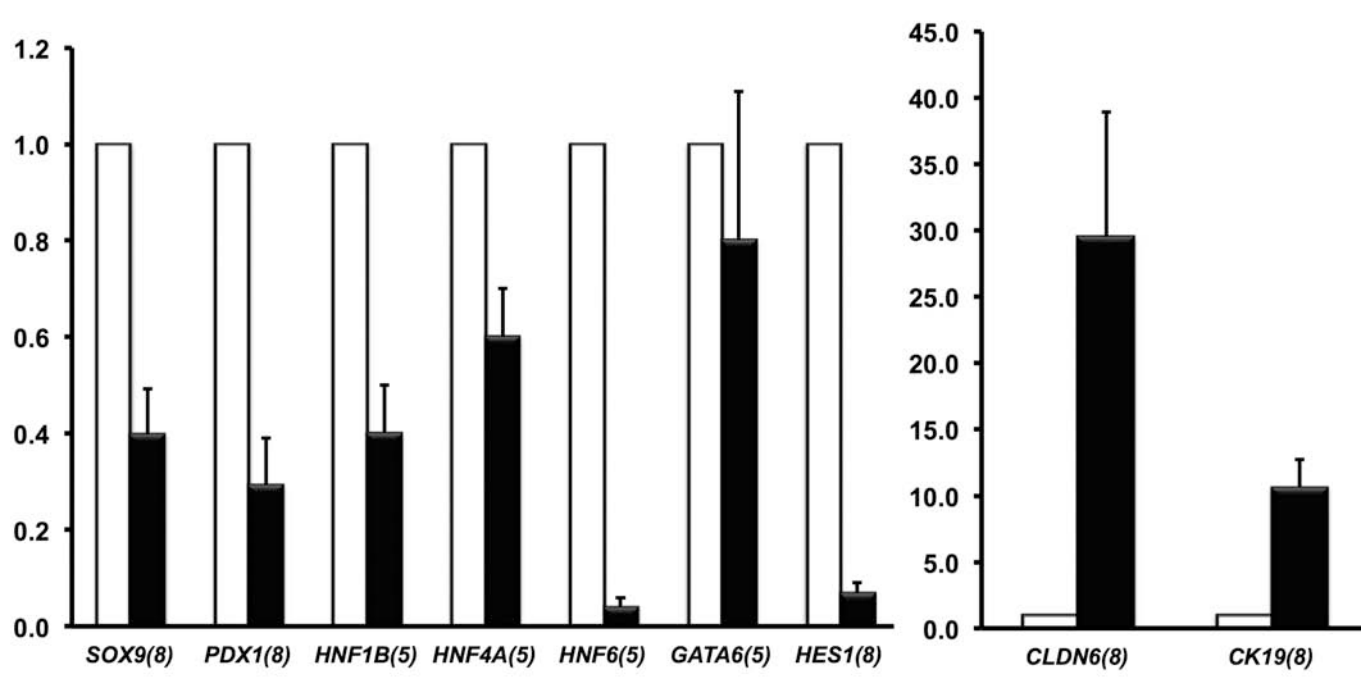

Figure S1 related to Figure 5. Purified human ducts lose ductal characteristics when cultured 1 week as monolayers. CA19.9 purified human ducts cultured as monolayer in CRML media for 1 wk (black bars) lose their ductal phenotype compared to the freshly isolated cells (white bars). The values are normalized to that of fresh ducts of the same donor; the number of donors in parentheses. The Ct values were: 20 for *HES*, 24 for *PDX*1, 20 for *SOX9* but embryonically expressed *claudin6* 25 for fresh but 19 for cultured. Mean±sem.

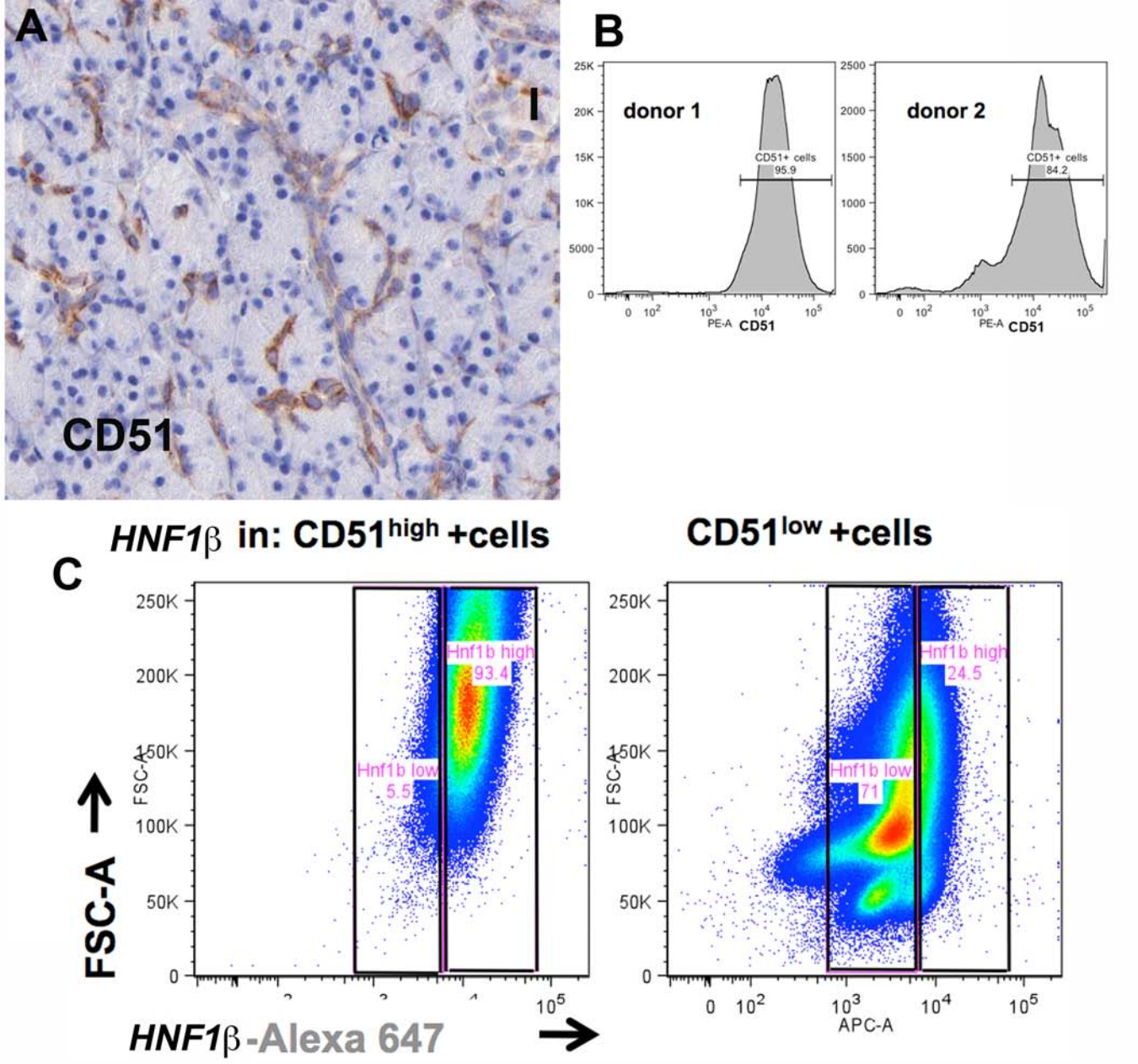

Figure S2 related to Figure 6. CD51 as second antibody for sorting human pancreatic duct cells. Based on our unpublished microarray data on human ducts (data not shown) and the immunostaining from the Human Protein Atlas (image from www.proteinatlas.org)**(A)**, CD51 had a specific but variable expression in ducts. We tested CD51 for sorting CA19-9 purified human pancreatic duct cells (**B**). As shown for two donors, the overlap was high with 95.9 and 84.2% overlap. **C.** Combined with immunostaining for HNF1β, CD51 expression largely overlapped with HNF1β expression: 93.4% of CD51^{high} cells expressed HNF1β^{high}, and 71% of CD51^{low} expressed HNF1β^{low}

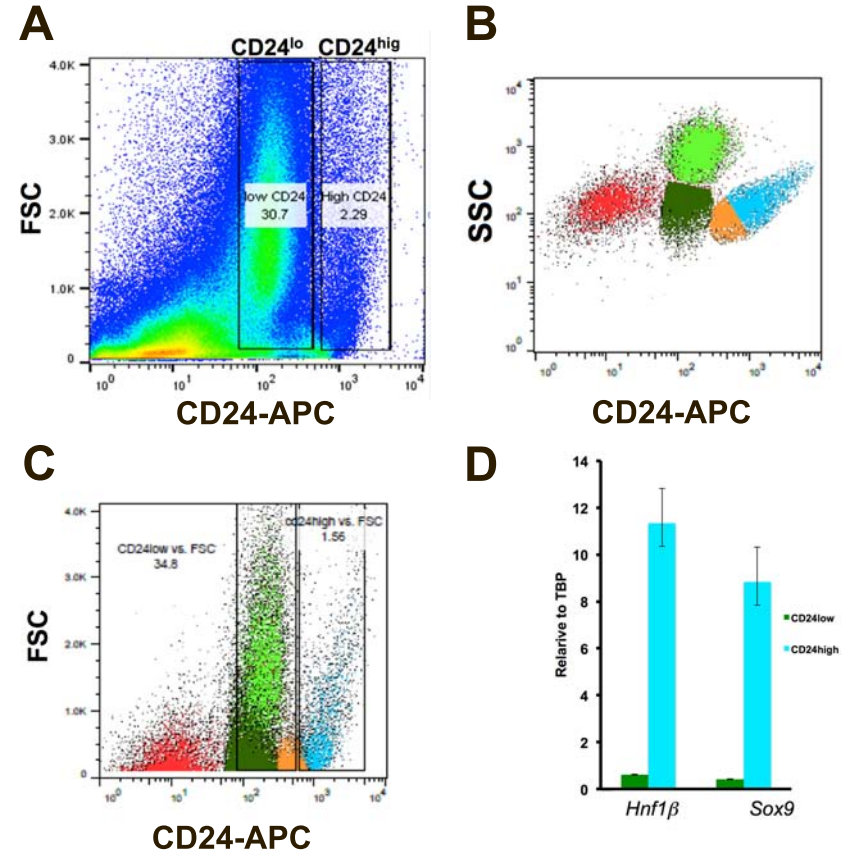

Figure S3 related to Figure 6. Alternate sorting strategy also indicates at least two populations. Mouse pancreatic ducts purified on basis of CD44+ using MACs were analyzed by FACS for the second antibody CD24. As shown in **A,** when plotted as increasing CD24 expression vs forward scatter FSC (size) as our usual protocol, we could "arbitrarily" assign high or low CD24 populations. **B** shows the same preparation but plotted as CD24 vs side scatter SSC (granularity) has clearly separate populations: red - $CD24^{negative}granularity^{low}$, dark green-$CD24^{low}granularity^{low}$, light green $CD24^{low}granularity^{high}$ and orange/aqua-$Cd24^{high}granularity^{low}$. **C** shows the overlap when these populations are plotted by FSC. When the cells were sorted on basis of CD24 vs SSC as either the green or aqua+orange populations, there was enrichment of *Hnf1β* and *Sox9* mRNA in the $CD24^{high}$ population (**D**). n= 3 experiments Mean±sem

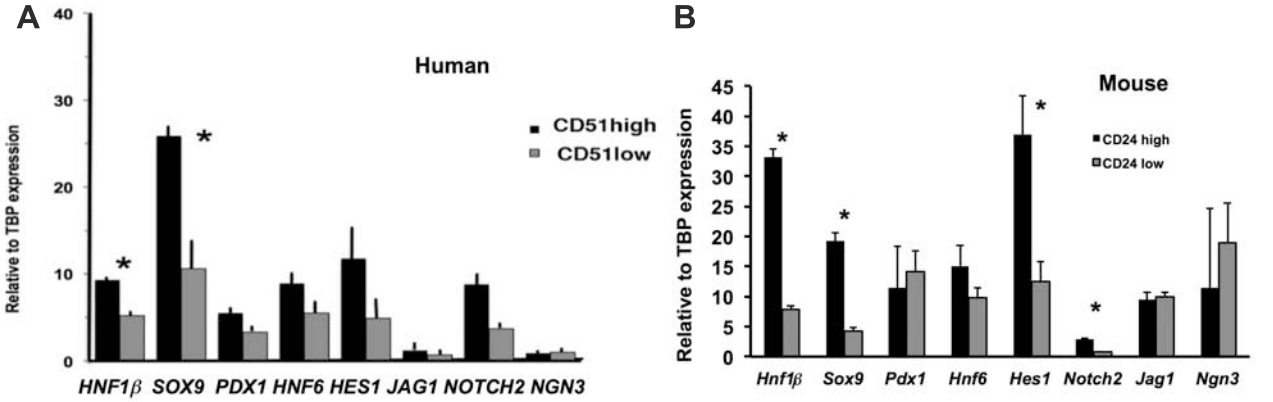

Figure S4 related to Figure 6. Gene expression in live pancreatic duct cells FACS sorted on the basis of 2 antibodies, first one to purify duct cells from islet-depleted tissue and second one to sort distinct subpopulations. **A.** Human duct cells were purified as CA19-9+ and subsequently incubated with CD51-PE and FACS sorted for $CD51^{high}$ and $CD51^{low}$ populations and finally their gene expression was analyzed by qPCR. Human pancreas donors(n=3), normalized to *TBP*. **B.** A second independent set of gene expression experiments on mouse duct cells purified by CD44 and the sorted for $CD24^{high}$ and $CD24^{low}$ using a different CD24-Pe antibody than in Figure 6. n=3 independent experiments, each pooled from 15 mouse pancreas; values normalized to *Tbp*. Mean ±sem

Table S1. Related to Experimental Procedures. Optimized organogenesis media for growing mouse pancreatic duct cell-derived organoids

Reagent	Concentration
Advanced DMEM/F12	50%
L-NRW cell line condition media	50%
Penicillin/Streptomycin	1X
FBS	10%
Glutamax	1X
FGF10	50 ng/ml
EGF	50 ng/ml
Nicotinamide	10 mM

Table S2. Related to Figure 6. Gating on CD24 expression of mouse CD44^{+} purified duct cells gave distinct subpopulations.

	CD24^{high}	**CD24^{low}**
% HNF1β ^{high}	73.8	55.8
%HNF1β ^{low}	24.7	34.7
%HNF1β ^{undetectable}	0.06	6.7
%SOX9 ^{high}	65.7	9.9
%SOX9 ^{low}	26.8	76.4
% SOX9 ^{undetectable}	0.05	9.6

Table S3. Related to Figure 6. $CD24^{low/high}$ subpopulations percentage between young and old mice. $CD24^{low}$ cells were twice as abundant in the younger animals.

Age	CD24 level	Percentage
Young (7-9 wks)	$CD24^{low}$	18.6
	$CD24^{high}$	1.2
Old (7-9 month)	$CD24^{low}$	8.7
	$CD24^{high}$	1.2
Old (1+year)	$CD24^{low}$	7.9
	$CD24^{high}$	2.5

Table S4. Related to Experimental Procedures. Primary antibodies used for different experiments

Application	Name	Derived species	Vendor	Catalogue #	Dilution (Method)
Duct cell isolation	CD24, clone M1/69	Rat	eBioscience	17-0242	1:100 (IMS)
	CD44	Rat	eBioscience	11-0441	1:100 (IMS)
	CA19-9	Mouse	Invitrogen	MA5-12421	1:100 (IMS)
FACS	HNF1β	Rabbit	Santa Cruz	SC8986	1:150 (PF)
	SOX9	Rabbit	Millipore	AB5535	1:1000 (PF)
	Fluorochrome-Conjugated-CD44clone IM7 PE	Rat	eBioscience	11-0441	1:100
	Fluorochrome-Conjugated-CA19-9	Mouse	Life Technologies	18-7240	1:200
	Fluorochrome-Conjugated-CD24, clone M1/69 APC	Rat	eBioscience	17-0242	1:100
	Fluorochrome-Conjugated- CD51	Mouse	eBioscience	12-0512	1:100
	CD16/CD32	Rat	BD	553142	1:100
IHC	HNF1β	Rabbit	Santa Cruz	SC22840; SC8986	1:150 (PF)
	HNF1β	Goat	Santa Cruz	SC7411	1:100 (PF)
	SOX9	Rabbit	Millipore	AB5535	1:1000 (PF)
	BrdU	Mouse	Dako	M0744	1:100 (PF)
	PanCK	Rabbit	Dako	Z0622	1:300 (PF)
Organoid staining	SOX9	Rabbit	Millipore	AB5535	1:200 (WM,Bio-SA)
	HNF1β	Rabbit	Santa Cruz	SC8986	1:200 (WM,Bio-SA)
	HNF6	Rabbit	Santa Cruz	13050	1:200 (WM,Bio-SA)
	Ki67	Mouse	BD Pharmigen	556003	1:200 (WM,FITC)

Abbreviations: IMS, Immunomagnetic Separation. Bio-SA, biotin-streptavidin-conjugated fluorescein amplification. PF, Paraffin Fixed. WM, Whole mount staining.

Table S5. Related to Experimental Procedures. MCDB131 media was supplemented with compounds listed for each stage (S1-S4) for differentiation of pancreatic duct cells-derived organoids to pancreatic progenitor cells.

Compound	Provider	Cat#	S1(d1-3)	S2(d3-6)	S3(d6-9)	S4 (d9-14)
Sodium bicarbonate	Sigma	S6297	2.5 g/l	2.5 g/l	1.5 g/l	1.5 g/l
Glutamax	Life Technologies	35050-061	1x	1x	1x	1x
Glucose	Sigma	G8769	10 mM	10 mM	20 mM	20 mM
BSA	Sigma	A2934	2%	2%	2%	2%
Ascorbic acid	Sigma	A4544	0.25 mM	0.25 mM	-	-
FGF7	R & D Systems	251-KG	50 ng/ml	2 ng/ml	-	-
SANT-1	Sigma	S4572	0.25 mM	0.25 mM	0.25 mM	-
Retinoic acid	Sigma	R2625	1 mM	0.1 mM	0.05 mM	-
Stemolecule™ LDN-193189	Stemgent	04-0019	100 nM	200 nM	100 nM	100 nM
Insulin-Transferrin-Selenium (ITS)	Life Technologies	51500056	1:200	1:200	1:200	1:200
PKC activator (TPB)	EMD Millipore	565740	200 nM	100 nM	-	-
3,3′5-Triiodo-l-thyronine sodium (T3)	Sigma	T6397	-	-	1mM	1mM
ALK5 inhibitor II	Enzo Life Sciences	AL X-270-445-M005	-	-	10 mM	10 mM
Zinc sulfate	Sigma	Z0251	-	-	10 mM	10 mM
Heparin	Sigma	H3149	-	-	10 mg/ml	10 mg/ml (d12-14)
γ-secretase inhibitor XX	EMD Millipore	565789	-	-	-	100 nM (d9-12)

Table S6. List of Primers, related to Experimental Procedures

Primer	**Sequence 5' to 3'** **forward**	**reverse**
Human		
SOX9	AAAGGCAACTCGTACCCAAATTT	AGTGGGTAATGCGCTTGGAT
PDX1	CTGGATTGGCGTTGTTTGTG	CCAAGGTGGAGTGCTGTAGGA
HNF1β	AGAAGCGTGCCGCTCTGT	GAATTGTCGGAGGATCTCTCGTT
HNF4a	CTGCAGGCTCAAGAAATGCTT	TCATTCTGGACGGCTTCCTT
GATA6	AGCGCGTGCCTTCATCA	GTGGTAGTTGTGGTGTGACAGTTG
HES1	TCCGGAGCTGGTGCTGAT	CCAGGACCAAGGAGAGAGGTAGA
CK19	GAAGGATGCTGAAGCCTGGTT	GCGACCTCCCGGTTCAAT
HNF6	GCAGGAAAACACCACTGGATCT	GCCAAGCACAGCGAGGAT
JAG1	AAAGATCTCAATTACTGTGGGACTCA	AGGGCCTGTGTTGCTACAAGTT
NGN3	TTTTGCGCCGGTAGAAAGG	CTCACGGGTCACTTGGACAGT
NOTCH 2	CCTTTTAGATGATAATGGACAACTATAGACTT	TGCCAAGAGCATGAATACAGAGA
CLDN6	GGA TCT TGA CAT GCC CAT CTT AG	CAA GCA GCC TCC GCA TTA G
RPLP0	CATCTACAACCCTGAAGTGCTTGA	ACCCTCCAGGAAGCGAGAAT
PPIA	GCGTCTCCTTTGAGCTGTTTG	CAGTGCTCAGAGCACGAAAATT
TBP	GCTTCCGCTGGCCCATA	ACGCCAAGAAACAGTGATGCT
Mouse		
Sox9	CGGCTCCAGCAAGAACAAG	GCGCCCACACCATGAAG
Pdx1	GATGTTGAACTTGACCGAGAGA	GTCCCGCTACTACGTTTCTTATC
Hnf1β	CCTCTCTCAACACCTCAACAAG	GAGGATCTCCCGTTGCTTTC
Hnf4a	CAAGAGGTCCATGGTGTTTAAGG	GTGCCGAGGGACGATGTAGT
Hes1	ACCCCAGCCAGTGTCAACA	TGTGCTCAGAGGCCGTCTT
Nkx6.1	CTTCTGGCCCGGAGTGATG	GGGTCTGGTGTGTTTTCTCTTC
CaII	CGATCCTTGCTCCCTTCTTC	CACGATCCAGGTCACACATT
Hnf6	AAGTTCCCTCACCAT CATCAC	AGTGTGAAACTACCGCTCAC
Pcna	CTCCGCCACCATGTTTGAG	CAGCACCTTCTTCAGGATGGA
Dclk1	CAGCCTGGACGAGCTGGTGG	TGACCAGTTGGGGTTCACAT
CD71	GAAGTCCAGTGTGGGAACAGGT	CAACCACTCAGTGGCACCAACA
Mafb	GCCTTCTTCTCCCAGCTTCA	ATCCAGTACAGGTCCTCGAGATG
Jag1	ACACAGGGATTGCCCACTTC	AGCCAAAGCCATAGTAGTGGTCAT
Ngn3	CAGTCACCCACTTCTGCTTC	GAGTCGGGAGAACTAGGATG
Notch 2	AGAGATGGAAGTGCCTATCACACA	GGACGGGACGTCTCTTCATTT
Tbp	CTGGCGGTTTGGCTAGGTT	TGGGCACTGCGGAGAAA