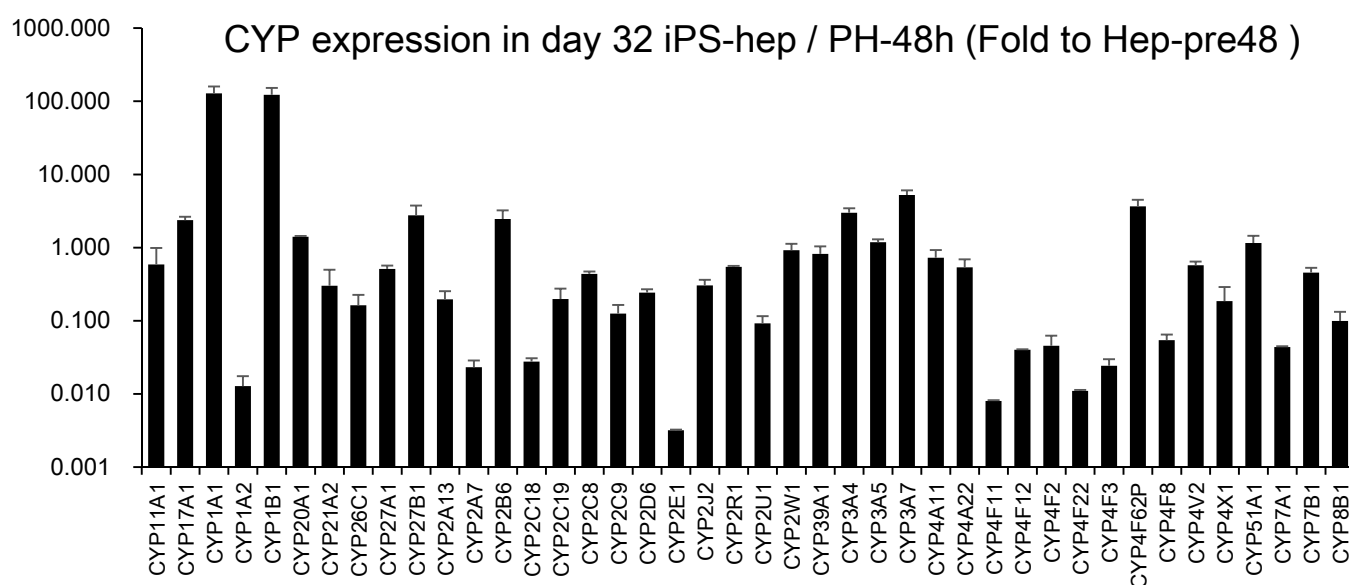


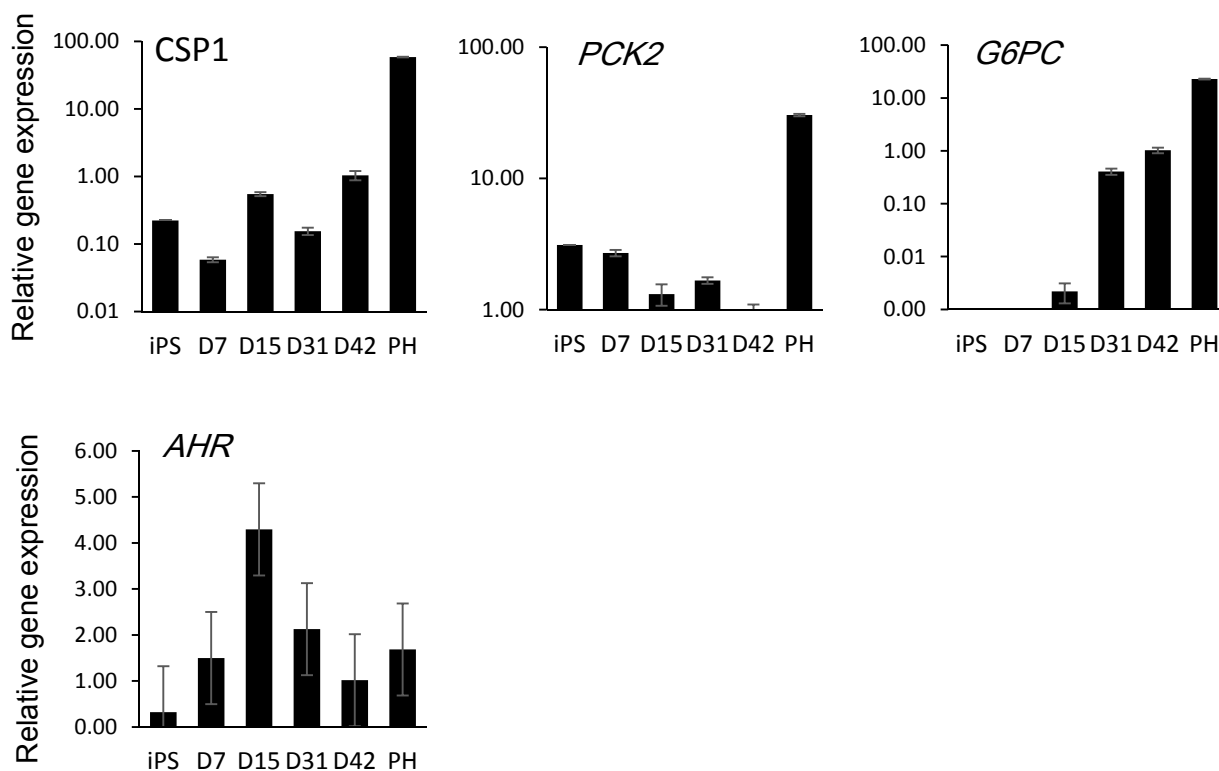
**Figure S1. Comparison among different thicknesses of the CV membrane used**

Human iPS cell-derived endoderm cells on day 3 were plated onto 0.5x, 1x, 2x, and 10x-fold thicknesses of CV membrane and cultured until day 32. Expression levels of various gene transcripts are quantified by real-time RT-PCR in the differentiated cells. Values represent means  $\pm$  S. D (n=3). Significance shown as \* $p$ <0.05 or \*\* $p$ <0.01, by two tailed Student's  $t$ -test, compared to those of hiPS-hep grown on 1x thickness of CV.



**Figure S2. CYP expression profiles revealed by gene array analysis of hiPS-hep on day 30 versus PH (pre-48h).**

CYP genes expressed in day 30 hiPS-hep are shown as fold changes to those expressed in PH (pre-48h). Values represent means  $\pm$  S. D (n=3).



**Figure S3. Expression levels of the hepatocyte zone 1 (CPS1, PCK2, G6PC) and 3 (AHR) genes.**

Expression levels of various gene transcripts quantified by real-time RT-PCR in undifferentiated hiPS cells (iPS) or D7, D15, D31 or D42 differentiated hiPS cells on the CV membrane are compared with that of the primary hepatocytes (PH, 0h; no pre-culture). Values represent means  $\pm$  S. D (n=3).

Table S1. Primers list used in PrimerArray for detecting human hepatic differentiation

Well ID	Symbol	Gene	Gene size	Position	Oligo dT	Random 6 mers	Exon Junction	Variants	Variants cover ratio
1	NANOG	NM 024865.2	2098	375	NG	OK	1/3		1/1
2	POU5F1	NM 002701.4	1411	508	OK	OK	2/4		1/3
3	SOX2	NM 003106.3	2520	1081	OK	OK	0/0		1/1
4	CD9	NM 001769	1321	238	OK	OK	1/7		1/1
5	DNMT3B	NM 0175948	2893	1257	NG	OK	1/21	NM 006892.NM 175849.NM 175850.NM 001207055.NM 001207056	6/6
6	GABRB3	NM 000814	5783	677	NG	OK	1/8	NM 021912.NM 001191320.NM 001191321	4/4
7	GAL	NM 015973	778	350	OK	OK	1/5		1/1
8	GDF3	NM 020634	1224	337	OK	OK	1/1		1/1
9	GRB7	NM 005310	2285	1112	OK	OK	1/14	NM 001030002.NM 001242442.NM 001242443	4/4
10	IFITM1	NM 003641	733	423	OK	OK	1/1		1/1
11	PODXL	NM 005397	5911	1269	NG	OK	1/7	NM 001018111	2/2
12	TDGF1	NM 001174136	1784	428	OK	OK	1/5	NM 003212	2/2
13	ZFP42	NM 174900	2646	308	NG	OK	1/3		1/1
14	SOX7	NM 031439.2	3219	280	NG	OK	1/1		1/1
15	LAMB1	NM 002291.2	5866	4733	OK	OK	1/33		1/1
16	HNF1B	NM 004558.2	2842	1787	OK	OK	1/8	NM 001165923	2/3
17	GSC	NM 173849.2	1217	800	OK	OK	1/2		1/1
18	NODAL	NM 018055.4	2086	946	OK	OK	1/2		1/1
19	FOXA2	NM 021784.4	2428	286	NG	OK	1/1	NM 153675	2/2
20	SOX17	NM 022454.3	2350	1548	OK	OK	0/1		1/1
21	CXCR4	NM 001008540	1912	992	OK	OK	0/0	NM 003467	2/2
22	GATA4	NM 002052.3	3419	1542	NG	OK	1/6		1/1
23	GATA6	NM 005257.4	3770	1890	NG	OK	1/6		1/1
24	AFP	NM 001134.1	2032	332	NG	OK	1/14		1/1
25	CYP3A7	NM 000765	2095	987	OK	OK	2/12		1/1
26	DLK1	NM 003336.5	1599	316	OK	OK	2/4		1/2
27	PROX1	NM 002763.3	3088	228	OK	OK	1/4		1/1
28	TBX3	NM 005996.3	4754	1887	NG	OK	1/6	NM 016569	2/2
29	SERPINA1	NM 000295.4	3220	1850	OK	OK	0/4	NM 001002235.NM 001002236.NM 001127700.NM 001127701.NM 001127702.NM 001127703.NM 001127704.NM 001127705.NM 001127706.NM 001127707	11/11
30	AHSG	NM 001622.2	1594	269	OK	OK	1/6		1/1
31	ALB	NM 000477.5	2264	310	NG	OK	1/14		1/1
32	APOA4	NM 000482.3	1460	258	OK	OK	1/2		1/1
33	ASGR1	NM 001671.4	1519	394	OK	OK	1/8	NM 001197216	2/2
34	KRT8	NM 001256282	1807	73	NG	OK	1/8		1/4
35	KRT18	NM 000224.2	1485	1259	OK	OK	1/6	NM 199187	2/2
36	FABP1	NM 001443.2	598	441	OK	OK	1/3		1/1
37	FCG	NM 000509.4	1865	1275	OK	OK	1/9	NM 021870	2/2
38	G6PC	NM 00151.2	1096	324	OK	OK	1/4		1/1
39	PCK2	NM 004563.2	2221	413	NG	OK	1/9	NM 001018073	2/2
40	RBPA	NM 006744.3	941	442	OK	OK	1/5		1/1
41	TAT	NM 000353.2	2757	1381	OK	OK	1/11		1/1
42	TF	NM 001063.3	2808	2009	OK	OK	1/16		1/1
43	TD02	NM 005651.3	1703	494	OK	OK	1/11		1/1
44	HHEX	NM 002729.4	1772	675	OK	OK	1/3		1/1
45	HNF1A	NM 000545.5	3241	2917	OK	OK	0/9		1/1
46	HNF4A	NM 178849.2	4707	905	NG	OK	1/9	NM 001030003.NM 001030004.NM 175914.NM 000457.NM 178850.NM 001258355	7/7
47	AHR	NM 001621.4	6247	1479	NG	OK	1/10		1/1
48	NR13	NM_001077473	1120	607	OK	OK	1/7	NM 001077480.NM 001077481.NM 001077482.NM 001077474.NM 001077475.NM 001077476.NM 001077477.NM 001077478.NM 001077479.NM 001077469.NM 005122.NM 001077470.NM 001077471.NM 001077472	15/15
49	NR12	NM 003889.3	4446	2680	NG	OK	1/8	NM 033013.NM 022020	3/3
50	NR1H3	NM 003693.3	1939	1250	OK	OK	1/9	NM 001130107.NM 001130102.NM 001251934.NM 001251935	5/5
51	NR1H4	NM 001206979	2325	1412	OK	OK	1/10	NM 001206977.NM 001206978.NM 005123.NM 001206993.NM 001206992	6/6
52	RXRRA	NM 002957.4	5547	180	NG	OK	1/9		1/1
53	NR3C1	NM_001204259	6801	2026	NG	OK	1/8	NM 001024094.NM 001204258.NM 001204260.NM 001204261.NM 001204262.NM 001204263.NM 001204264.NM 001204265.NM 000176.NM 001018074.NM 001018075.NM 001018076.NM 001018077.NM 001020825	15/15
54	PPARA	NM 001001928	9965	552	NG	OK	1/7	NM 005036	2/2
55	CYP1A1	NM 000499	2608	1971	OK	OK	0/6		1/1
56	CYP1A2	NM 000761	3127	969	NG	OK	0/6		1/1
57	CYP2A6	NM 000762	1775	1212	OK	OK	1/8		1/1
58	CYP2C8	NM 000770	1924	1389	OK	OK	1/8	NM 001198853.NM 001198854.NM 001198855	4/5
59	CYP2E1	NM 000773	1667	1026	OK	OK	1/8		1/1
60	CYP2D6	NM 000106	1673	1419	OK	OK	1/8	NM 001025161	2/2
61	CYP3A4	NM 017460	2792	1596	OK	OK	1/12	NM 001202855	2/4
62	CYP7A1	NM 000780	2875	235	NG	OK	1/5		1/1
63	ABCB4	NM 000443.3	8687	2627	OK	OK	1/27	NM 018850.NM 018849	3/3
64	ABCC1	NM 004996.3	6584	1681	NG	OK	1/30		1/1
65	ABCC3	NM 003786.3	5183	1530	NG	OK	1/30	NM 001144070	2/4
66	ABCC2	NM 004827.2	4445	1394	NG	OK	2/15	NM 001257386	2/2
67	SLC10A1	NM 003049.3	1631	524	OK	OK	1/4		1/1
68	SLC22A1	NM 003057.2	1921	790	OK	OK	1/10	NM 153187	1/1
69	SLC22A2	NM 003058.3	2512	1464	OK	OK	1/10		2/2
70	SLCO1B1	NM 006446.4	2800	1577	OK	OK	1/14		1/1
71	SLCO1B3	NM 019844.3	3014	2007	OK	OK	1/15		1/1
72	SLCO2B1	NM 007256.4	4362	3036	OK	OK	0/13	NM 001145211.NM 001145212	3/3
73	UGT1A1	NM 000463.2	2357	430	NG	OK	0/4		1/1
74	GSTA2	NM 000846.4	1320	432	OK	OK	1/6		1/1
75	ATP5G1	NM_005175.2	663	455	OK	OK	1/4	NM_001002027	2/2
76	POLG	NM 002693.2	4464	3732	OK	OK	1/22	NM 001126131	2/2
77	PPARGCIA	NM 013281.3	6318	521	NG	OK	1/12		1/1
78	UCP2	NM 003355.2	1646	1209	OK	OK	1/7		1/1
79	ARG1	NM 001244438	1499	663	OK	OK	1/7	NM 000045	2/2
80	ASL	NM 001024943	2061	1295	OK	OK	1/15	NM 000048.NM 001024944.NM 001024946	4/4
81	ASS1	NM 000050.4	1863	1356	OK	OK	1/15	NM 054012	2/2
82	CPS1	NM 001122633	5738	4340	OK	OK	1/38	NM 001875.NM 001122634	3/3
83	NAGS	NM 153006.2	2086	1459	OK	OK	1/6		1/1
84	OTC	NM 000531.5	1647	67	NG	OK	0/9		1/1
85	KRT19	NM 002276.4	1490	840	OK	OK	1/5		1/1
86	KRT7	NM 003556.3	1753	1008	OK	OK	1/8		1/1
87	AQP1	NM 19398.2	2807	773	NG	OK	1/3	NM 001185060.NM 001185061.NM 001185062	3/3
88	HES1	NM 005524.3	1475	618	OK	OK	1/17		1/1
89	GUSB	NM 000181	2321	1773	OK	OK	1/11		1/1
90	HPRT1	NM 000194	1435	684	OK	OK	2/8		1/1
91	PGK1	NM 000291	2439	1086	OK	OK	1/10		1/1
92	ACTB	NM 001101.3	1852	1135	OK	OK	1/5		1/1
93	GAPDH	NM 002046.4	1401	415	OK	OK	1/8	NM 001256799	2/2
94	TBP	NM 003194	1921	954	OK	OK	1/7	NM 001172085	2/2
95	B2M	NM 004048	987	122	OK	OK	1/3		1/1
96	PP1A	NM 021130	2276	522	NG	OK	1/4		1/3

**Table S2. Top 10 gene categories of the up- or down-regulated genes in hiPS-hep compared to undifferentiated iPSCs**

up-regulated gene in hiPS-hep	
Category	<i>P</i> -value
Lipid Metabolism	1.3E-28
Metabolic Disease	6.53E-23
Vitamin and Mineral Metabolism	6.53E-23
Inflammatory Response	6.53E-23
Gastrointestinal Disease	8.32E-19
Cellular Movement	3.15E-17
Digestive System Development and Function	4.37E-16
Cell Death and Survival	2.27E-14
Carbohydrate Metabolism	1.03E-13
Protein Synthesis	1.49E-13
down-regulated gene in hiPS-hep	
Category	<i>P</i> -value
Cancer	1.02E-19
Cellular Development	1.42E-13
Cell Cycle	1.45E-13
Cellular Assembly and Organization	7.72E-12
DNA Replication, Recombination, and Repair	6.38E-11
Cell Morphology	2.29E-10
Tissue Development	8.23E-10
Cell-To-Cell Signaling and Interaction	9.23E-10
Cellular Function and Maintenance	3.26E-09
Organismal Survival	9.43E-09

**Table S3. Top 100 up-regulated genes in hiPS-hep compared to undifferentiated hiPSCs**

Gene Name	Fold Change	Gene Name	Fold Change
albumin	17.8	tryptophan 2,3-dioxygenase	12
haptoglobin-related protein	16.7	cytochrome P450 family 3 subfamily A member 5	11.9
aldolase, fructose-bisphosphate B	16.2	annexin A8 like 1	11.9
apolipoprotein H	15.8	arylacetamide deacetylase	11.8
apolipoprotein C3	15.8	fibrinogen beta chain	11.8
serum amyloid A1	15.6	glutathione S-transferase alpha 2	11.8
haptoglobin	15.5	ubiquitin D	11.8
fibrinogen like 1	14.7	alpha-2-macroglobulin	11.7
serpin family A member 3	14.7	vanin 1	11.7
transmembrane 4 L six family member 4	14.2	inter-alpha-trypsin inhibitor heavy chain 1	11.7
apolipoprotein C2	14.1	complement factor H	11.7
defensin beta 1	14	arginase 1	11.7
inter-alpha-trypsin inhibitor heavy chain 3	13.9	N-acetyltransferase 8 (putative)	11.6
serum amyloid A4, constitutive	13.9	hydroxyacid oxidase 1	11.6
serum amyloid A2	13.9	UDP glucuronosyltransferase family 1 member A10	11.5
aldo-keto reductase family 1 member B10	13.6	aldo-keto reductase family 1 member C2	11.5
nicotinamide N-methyltransferase	13.6	histidine rich glycoprotein	11.5
serpin family A member 1	13.5	coagulation factor II, thrombin	11.5
alcohol dehydrogenase 1C (class I), gamma polypeptid	13.2	HNF1 homeobox A	11.4
galectin 4	13.2	hydroxysteroid 17-beta dehydrogenase 2	11.4
transmembrane 4 L six family member 5	13	orosomuroid 1	11.4
serpin family C member 1	13	complement component 4 binding protein beta	11.4
hydroxysteroid 11-beta dehydrogenase 1	12.8	alpha 2-HS glycoprotein	11.4
retinol binding protein 4	12.7	complement factor I	11.3
carboxylesterase 1	12.7	caspase 1	11.3
cytochrome P450 family 8 subfamily B member 1	12.6	fibrinogen alpha chain	11.3
GC, vitamin D binding protein	12.6	vitronectin	11.3
fatty acid binding protein 1	12.5	sulfotransferase family 2A member 1	11.3
angiotensinogen	12.5	solute carrier family 40 member 1	11.2
aldo-keto reductase family 1 member B15	12.5	apolipoprotein B	11.2
acyl-CoA synthetase medium chain family member 2E	12.5	aldehyde dehydrogenase 1 family member A1	11.2
UDP glucuronosyltransferase family 2 member B7	12.4	flavin containing monooxygenase 3	11.2
apolipoprotein A1	12.4	complement component 4 binding protein alpha	11.1
cytochrome P450 family 2 subfamily C member 9	12.4	hyaluronan binding protein 2	11
cytochrome P450 family 2 subfamily C member 18	12.3	baculoviral IAP repeat containing 3	10.9
cytochrome P450 family 3 subfamily A member 7	12.3	complement C8 alpha chain	10.9
UDP glucuronosyltransferase family 2 member B4	12.3	orosomuroid 2	10.8
aldo-keto reductase family 1 member C4	12.2	APOBEC1 complementation factor	10.8
UDP glucuronosyltransferase family 2 member B10	12.2	secretory leukocyte peptidase inhibitor	10.7
asialoglycoprotein receptor 2	12.2	lipopolysaccharide binding protein	10.7
fibrinogen gamma chain	12.2	transmembrane 4 L six family member 1	10.7
inter-alpha-trypsin inhibitor heavy chain 2	12.2	UDP glucuronosyltransferase family 2 member B15	10.7
glutathione S-transferase alpha 5	12.2	serpin family A member 4	10.6
transferrin	12.2	cadherin related family member 2	10.6
bile acid-CoA:amino acid N-acyltransferase	12.1	acyl-CoA synthetase long chain family member 5	10.6
4-hydroxyphenylpyruvate dioxygenase	12.1	transthyretin	10.6
UDP glucuronosyltransferase family 1 member A6	12.1	PDZK1 interacting protein 1	10.5
amyloid P component, serum	12.1	cytochrome P450 family 4 subfamily A member 11	10.5
plasminogen	12.1	ATP binding cassette subfamily C member 3	10.5
UDP glucuronosyltransferase family 2 member B11	12.1	hepcidin antimicrobial peptide	10.5