

Supplementary Materials for

In Vivo Liver Regeneration Potential of Human Induced Pluripotent Stem Cells from Diverse Origins

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Table S3. Genes differentially methylated between mesoderm cells (all iPSCmes and their parental cells) versus all other cell lines (all other iPSCs, other parental cells, and ESCs).

Table S4. Genes differentially methylated between ectoderm cells (iPSCect and their parental cells) versus all other cell lines (all other iPSCs, other parental cells, and ESCs).

SUPPLEMENTAL MATERIALS

Supplemental methods

Retroviral production and reprogramming of keratinocytes.

Retroviruses for the four factors were independently produced after co-transfecting the 293T cell line with pMX retroviral vectors expressing Oct4, Sox2, Klf4 or c-Myc (Addgene) and helper plasmids as previously described (10, 23). A 1:1:1:1 mix of retroviruses containing Oct4, Sox2, Klf4 and c-Myc was added to keratinocytes (passages 1) in the presence of 8 ug/ml polybrene. After incubating for 3 days in the serum free gold media (Lonza), media was replaced with 0.5mM NaB containing hESC medium. After transformed colonies were observed in the reprogramming plates, a pluripotent stem cell marker, TRA-1-60 antibody (1:200 dilution, Millipore) and Alexa555 conjugated anti-mouse IgM antibody (1:500 dilution, Invitrogen) were added into live cell culture (without fixation) and incubated for 1 hour at 37°C, to distinguish the iPSC from non-iPSC colonies. TRA-1-60 positive colonies appeared in about 6 days after retroviral transduction, and individual TRA-1-60 positive colonies were picked onto MEF coated plates.

Embryoid body formation and spontaneous differentiation into three germ layer cells.

Human iPSCs (iK1, iK2, and iK3) were dissociated by collagenase IV digestion and plated in ultra low attachment plates (Corning) at the density of $\sim 1 \times 10^6$ cells/well in the presence of

differentiation medium (DMEM supplemented with 20% FBS, L-glutamine, β -mercaptoethanol, and Non-essential amino acids). 50% of the medium was replaced with fresh medium every 2 days. After 7 days the embryoid bodies were transferred to 0.1% gelatin-coated culture dishes and cultured for additional 3 days before fixation and staining. Antibodies against Tuj1 (Covance, 1:500), α -fetoprotein (Dako, 1:200), or SMA (DAKO, 1:100) were used to detect the spontaneously differentiated cells from EBs.

Teratoma formation.

Ten-week-old male NSG mice (Jackson Laboratories) were anesthetized and \sim 1 million iPSCs, resuspended in 20-40 μ l of 50% matrigel, were injected subcutaneously. Mice were euthanized 8 to 12 weeks after cell injection and tumors were analyzed following H&E protocol. All animal experiments were conducted following experimental protocols previously approved by Johns Hopkins IACUC.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde. The following antibodies were used: TRA-1-60 (Millipore, 1:100); SSEA-4 (Cell Signaling, 1:200); Tuj1 (Covance, 1:500), α -fetoprotein (Dako, 1:200), SMA (DAKO, 1:100), OCT-4 (Millipore, 1:100), NANOG (BD, 1:200). Secondary antibodies used were all of the Alexa Fluor Series from Invitrogen.

RNA isolation and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from undifferentiated ESC/iPSCs cultured on matrigel and genomic DNA was removed using RNAqueous® -PCR kit (Applied Biosystems). 2 ug total RNA from each sample was subjected to cDNA using High Capacity RNA-to-cDNA kit (Applied Biosystems). cDNA products were diluted 1:100 for *Oct4* and 1:1000 for 18s rRNA in water, and 4 ul of each cDNA with TaqMan® Universal PCR Master Mix and TaqMan® Gene Expression Assays for each gene (Applied Biosystems) were used for real time PCR. Reactions were carried out in triplicate and analyzed on StepOnePlus Real-Time PCR System (Applied Biosystems).

Detection of Human DNA within Transplanted Mouse Livers

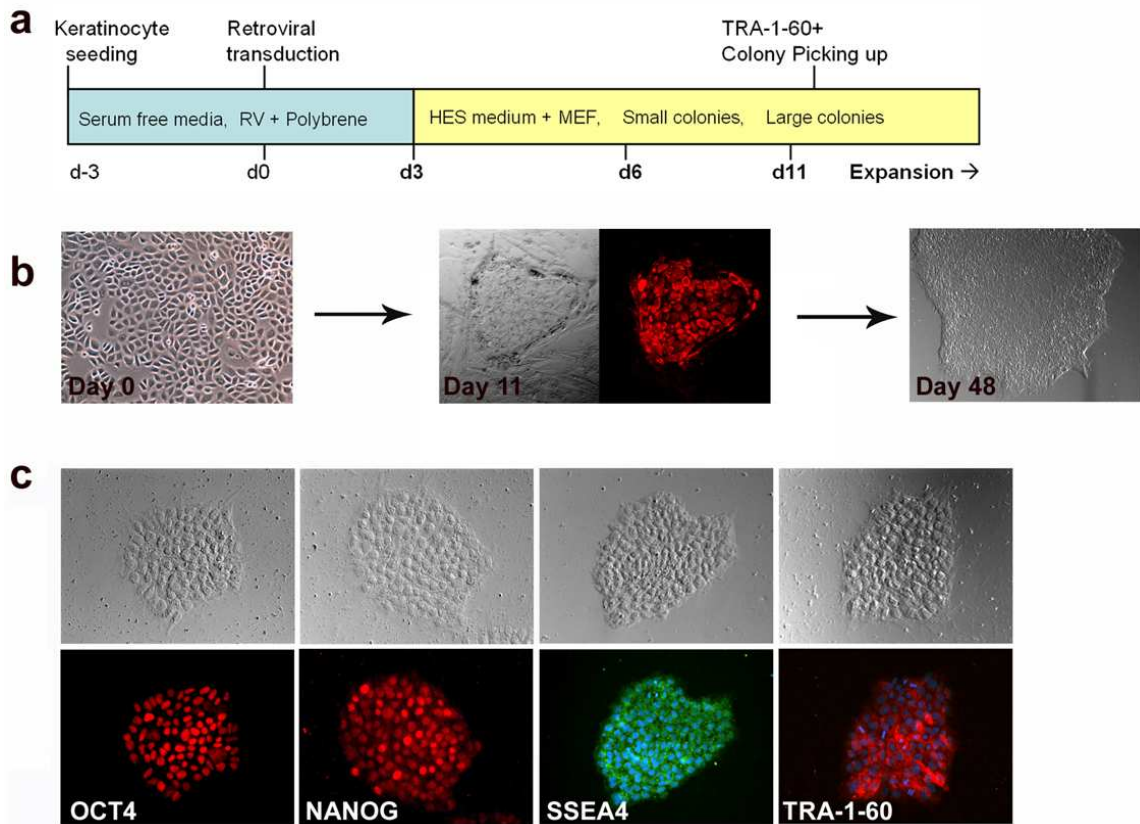
Human genomic DNA in the chimeric liver was detected by amplifying the human specific *Alu* gene (12). Genomic DNA samples were isolated from the liver using the DNeasy tissue kit, and used as templates to amplify the *Alu* repeats with human *Alu* specific primers (aatatggcccaactgcagaa; catcgcattttcacatccaa, product = 182bp) and the PCR Master Mix (Promega, Madison, WI). The liver tissues of control (nontransplanted) mice and human primary hepatocytes served as negative and positive controls.

Laser capture microdissection analysis

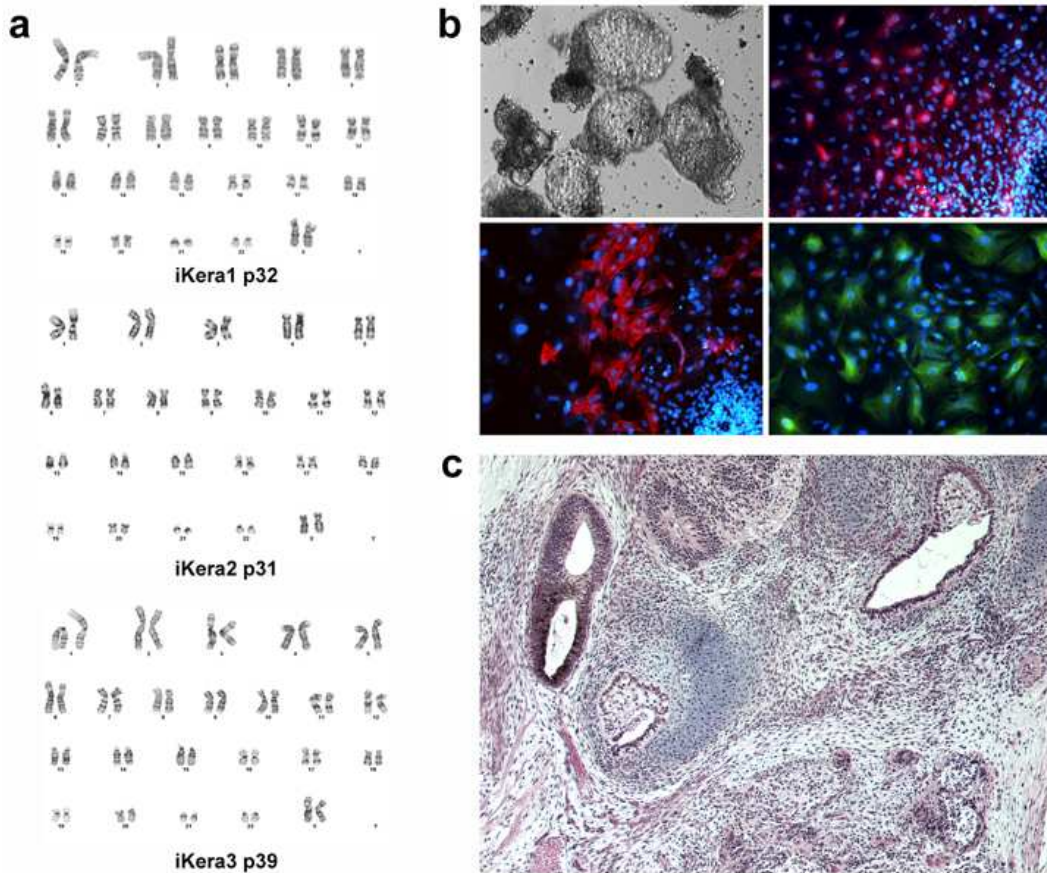
Approximately 500 human ALB positive cells from transplanted mouse liver sections were collected by laser pulse capture (Zeiss P.A.L.M MicroLaser systems). Genomic DNA was purified from the collected human ALB positive cells and amplified by PCR using oligonucleotides from

mouse hypoxanthine-guanine phosphoribosyltransferase (HPRT) (agcgcaagttgaatctgc, agcgacaatctaccagag, product = 219bp) sequences.

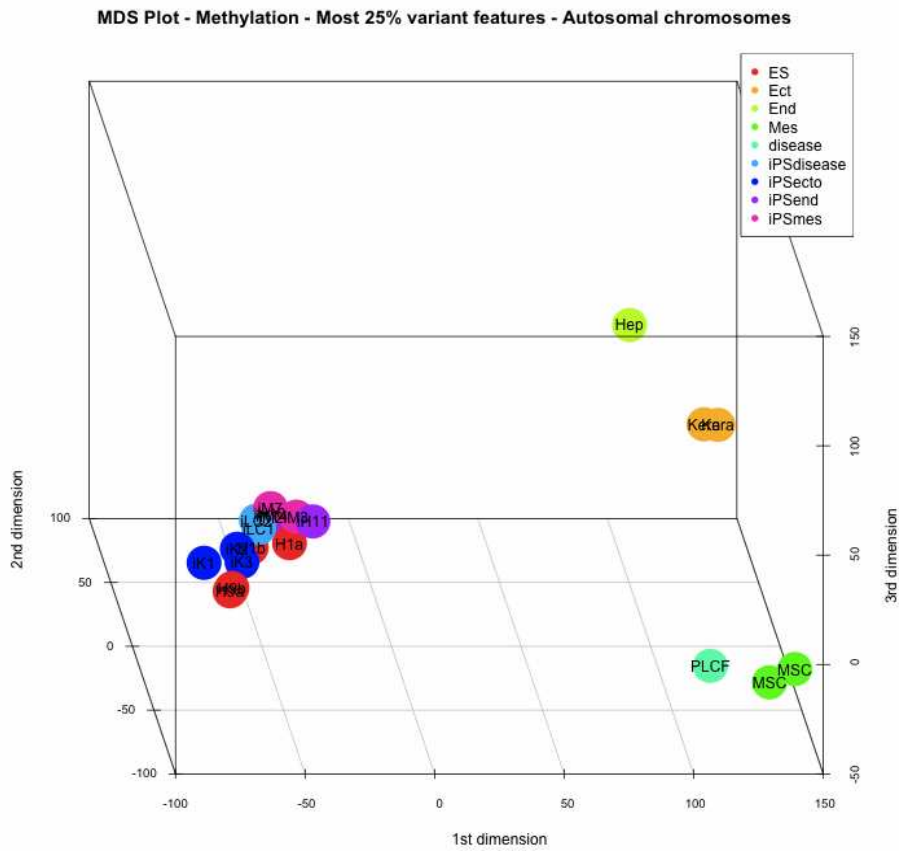
Supplemental Figure 1. Human keratinocyte-derived iPSC (iKera) colony formation and characterization. (a) A diagram of keratinocyte derived iPSC generation protocol. (b) A phase contrast image of primary human keratinocytes before and after iPSC reprogramming. (c) Representative immunofluorescence analysis of one iPSC line derived from keratinocytes (iKera1) growing on Matrigel. Clear expression of the ESC surface antigens SSEA4 and TRA-1-60, and the nuclear transcription factors OCT4 and NANOG are observed.



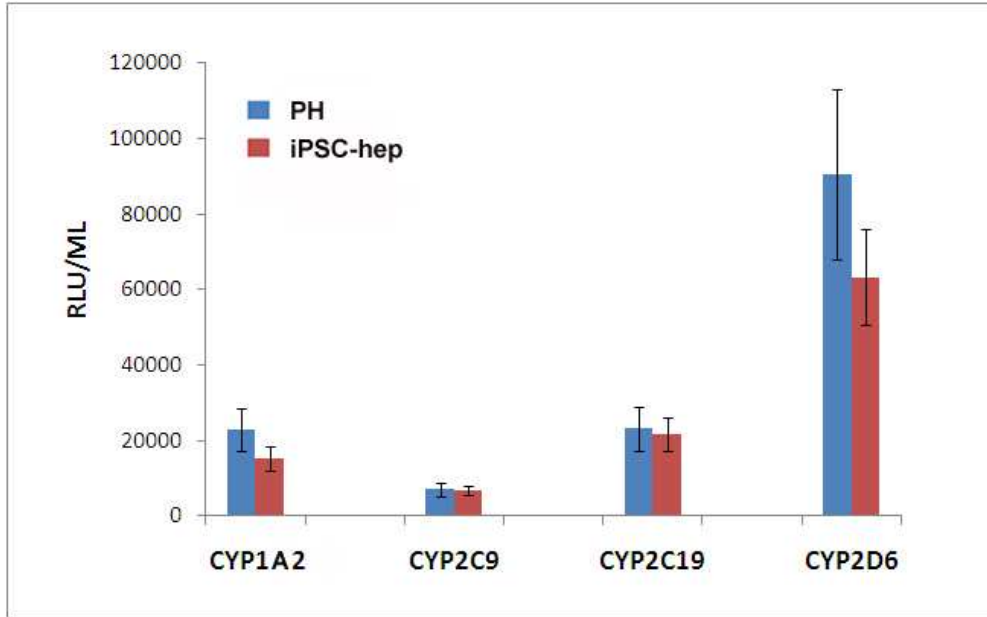
Supplemental Figure 2: iKera cells can differentiate into all three primary germ layers in vitro and in vivo. (a) Karyotyping of iKera cells. After 30 passages, these iPSCs (including iK1, iK2, and iK3 lines shown here) showed normal karyotypes. (b) Embryoid bodies derived from the iK1 line and in vitro differentiation into all three primary germ cell layers. After generation of embryoid bodies (left above) iK1 cells spontaneously differentiated into endoderm (AFP+, red, right above), mesoderm (SMA+, red, left below) and ectoderm (TuJ1+, green, right below). Blue nuclear staining is DAPI. (c) Spontaneous differentiation into all three germ layers including glandular epithelium, cartilages, pigmented epithelium is evident in teratomas.



Supplemental Figure 3. Multidimensional scaling plot based on genome-scale DNA methylation analysis of human iPSCs and their parental cells, and hESCs. Euclidian distance and 25% most varying autosomal loci across all samples analyzed were used. Color code for each group of cell lines analyzed is shown in the figure. iM2, iM3 and iM7 are derived from bone marrow mesenchymal stem cells (MSC). iH11, iH14 and iH10 are from primary hepatocytes (Hep). iK1, iK2, and iK3 are from keratinocytes (Kera). iLC1 and iLC2 are from fibroblasts (PLCF). Both H1 and H9 are hESC lines.

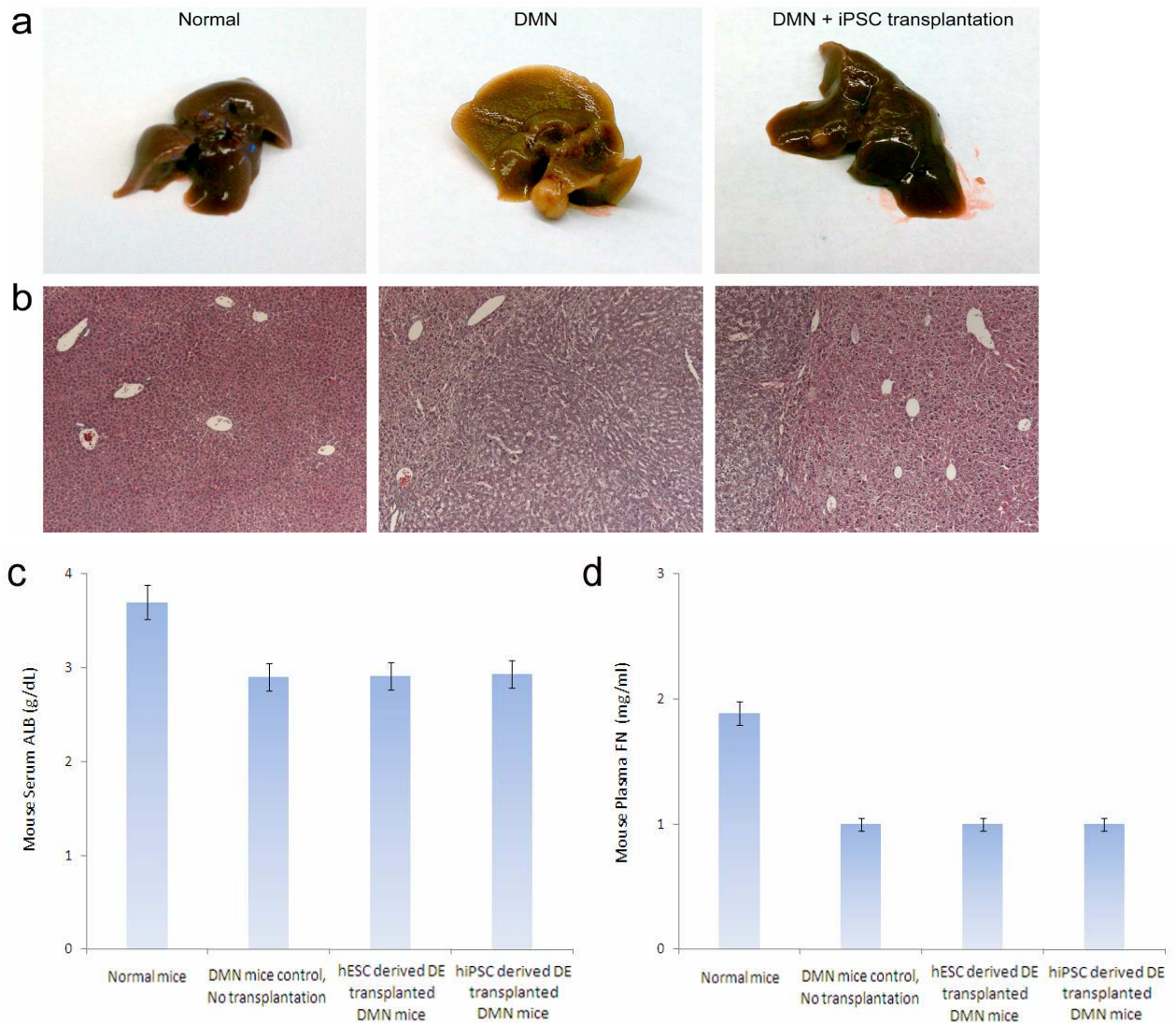


Supplemental Figure 4. Multiple CYP450 enzyme activity analyses for human iPSC-derived hepatocytes. CYP1A2, CYP2C9, CYP2C19, and CYP2D6 were further tested in iPSC derived hepatocytes (iPSC-hep). Levels of all of these key CYP enzyme activities of iPSC derived hepatocytes were comparable to human primary hepatocytes (PH), (n=3, Mean± SEM).



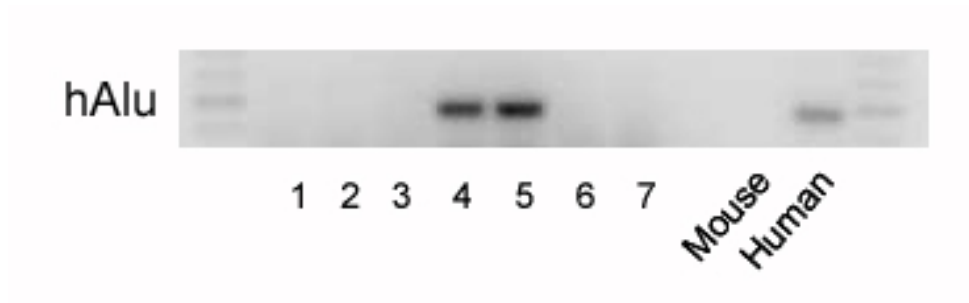
Supplemental Figure 5. DMN mouse liver histology and function before and after the transplantation of human iPSC-derived hepatic cells

(a) Representative pictures from each group (normal NSG mouse liver before transplantation, DMN 4 week treated NSG mouse liver, and a DMN treated NSG mouse liver transplanted with human iPSC derived hepatic cells) are shown. (b) Histological analyses of each liver shown in (a) (hematoxylin and eosin staining, magnification x400). (c, d) Levels of mouse serum albumin (ALB) and fibrinogen (FN) in the DMN treated mice with and without transplantation of human stem cell derived hepatic cells. Mouse serum albumin and plasma fibrinogen levels were measured using the ELISA kits (Bethyl lab E99-134, GenWay 40-374-130050). The bars indicate the means \pm SD (n=3).



Supplemental Figure 6. Detection of human DNA within transplanted mouse livers.

PCR analyses using primers that specifically recognize human *Alu* sequences on genomic DNA isolated from mouse livers injected with human iPSC derived definitive endoderm cells. Human *Alu* DNA bands were detected in at least one or two tissue pieces (among multiple different portions measured) of recipient mouse livers which had shown 10 to 15% of human engraftment by human ALB staining. A typical result is shown here with iK1 transplanted mouse (1-7, seven different portions of the recipient liver tissue). The liver tissues of control (non-transplanted) mice served as a negative control and human cells (primary hepatocytes, PH) were used as a positive control.



Supplemental Figure 7. Mouse gene analysis in engrafted human albumin-positive cells

PCR analyses for detecting mouse genes (HPRT) from the engrafted human ALB+ cells in mouse liver tissue. Human ALB+ cells were precisely collected from transplanted mouse liver tissues by laser capture microdissection and genomic DNA was purified for PCR. There was no mouse gene detected in the human ALB+ cells isolated from either human ESC-DE transplanted mice (hES) or human iPSC-DE transplanted mice (iH12, iM9, iK1). The mouse liver tissue control (mliver) contained the only cells expressing the mouse gene. This result indicates that the engrafted human hepatic cells did not undergo fusion with mouse liver cells.



Supplemental Figure 8. Effect of passaging on pluripotency gene *OCT4* expression.

Quantification of the expression level of *OCT4* in multiple origin human iPSC lines harvested at early (p7-12) and later passages (p34-40) by quantitative PCR. H1 and H9 ESCs were used as controls as shown in the figure. No significant differences were observed between early and later passage iPSCs of different origin and the values were similar to hESCs. The values were normalized by 18S expression and expressed as relative quantitation (RQ), the error bars depict the SD (n=3).

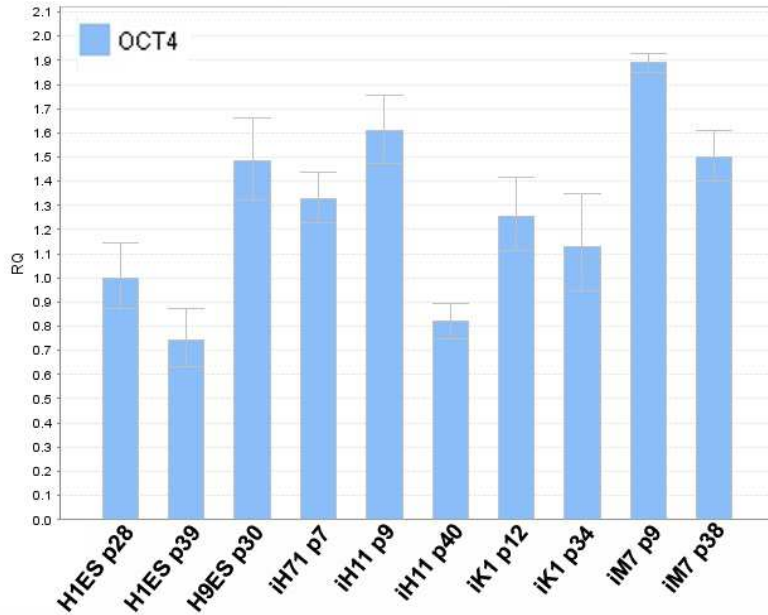


Table S1. Human iPSCs and ESCs used for this study and their pluripotency

Cell lines	Cell sources	Germ layer of original cells	Pluripotency marker expression	EB formation /3 germ layer differentiation in vitro	Teratoma formation /In vivo pluripotency
iH6, 10, 11, 12, 14, 71	Hepatocytes	Endoderm	Y	Y	Y
iM2, 3, 7, 9	BM MSCs	Mesoderm	Y	Y	Y
iLC1, 2	Liver fibroblasts	Mesoderm	Y	Y	Y
iK1, 2, 3	Keratinocytes	Ectoderm	Y	Y	Y
H1 ES	Inner Cell Mass	Blastocyst	Y	Y	Y
H9 ES	Inner Cell Mass	Blastocyst	Y	Y	Y

Table S2. Genes differentially methylated between endoderm cells (iPSCend and their parental cells) versus all other cell lines (all other iPSCs, other parental cells, and ESCs), q-value < 0.001

Table S3. Genes differentially methylated between mesoderm cells (all iPSCmes and their parental cells) versus all other cell lines (all other iPSCs, other parental cells, and ESCs), q-value < 0.001

Table S4. Genes differentially methylated between ectoderm cells (iPSCect and their parental cells) versus all other cell lines (all other iPSCs, other parental cells, and ESCs), q-value < 0.001

Table S2-S4; These analyses were carried on using the log₂ ratio between un-methylated and methylated probes, as well as the beta statistics with overlapping results.

SYMBOL: Gene symbol of the neighboring gene; **t:** moderated t-statistics;

Tables S2-S4 are separately uploaded as supporting online material-2.

Table S2. END vs REST

NCBI Entrez Gene identifiers	SYMBOL	t
9121	SLC16A5	22.2
57124	CD248	21.3
84225	ZMYND15	20.9
23098	SARM1	20.7
57678	GPAM	20
400931	FLJ27365	18.3
9555	H2AFY	17.8
22906	TRAK1	16.5
55267	FLJ10945	16.5
114904	C1QTNF6	16.3
54905	CYP2W1	16
4056	LTC4S	15.9
23543	RBM9	15.6
4237	MFAP2	15.6
83871	RAB34	15.1
79652	C16orf30	14.9
81621	KAZALD1	14.8
27019	DNAI1	14.8
5799	PTPRN2	14.7
221091	LOC221091	14.3
90226	UCN2	14.2
1307	COL16A1	14.1
401459	FLJ46365	14
632	BGLAP	12.9
284358	FLJ36070	12.8
9196	KCNAB3	12.7
7465	WEE1	12.7
10962	MLLT11	12.5
64129	TINAGL1	12.5
1003	CDH5	12.5
23371	TENC1	12.3
116983	CENTB5	12.1
54471	FLJ20232	12.1
27350	APOBEC3C	12
9289	GPR56	11.7
10578	GPLY	11.7
57111	RAB25	11.6
115761	ARL11	11.6

Table S3 MES vs REST

NCBI Entrez Gene identifiers	SYMBOL	t
2875	GPT	27.2
2886	GRB7	22.5
2099	ESR1	17.7
60529	ALX4	17.3
57402	S100A14	16.6
2202	EFEMP1	16.3
5524	PPP2R4	16.1
57167	SALL4	15.7
200634	KRTCAP3	15.6
3918	LAMC2	15.5
284723	SLC25A34	15.4
29775	CARD10	15.4
3233	HOXD4	15.3
64220	STRA6	15.2
5308	PITX2	14.7
80004	RBM35B	14.7
116983	CENTB5	14.2
3199	HOXA2	14.1
23367	LARP1	14
4803	NGFB	13.7
2812	GP1BB	13.6
3083	HGFAC	13.5
9208	LRRFIP1	13.5
150135	C21orf129	13.2
6242	RTKN	13
164284	FLJ90166	12.8
1018	CDK3	12.4
23616	SH3BP1	12.3
1907	EDN2	12.1
6935	TCF8	11.9
51171	DHRS10	11.8
84951	TNS4	11.8
388428	FLJ44861	11.7
104	ADARB1	11.5
6910	TBX5	11.5
90050	C14orf152	11.4
3159	HMGA1	11.4
11259	DOC1	11.3

Table S4 ECT vs REST

NCBI Entrez Gene identifiers	SYMBOL	t
3633	INPP5B	22.5
25900	103	20.9
9891	NUAK1	19.1
64405	CDH22	17.8
374872	C19orf35	17.7
84617	TUBB6	17.2
5083	PAX9	17
57125	PLXDC1	16.9
80341	BPIL1	16.6
8309	ACOX2	16.5
399717	FLJ45983	16.3
30845	EHD3	16.2
53947	A4GALT	16.2
221421	C6orf206	15.8
84618	NT5C1A	15.5
83594	NUDT12	15.4
54790	FLJ20032	15.3
114757	CYGB	14.9
353131	LCE1A	14.6
91373	UAP1L1	14.5
83595	SOX7	14.3
257236	FLJ90575	14.2
9450	LY86	14.2
10747	MASP2	13.9
114902	C1QTNF5	13.7
51042	ZNF593	13.7
84647	PLA2G12B	13.7
7380	UPK3A	13.7
171019	ADAMTS19	13.3
11189	TNRC4	13.2
3131	HLF	13.2
3699	ITIH3	13.2
115701	HAK	13.1
9021	SOCS3	13.1
10485	C1orf61	13.1
50507	NOX4	13.1
148738	HFE2	13.1
1903	EDG3	13

90678	LRSAM1	11.5
3171	FOXA3	11.4
284837	LOC284837	11.3
10801	9-Sep	11.3
128876	C20orf128	11.2
1128	CHRM1	11.2
23413	FREQ	11.1
53822	FXYP7	11.1
9051	PSTPIP1	11
91663	MYADM	11
54106	TLR9	11
343035	C1orf36	11
27134	TJP3	11
6614	SN	10.9
65094	JMJD4	10.9
339665	SLC35E4	10.9
4793	NFKBIB	10.8
83742	MARVELD1	10.8
6776	STAT5A	10.7
1638	DCT	10.7
3866	KRT15	10.6
90199	WFDC8	10.6
6385	SDC4	10.5
56302	TRPV5	10.4
81875	ISG20L2	10.4
23061	KIAA0676	10.4
7462	LAT2	10.3
338773	TMEM119	10.3
6398	SECTM1	10.3
80781	COL18A1	10.2
84948	TIGD5	10.2
390212	GPR152	10.1
26	ABP1	10.1
2626	GATA4	10.1
162514	TRPV3	10
22924	MAPRE3	9.98
284114	TMEM102	9.97
9002	F2RL3	9.95
1454	CSNK1E	9.81
4261	CIITA	9.81
1760	DMPK	9.81
2246	FGF1	9.77
11322	TMC6	9.74
146802	FLJ31196	9.74

55103	RALGPS2	11.2
83597	RTP3	11.2
4773	NFATC2	11.2
26007	DAK	11
3914	LAMB3	11
4487	MSX1	10.9
3856	KRT8	10.9
2706	GJB2	10.9
5777	PTPN6	10.8
1	A1BG	10.8
221833	SP8	10.8
203100	HTRA4	10.8
128602	C20orf85	10.7
1776	DNASE1L3	10.7
122416	ANKRD9	10.6
2041	EPHA1	10.6
55615	PRR5	10.5
3898	LAD1	10.5
9750	C6orf32	10.5
151011	10-Sep	10.4
9249	DHRS3	10.4
4320	MMP11	10.3
780	DDR1	10.3
5265	SERPINA1	10.3
9289	GPR56	10.1
5083	PAX9	10
8424	BBOX1	10
8710	SERPINB7	10
55620	STAP2	10
85004	RERG	9.91
345	APOC3	9.88
338442	GPR109A	9.71
1030	CDKN2B	9.59
10672	GNA13	9.46
9028	RHBDL1	9.4
54970	TTC12	9.37
7253	TSHR	9.32
5306	PITPNA	9.26
81029	WNT5B	9.25
1044	CDX1	9.22
8673	VAMP8	9.21
843	CASP10	9.18
9048	ARTN	9.17
2155	F7	9.14

221	ALDH3B1	13
63924	CIDEC	12.7
5867	RAB4A	12.6
1280	COL2A1	12.6
113220	KIF12	12.6
90050	C14orf152	12.5
6770	STAR	12.4
4313	MMP2	12.4
283600	C14orf68	12.2
3386	ICAM4	12.1
11117	EMILIN1	12
22981	KIAA0980	12
10864	SLC22A7	12
3855	KRT7	11.9
55237	C14orf115	11.9
6236	RRAD	11.9
1	A1BG	11.6
2030	SLC29A1	11.6
5575	PRKAR1B	11.6
6927	TCF1	11.6
25999	CLIPR-59	11.5
60436	TGIF2	11.5
3083	HGFAC	11.4
219928	MRGPRF	11.4
4762	NEUROG1	11.3
718	C3	11.1
3697	ITIH1	11
23231	KIAA0746	10.9
335	APOA1	10.9
4625	MYH7	10.8
9253	NUMBL	10.7
81493	SYNC1	10.7
866	SERPINA6	10.7
80723	TMEM22	10.7
222487	GPR97	10.6
2662	GDF10	10.6
3929	LBP	10.5
761	CA3	10.5
29993	PACSIN1	10.5
23037	PDZK3	10.5
29881	NPC1L1	10.4
51156	SERPINA10	10.4
7490	WT1	10.4
1749	DLX5	10.4

10614	HEXIM1	9.71
8435	SOAT2	9.7
79748	LMAN1L	9.69
340205	TREML1	9.66
55205	ZNF532	9.64
55287	TMEM40	9.64
84985	FAM83A	9.63
2694	GIF	9.58
9362	CPNE6	9.49
79690	GAL3ST4	9.37
340061	LOC340061	9.33
148423	C1orf52	9.31
359710	C20orf185	9.3
2950	GSTP1	9.26
6275	S100A4	9.24
83401	ELOVL3	9.23
2709	GJB5	9.22
2214	FCGR3A	9.22
2125	EVPL	9.2
116840	CNTROB	9.2
6273	S100A2	9.09
5159	PDGFRB	9.08
3036	HAS1	9.04
80097	FLJ14346	9
54578	UGT1A6	9
6696	SPP1	8.99
6358	CCL14	8.97
5753	PTK6	8.95
81607	PVRL4	8.91
388533	UNQ467	8.9
80736	SLC44A4	8.86
6348	CCL3	8.82
137797	LYPD2	8.79
56833	SLAMF8	8.77
10544	PROCR	8.76
11117	EMILIN1	8.65
136371	ASB10	8.64
85456	TNKS1BP1	8.63
23500	DAAM2	8.63
126353	C19orf21	8.62
3590	IL11RA	8.59
79413	ZBED2	8.57
147495	APCDD1	8.56
23650	TRIM29	8.55

64866	CDCP1	9.11
146456	TMED6	9.06
1305	COL13A1	9.05
115572	FAM46B	8.96
7022	TFAP2C	8.95
10110	SGK2	8.92
5443	POMC	8.92
65012	SLC26A10	8.91
8614	STC2	8.87
131669	UROC1	8.86
114038	C21orf84	8.84
57533	TBC1D14	8.82
421	ARVCF	8.8
9020	MAP3K14	8.8
4081	MAB21L1	8.78
6615	SNAI1	8.74
222894	FERD3L	8.71
5493	PPL	8.7
2300	FOXL1	8.68
54436	SH3TC1	8.68
90990	KIFC2	8.6
8416	ANXA9	8.6
83541	C20orf55	8.58
54682	MANSC1	8.57
151278	FLJ32447	8.54
5077	PAX3	8.53
27111	SDCBP2	8.39
79574	EPS8L3	8.38
2027	ENO3	8.28
58473	PLEKHB1	8.23
1775	DNASE1L2	8.2
3909	LAMA3	8.2
1244	ABCC2	8.19
3294	HSD17B2	8.19
400830	DEFB32	8.12
9022	CLIC3	8.12
2672	GFI1	8.07
2147	F2	8.02
2877	GPX2	8.02
8431	NROB2	7.98
26232	FBXO2	7.97
3960	LGALS4	7.97
7850	IL1R2	7.96
50619	DEF6	7.93

54810	GIPC2	10.3
6484	ST3GAL4	10.3
5648	MASP1	10.3
4490	MT1B	10.3
57168	ASPHD2	10.3
54751	FBLIM1	10.2
7416	VDAC1	10.2
79574	EPS8L3	10.1
64757	MOSC1	10.1
6506	SLC1A2	10
43847	KLK14	10
7802	DNALI1	9.96
108	ADCY2	9.95
9970	NR1I3	9.94
3857	KRT9	9.92
23526	HMHA1	9.91
8525	DGKZ	9.85
1812	DRD1	9.84
25956	SEC31L2	9.84
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7480	WNT10B	6.45
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197407	ZNF553	6.41
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8718	TNFRSF25	6.37
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1357	CPA1	5.53
54	ACP5	5.5
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3000	GUCY2D	5.85
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5795	PTPRJ	5.83
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3373	HYAL1	-5.4
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9918	CNAP1	-5.4
9153	SLC28A2	-5.4
6037	RNASE3	-5.4
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3265	HRAS	-5.4
6749	SSRP1	-5.4
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5947	RBP1	-6
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7179	TPTE	-6
5313	PKLR	-6
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6690	SPINK1	-6
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5959	RDH5	-5.8
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60676	PAPPA2	-5.8
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55423	SIRPB2	-5.8
55898	UNC45A	-5.8
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3744	KCNA10	-5.9
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686	BTD	-6.6
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284723	SLC25A34	-21
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