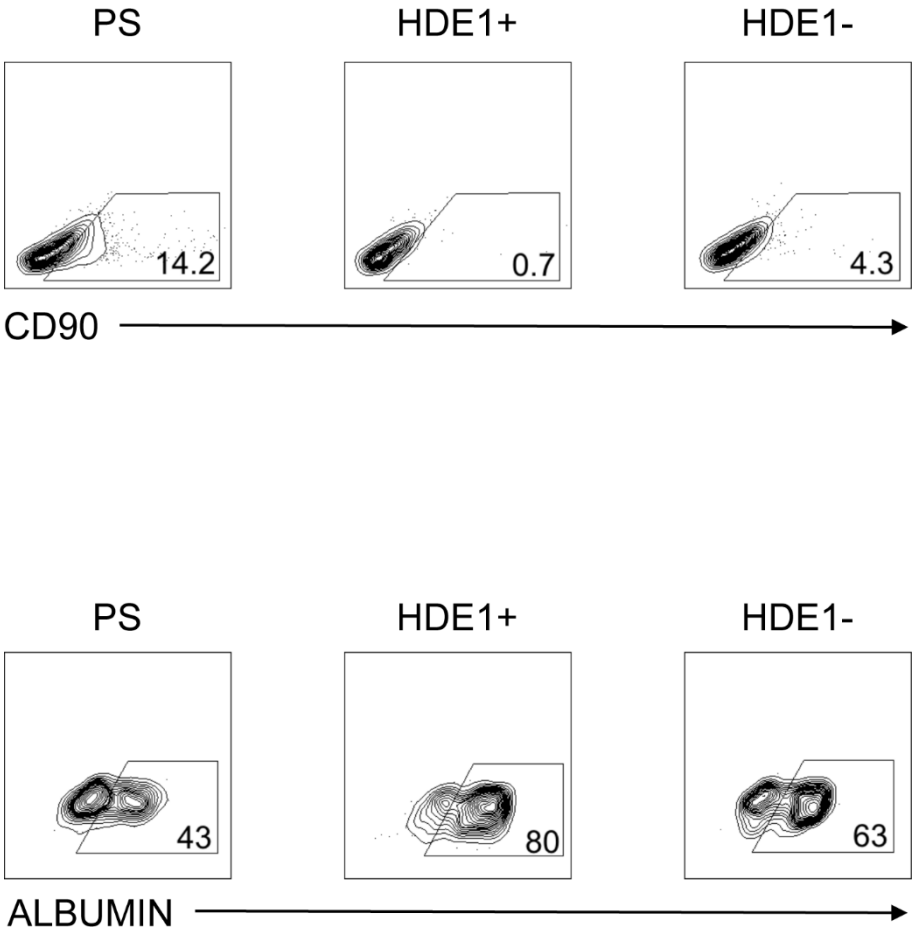

Figure S1. Analysis of the proportion of CD90$^+$ and ALB$^+$ cells in HDE1$^+$ and HDE1$^-$ -derived populations. Representative flow cytometric analyses of CD90 and ALB staining in populations derived from presort (PS), HDE1$^+$ and HDE2$^+$ cells following 28 days of culture as described in figure 3B.

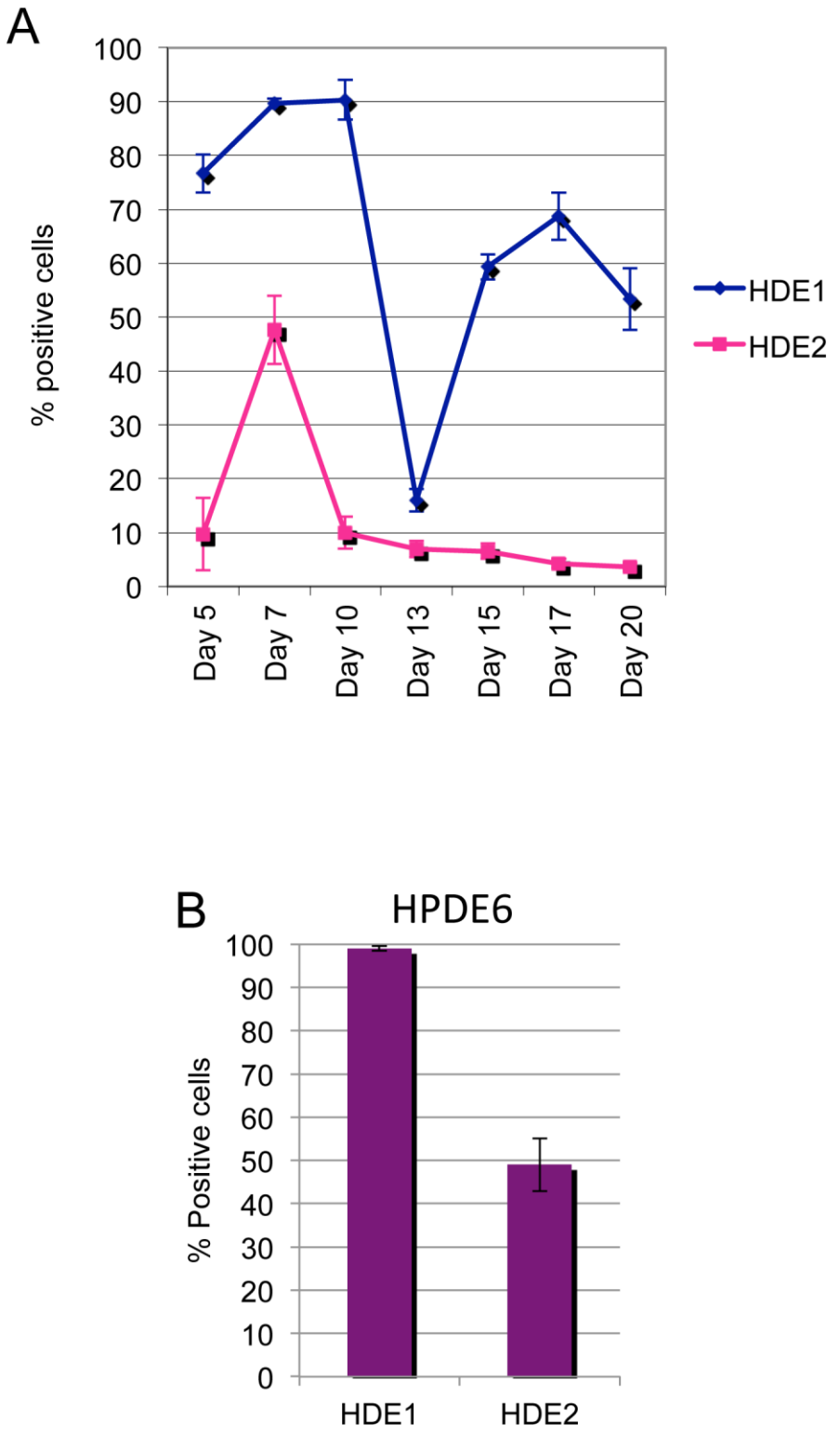

Figure S2. HDE1 and HDE2 staining patterns on hESCs-derived pancreatic populations and on HPDE6 cells. (A) Flow cytometric analyses of HDE1 and HDE2 staining at the indicated time of pancreatic differentiation of HES2 hESCs (n=3, data are represented as mean +/- SEM). (B) Flow cytometric analyses of HDE1 and HDE2 staining of the pancreatic duct epithelial cell line HPDE6 (n=3, data are represented as mean +/- SEM).

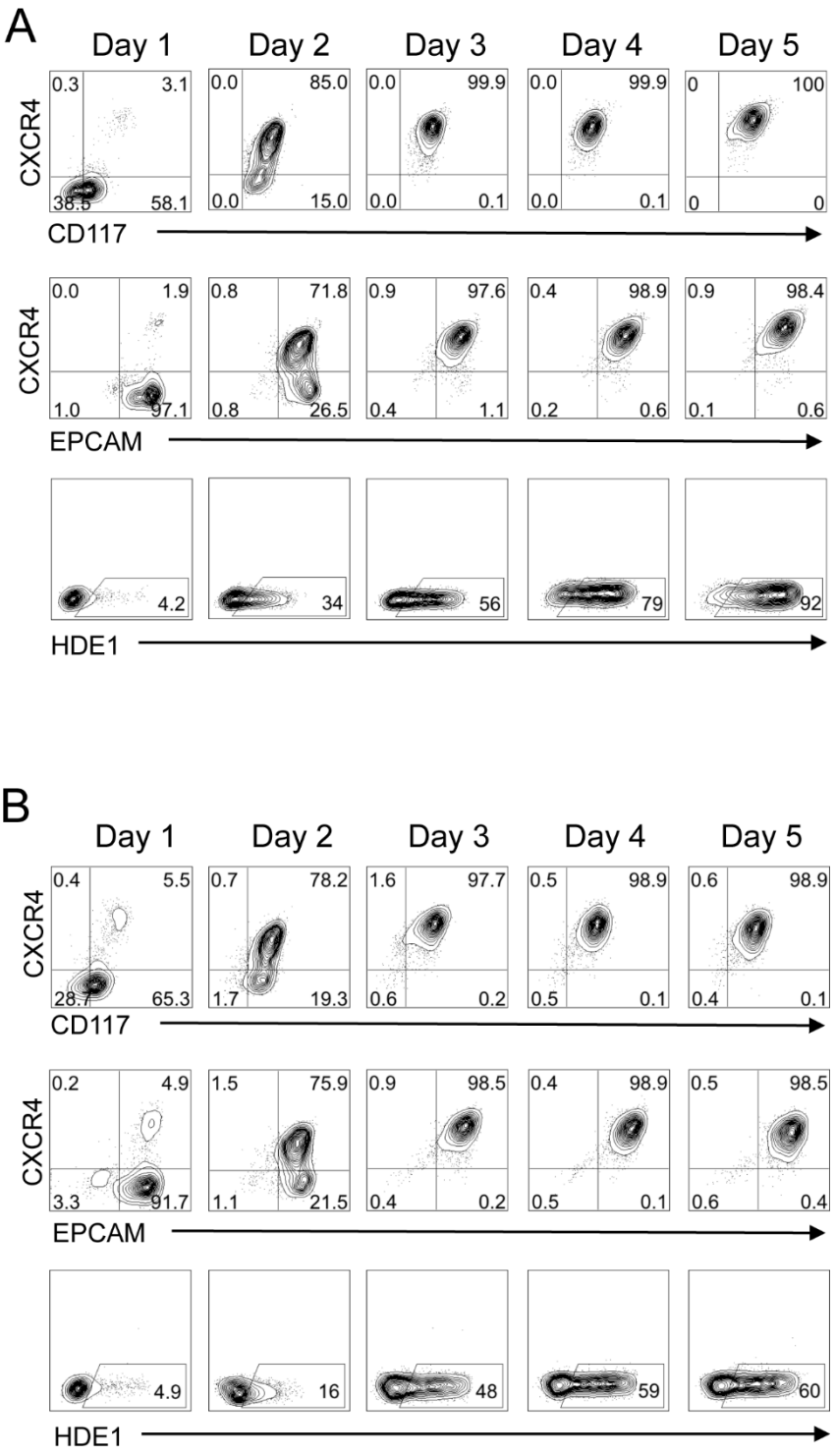

Figure S3. HDE1 and HDE2 staining patterns of definitive endoderm induced in monolayer cultures. (A) Representative flow cytometric analysis of CXCR4, CD117, EPCAM and HDE1 staining of the emerging HES2 hESC-derived endoderm populations induced in monolayer cultures. (B) Representative flow cytometric analysis of CXCR4, CD117, EPCAM and HDE1 staining of the emerging H1 hESC-derived endoderm populations induced in monolayer culture. All FACS plots are representative of the results from 3 independent experiments.

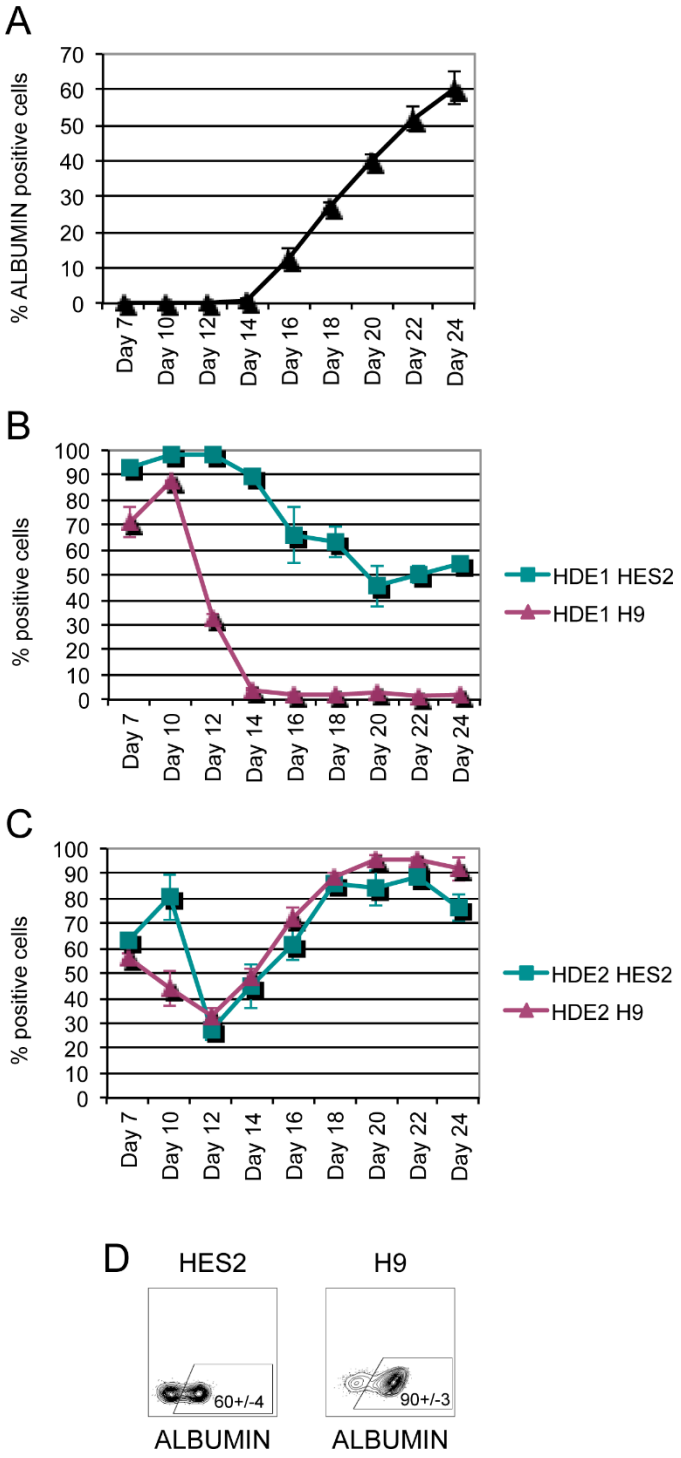

Figure S4. HDE1 and HDE2 staining patterns of HES2 and H9-derived populations at different stages of hepatic development. (A) Intra-cellular flow cytometric analysis of ALB staining at the indicated days of hepatic differentiation of HES2 hESCs (n=3, data are represented as mean +/- SEM). (B) Flow cytometric analysis of HDE1 staining at the indicated days of hepatic differentiation of HES2 and H9 hESCs (n=3, data are represented as mean +/- SEM). (C) Flow cytometric analysis of HDE2 staining at the indicated days of hepatic differentiation of HES2 and H9 hESCs (n=3, data are represented as mean +/- SEM). (D) Percentage of ALB^{+} cells in day 24 hepatocyte cultures generated from either HES2 (left panel) or H9 (right panel) hESCs. Values were determined by intra-cellular flow cytometric analyses (n=3, data are represented as mean +/- SEM)

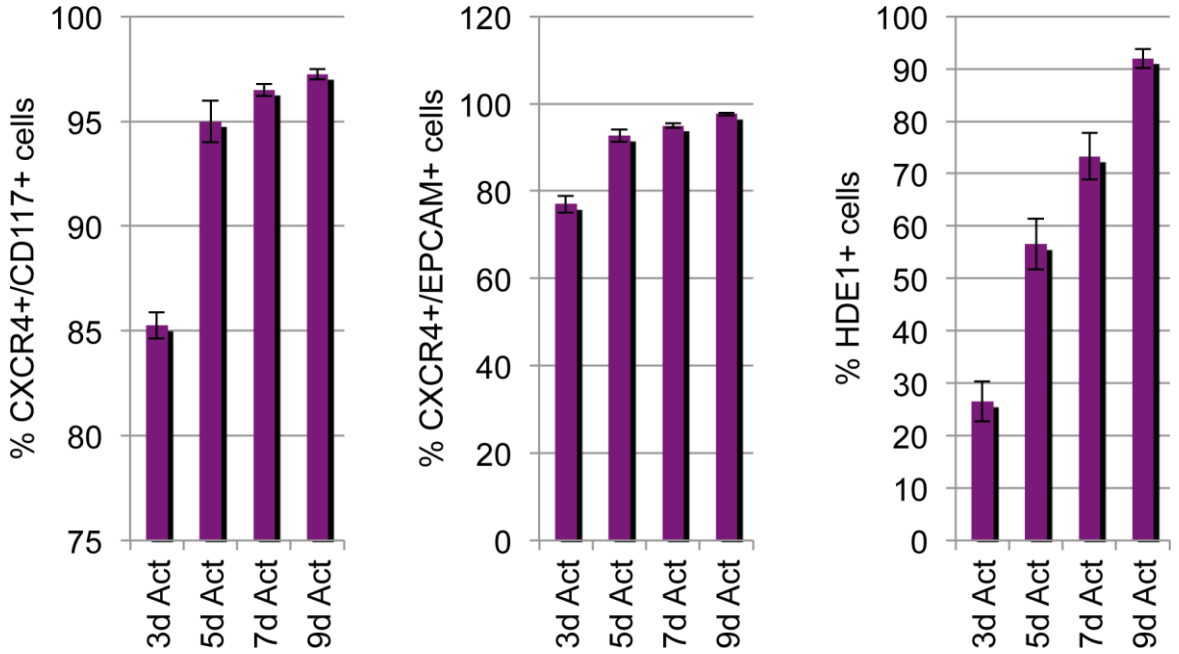

Figure S5. Analyses of H9 hESC-derived endoderm populations induced with activin A for different periods of time. Flow cytometric analysis showing the proportion of $CXCR4^{+}CD117^{+}$ cells, $CXCR4^{+}EPCAM^{+}$ cells and $HDE1^{+}$ cells in H9 hESC-derived populations induced with activin A for the indicated periods of time. (n=3, data are represented as mean +/- SEM).

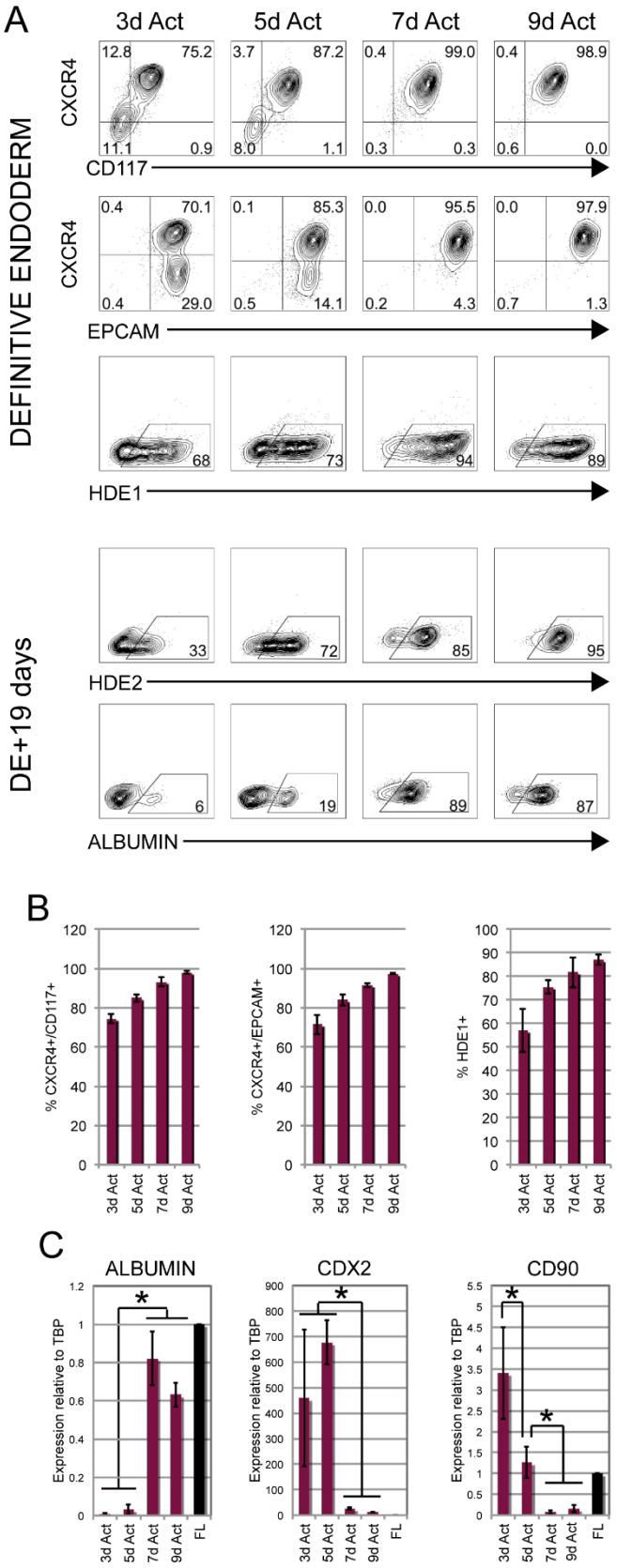

Figure S6. Hepatic potential of BJ hIPSC-derived endoderm correlates with HDE1 staining patterns. (A) Upper three rows: representative flow cytometric analysis showing the proportion of CXCR4$^+$, CD117$^+$ EPCAM$^+$ and HDE1$^+$ cells in BJ hIPSC-derived endoderm populations induced with activin A for the indicated period of time (days). Lower two rows:

representative flow cytometric analysis showing the proportion of $HDE2^+$ and ALB^+ cells in hepatic cultures generated from endoderm induced for the indicated periods of time. Cells were analyzed 19 days following the endoderm stage (DE+19 days). (B) Flow cytometric analysis showing the proportion of $CXCR4^+CD117^+$ cells, $CXCR4^+EPCAM^+$ cells and $HDE1^+$ cells in BJ hIPSC-derived populations induced with activin A for the indicated periods of time. (n=3, data are represented as mean +/- SEM). (C) RT-qPCR analyses of *ALB*, *CDX2* and *CD90* expression in the BJ hIPSC-derived hepatic populations (DE+19) generated from the endoderm induced with activin A for different periods of time. (FL=Fetal Liver). Values were determined relative to TBP and compared to fetal liver (FL set at 1, n=3, data are represented as mean +/- SEM. * indicates $p<0.05$).

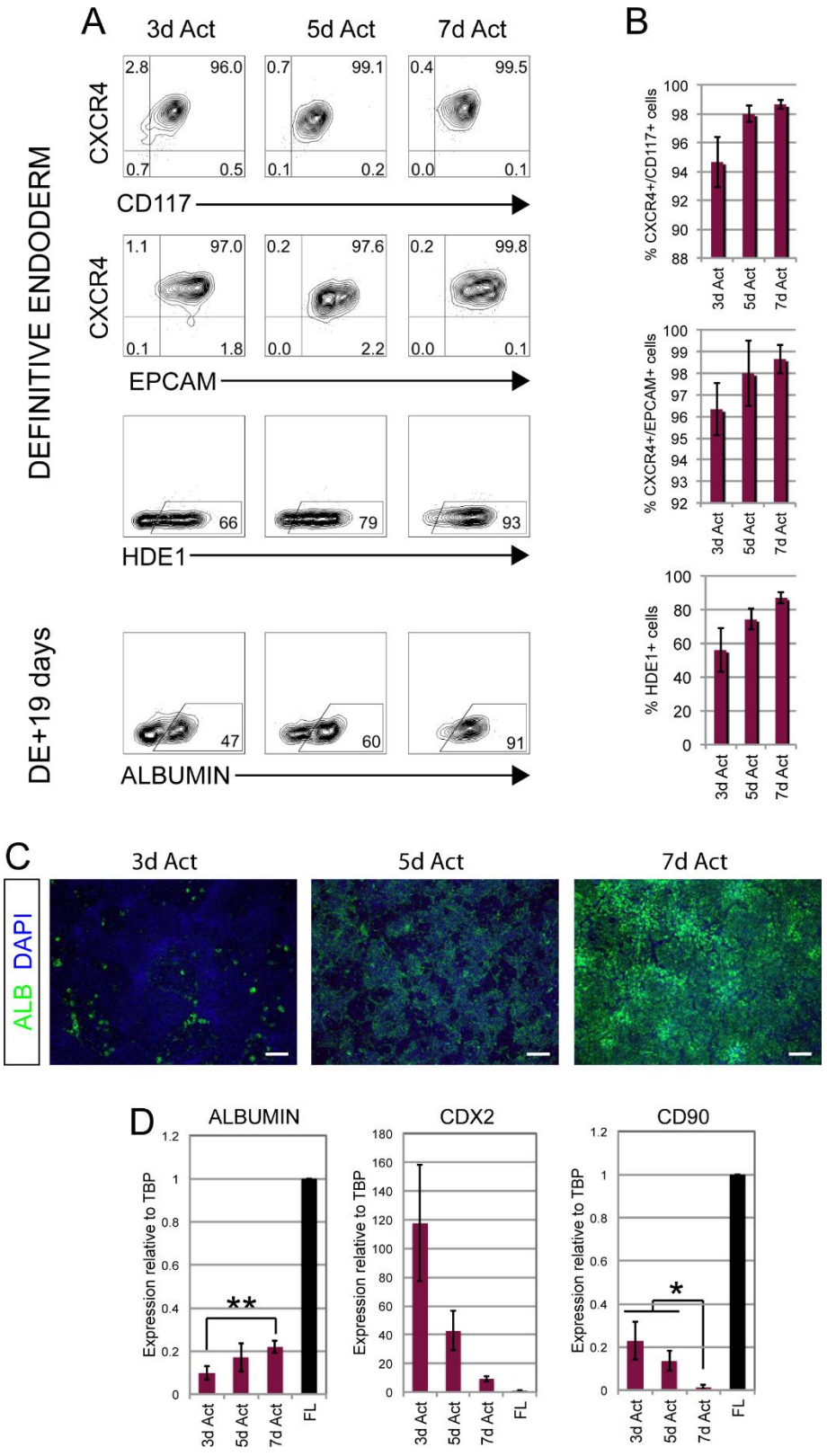

Fig. S7. Hepatic potential of MSC-iPS1 hiPSC-derived endoderm correlates with HDE1 staining patterns. (A) Upper three rows: representative flow cytometric analysis showing the proportion of $CXCR4^+$, $CD117^+$ $EPCAM^+$ and $HDE1^+$ cells in MSC-iPS1 hIPSC-derived endoderm populations induced with activin A for the indicated period of time (days). Lower row: representative flow cytometric analysis showing the proportion of and ALB^+ cells in hepatic cultures generated from endoderm induced for the indicated periods of time. Cells

were analyzed 19 days following the endoderm stage (DE+19 days). (B) Flow cytometric analysis showing the proportion of $CXCR4^{+}CD117^{+}$ cells, $CXCR4^{+}EPCAM^{+}$ cells and $HDE1^{+}$ cells in MSC-iPS1 hIPSC-derived populations induced with activin A for the indicated periods of time. (n=3, data are represented as mean +/- SEM). (C) Immunocytochemistry analyses of ALB (green) expression in MSC-iPS1 hiPSC-derived hepatic populations following 19 days of culture of definitive endoderm stage induced with activin A for the indicated period of time (days). Scale bars: 200 um. (D) RT-qPCR analyses of *ALB*, *CDX2* and *CD90* expression in the MSC-iPS1 hIPSC-derived hepatic populations (DE+19) generated from the endoderm induced with activin A for the indicated periods of time. (FL=Fetal Liver). Values were determined relative to TBP and compared to fetal liver (FL set at 1, n=3, data are represented as mean +/- SEM. * indicates $p<0.05$. ** indicates $p<0.01$).

Table S1. Primary antibody list

Antibody	Company	Catalogue number	Ig Species	Conjugate	Dilution
HDE1	n/a	n/a	Mouse IgG1	none	1:100 (flow and IHC)
HDE2	n/a	n/a	Mouse IgG1	None	1:100 (flow and IHC)
SOX17	R&D	MAB1924	Mouse	None	1:40
CXCR4 (CD184)	BD	555976	Mouse	APC	1:50
CD117	BD	340529	Mouse	PE	1:25
EPCAM (CD326)	eBioscience	12-9326-42	Mouse	PE	1:100
KDR	R&D	FAB357A	Mouse	APC	15:100
KDR	R&D	FAB357P	Mouse	PE	15:100
PDGFRa (CD140a)	BD	556002	Mouse	PE	1:20
CD56	BD	555518	Mouse	APC	1:20
ALBUMIN	Bethyl	A80-129A	Goat	None	1:200 (flow) 1:400 (IHC)
ALBUMIN	DAKO	A0001	Rabbit	None	1:400 (flow) 1:4000 (IHC)
AFP	DAKO	A00008	Rabbit	None	1:2000
CD90	Biolegend	328110	Mouse	PE	1:400
5H10	Streeter lab	n/a	Mouse IgM	None	50ul (supernatant)
HICO 3-C5	Streeter lab	n/a	Mouse IgM	None	50ul (supernatant)
c-peptide	Beta Cell Biology Consortium	AB1921	Rat	None	1:300 (flow) 1:1000 (IHC)

Table S2. IgG control list

Antibody	Company	Catalogue number	Conjugate	Stock concentration
Goat IgG	Sigma	I5256	none	1mg/ml
Rabbit IgG	Jackson Immunoresearch	001-000-003	None	11mg/ml
Mouse IgG1	Life technologies	MG105	APC	4.1mg/ml

Table S3. Secondary antibody list

Antibody	Company	Product Code	Conjugate	Dilution
Goat anti-Mouse IgG	Jackson immunoresearch	115-115-164	PE	1:200
Goat anti-Mouse IgM	Jackson immunoresearch	115-096-075	FITC	1:100
Goat anti-Mouse IgG	Jackson immunoresearch	115-165-164	Cy3	1:200
Donkey anti-Rat IgG	Life technologies	A21208	Alexa488	1:400
Donkey anti-Goat IgG	Life technologies	A11055	Alexa488	1:400
Donkey anti-Rabbit IgG	Life technologies	A11008	Alexa488	1:400
Donkey anti-Rabbit IgG	Jackson immunoresearch	711-165-152	Cy3	1:300

Table S4. RT-qPCR primer list

GENE	Forward sequence	Reverse sequence
ALBUMIN	5'-GTGAAACACAAGCCCAAGGCAACA-3'	5'-TCAGCCTTGCAGCACTTCTCTACA-3'
CD90	5'-ATACCAGCAGTTCACCCATTCAGT-3'	5'-AATTGCTGGTGAAGTTGGTTCGGG-3'
CER	5'-CTTGTCTCAGCTCTGCCACTAACT-3'	5'-TCATCTAGGTCGGGTCCGTCATTT-3'
CXCR4	5'-AGGGAACTGAACATTCCAGAGCGT-3'	5'-AAACGTTCCACGGGAATGGAGAGA-3'
FOXA2	5'-GCATTCCCAATCTTGACACGGTGA-3'	5'-GCCCTTGCAGCCAGAATACACATT-3'
MEOX1	5'-TGAGGACTGATGGCCAAAGAGCAT-3'	5'-ATCCAAACTCACGTTGACCTCCCT-3'
MESP1	5'-AGCCCAAGTGACAAGGGACAACT-3'	5'-AAGGAACCACTTCGAAGGTGCTGA-3'
OCT4	5'-ATGCATTCAAACTGAGGTGCCTGC-3'	5'-CCACCCTTTGTGTTCCCAATTCCT-3'
SOX17	5'-AGGAAATCCTCAGACTCCTGGGTT-3'	5'-CCCAAACTGTTCAAGTGGCAGACA-3'
SOX7	5'-AGCATGCTTCCTTTAGCTGCTGTG-3'	5'-TTGCTCTAAAGCACTGGCTGAGGA-3'
TBP	5′-GCGCAAGGGTTTCTGGTTTGCC-3′	5′-AGGGATTCCGGGAGTCATGGC-3′
ZIC1	5′-GTCTTCGCGCGCTCCGAGAATT-3′	5′-CTTGCGGTCGCTGCTGTTAGCG-3′