

Table S1. Gene expression levels from Affymetrix microarray data

Gene	UniGene	GeneBank	hESC	hESC-MEC	FH-Ep-P3	FH-Ep-PP
OCT4	Hs.249184	NM_002701	10.8	6.8	5.4	5.5
NANOG	Hs.661360	NM_024865	9.0	5.8	5.2	5.4
SOX2	Hs.518438	NM_003106	9.6	5.6	5.1	5.0
KLF4	Hs.376206	NM_004235	6.7	9.6	8.9	8.0
SOX17	Hs.98367	NM_022454	4.7	5.1	5.7	4.9
HNF3a	Hs.163484	NM_004496	4.4	4.2	3.8	4.9
HNF1a	Hs.654455	NM_000545	6.7	6.8	6.6	6.9
HNF1b	Hs.191144	NM_000458	6.4	6.6	6.4	7.0
HNF4a	Hs.116462	NM_178849	6.5	6.7	5.6	6.3
HNF6	Hs.658573	NM_004498	6.0	6.2	5.1	5.3
CEBPA	Hs.699463	NM_004364	7.9	7.4	6.5	9.4
GATA4	Hs.243987	NM_002052	6.2	6.6	6.4	6.3
GATA6	Hs.514746	NM_005257	5.3	7.6	7.8	6.4
TGFBR1	Hs.494622	NM_004612	8.0	8.0	8.6	6.8
TGFBR2	Hs.82028	NM_003242	6.1	8.5	7.9	6.8
SMAD4	Hs.75862	NM_005359	6.6	6.5	5.7	5.8
AFP	Hs.518808	NM_001134	6.4	7.0	5.5	13.5
ALBUMIN	Hs.418167	NM_000477	4.8	4.5	4.3	11.7
ASGR1	Hs.12056	NM_001671	7.5	6.2	5.6	11.2
SERPINA1	Hs.525557	NM_000295	5.8	6.1	5.1	10.8
VIMENTIN	Hs.642813	NM_003380	8.1	10.8	10.0	7.4
G6PC	Hs.212293	NM_000151	6.1	5.0	4.3	6.7
CK19	Hs.654568	NM_002276	6.9	6.1	5.3	5.2
CK8	Hs.533782	NM_002273	8.8	6.7	8.0	8.4
TAT	Hs.161640	NM_000353	6.8	6.6	5.6	6.0
APOE	Hs.654439	NM_000041	11.4	8.1	7.4	12.0
TDO2	Hs.183671	NM_005651	4.9	5.3	4.3	7.8
TTR	Hs.427202	NM_000371	7.8	8.5	7.0	13.3
CDH1	Hs.461086	NM_004360	9.5	4.4	4.4	7.7
CYP3A4	Hs.654391	NM_017460	7.0	6.8	6.0	8.1
CYP2C9	Hs.282624	NM_000771	6.3	6.4	5.4	6.1
CYP7A1	Hs.1644	NM_000780	3.5	3.5	3.1	3.0
BRACHYURY	Hs.38945	NM_003181	8.0	8.3	6.7	6.8
GAPDH	Hs.544577	NM_002046	13.8	14.1	13.4	12.5

Table S2. Normalized gene expression relative to GAPDH from Affymetrix microarray data

Gene	UniGene	GeneBank	hESC/ hESC-MEC	FH-Ep-P3/ hESC-MEC	FH-Ep-PP/ hESC-MEC
OCT4	Hs.249184	NM_002701	1.6	0.8	0.9
NANOG	Hs.661360	NM_024865	1.6	1.0	1.1
SOX2	Hs.518438	NM_003106	1.8	1.0	1.0
KLF4	Hs.376206	NM_004235	0.7	1.0	0.9
SOX17	Hs.98367	NM_022454	1.0	1.2	1.1
HNF3a	Hs.163484	NM_004496	1.0	1.0	1.3
HNF1a	Hs.654455	NM_000545	1.0	1.0	1.1
HNF1b	Hs.191144	NM_000458	1.0	1.0	1.2
HNF4a	Hs.116462	NM_178849	1.0	0.9	1.1
HNF6	Hs.658573	NM_004498	1.0	0.9	1.0
CEBPA	Hs.699463	NM_004364	1.0	0.9	1.4
GATA4	Hs.243987	NM_002052	1.0	1.0	1.1
GATA6	Hs.514746	NM_005257	0.7	1.0	1.0
TGFBR1	Hs.494622	NM_004612	1.0	1.1	1.0
TGFBR2	Hs.82028	NM_003242	0.7	1.0	0.9
SMAD4	Hs.75862	NM_005359	1.0	0.9	1.0
AFP	Hs.518808	NM_001134	0.9	0.8	2.2
ALBUMIN	Hs.418167	NM_000477	1.1	1.0	2.9
ASGR1	Hs.12056	NM_001671	1.3	0.9	2.1
SERPINA1	Hs.525557	NM_000295	1.0	0.9	1.0
VIMENTIN	Hs.642813	NM_003380	0.8	1.0	0.8
G6PC	Hs.212293	NM_000151	1.3	0.9	1.5
CK19	Hs.654568	NM_002276	1.2	0.9	1.0
CK8	Hs.533782	NM_002273	1.3	1.3	1.4
TAT	Hs.161640	NM_000353	1.0	0.9	1.0
APOE	Hs.654439	NM_000041	1.4	1.0	1.7
TDO2	Hs.183671	NM_005651	0.9	0.8	1.6
TTR	Hs.427202	NM_000371	0.9	0.9	1.8
CDH1	Hs.461086	NM_004360	2.2	1.1	2.0
CYP3A4	Hs.654391	NM_017460	1.0	0.9	1.3
CYP2C9	Hs.282624	NM_000771	1.0	0.9	1.1
CYP7A1	Hs.1644	NM_000780	1.0	0.9	1.0
BRACHYURY	Hs.38945	NM_003181	1.0	0.9	0.9
GAPDH	Hs.544577	NM_002046	1.0	1.0	1.0

Table S3. Efficacy of cell culture manipulations to advance hepatic differentiation in hESC-MEC

Gene or protein	Untreated controls	Activin A, aFGF, HGF, OSM, DKK-1, γ -Secretase, Trichostatin A	Activin A, aFGF, HGF, OSM, DKK-1, γ -Secretase	Activin A, aFGF, HGF, OSM, DKK-1	Activin A, aFGF, HGF, OSM, γ -Secretase	Activin A, aFGF, HGF, OSM, Trichostatin A	Activin A, aFGF, HGF, OSM, DKK-1, Trichostatin A	Activin A, aFGF, HGF, OSM, γ -Secretase, Trichostatin A	HGF, OSM
†Alb	+	+	+	+	+	+	+	+	+
†AFP	-	-	-	-	-	-	-	-	-
†CK19	+	+	+	+	+	+	+	+	+
†Vim	+	+	+	+	+	+	+	+	+
† α SMA	+	+	+	+	+	+	+	+	+
*Glycogen	+	+	++	++	+	+	++	+	+
*DPPIV	+	-	-	-	-	+	-	-	+
*GGT	+	-	-	-	+	++	++	-	+

Abbreviations: aFGF, acidic fibroblast growth factor; HGF, hepatocyte growth factor; OSM, oncostatin M; DKK-1, dickkopf-1; Alb, albumin; AFP, alpha-fetoprotein; CK19, cytokeratin 19; Vim, vimentin; α SMA, alpha-smooth muscle actin.

†RT-PCR; *cytochemical staining. Gene expression graded from absent (-) to maximum of 3+

Supplementary Table 4

Gene expression expressed in fold of healthy NOD/SCID mice after ALF induced by Rif-Phen-MCT with either sham-treatment or transplantation of hESC-MEC

Gene description	Gene Symbol	Sham-3d	Sham-7d	hESC-MEC 3d	hESC-MEC 7d
Oxidative or Metabolic Stress					
Crystallin, alpha B	Cryab	-1.2	1.6	1.1	1.1
Cytochrome P450, family 1, subfamily a, polypeptide 1	Cyp1a1	-3.7	-4.9	-2.3	-2.0
Cytochrome P450, family 1, subfamily b, polypeptide 1	Cyp1b1	-3.4	1.5	-2.6	-1.5
Cytochrome P450, family 2, subfamily a, polypeptide 5	Cyp2a5	-6.7	-5.1	-1.6	-4.7
Cytochrome P450, family 2, subfamily b, polypeptide 10	Cyp2b10	-1.2	1.1	-1.7	-1.0
Cytochrome P450, family 2, subfamily b, polypeptide 9	Cyp2b9	-7.3	-7.2	-5.0	-12.8
Cytochrome P450, family 2, subfamily c, polypeptide 29	Cyp2c29	-46.3	-106.9	-6.8	-3.6
Cytochrome P450, family 3, subfamily a, polypeptide 11	Cyp3a11	-13.8	-27.7	-21.5	-6.3
Cytochrome P450, family 4, subfamily a, polypeptide 10	Cyp4a10	1.6	-5.9	-7.9	-8.9
Cytochrome P450, family 4, subfamily a, polypeptide 14	Cyp4a14	7.8	-2.0	-7.8	-58.7
Cytochrome P450, family 7, subfamily a, polypeptide 1	Cyp7a1	-32.9	-100.2	-15.4	-2.2
Epoxide hydrolase 2, cytoplasmic	Ephx2	-4.1	-10.4	-3.6	-2.9
Flavin containing monooxygenase 1	Fmo1	-15.6	-2.3	-1.1	-1.0
Flavin containing monooxygenase 4	Fmo4	-1.2	-2.0	1.0	1.1
Flavin containing monooxygenase 5	Fmo5	1.7	-1.3	-1.1	-1.1
Glutathione peroxidase 1	Gpx1	-3.2	-3.1	-1.4	-1.3
Glutathione peroxidase 2	Gpx2	-1.6	-1.1	-1.9	1.3
Glutathione reductase	Gsr	-1.4	-1.4	-1.4	-1.2
Glutathione S-transferase, mu 1	Gstm1	-3.8	-10.6	-4.2	-1.6
Glutathione S-transferase, mu 3	Gstm3	-3.9	-5.8	-3.2	1.2
Heme oxygenase (decycling) 1	Hmox1	1.1	3.5	1.1	1.0
Heme oxygenase (decycling) 2	Hmox2	-1.2	-1.6	-1.2	-1.1
Metallothionein 2	Mt2	5.7	7.8	4.9	4.0
Polymerase (RNA) II (DNA directed) polypeptide K	Polr2k	-1.5	-1.2	-1.2	-1.1
P450 (cytochrome) oxidoreductase	Por	-1.7	-5.9	-1.2	-2.0
Superoxide dismutase 1, soluble	Sod1	-2.7	-3.6	-1.9	-1.7
Superoxide dismutase 2, mitochondrial	Sod2	-4.4	-5.4	-1.4	-1.5
Heat Shock					
DnaJ (Hsp40) homolog, subfamily A, member 1	Dnaja1	-1.9	-2.8	-1.0	-1.2
Heat shock factor 1	Hsf1	-1.4	-1.7	-1.2	-1.0
Heat shock protein 1B	Hspa1b	-1.9	-1.1	-1.0	-1.9
Heat shock protein 1-like	Hspa1l	-2.8	-4.0	-1.9	-1.6
Heat shock protein 4	Hspa4	-1.5	-3.3	1.3	1.2
Heat shock protein 5	Hspa5	1.9	1.5	2.5	1.9
Heat shock protein 8	Hspa8	-1.0	-1.2	1.8	1.3
Heat shock protein 1	Hspb1	-1.2	1.8	-1.2	-1.5
Heat shock protein 1 (chaperonin)	Hspd1	-1.6	-2.4	-1.3	-1.1
Heat shock protein 1 (chaperonin 10)	Hspe1	-1.7	-1.7	-1.1	-1.1
Proliferation and Carcinogenesis					
Colony stimulating factor 2 (granulocyte-macrophage)	Csf2	-1.2	2.6	1.0	-1.0
Cyclin C	Ccnc	-1.7	-1.7	1.0	1.0

Cyclin D1	Ccnd1	-1.1	1.8	2.9	3.4
Cyclin G1	Ccng1	1.4	2.4	1.7	1.7
E2F transcription factor 1	E2f1	-1.0	2.2	2.0	2.3
Early growth response 1	Egr1	4.2	15.3	1.3	-1.5
Proliferating cell nuclear antigen	Pcna	-1.3	1.4	1.1	1.4
Growth Arrest and Senescence					
Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	105.0	92.0	45.2	40.6
DNA-damage inducible transcript 3	Ddit3	1.2	3.9	2.0	-1.0
Growth arrest and DNA-damage-inducible 45 alpha	Gadd45a	-3.1	1.6	-1.6	-2.8
Insulin-like growth factor binding protein 6	Igfbp6	-10.2	-3.5	-2.9	-1.7
Transformed mouse 3T3 cell double minute 2	Mdm2	-1.0	1.8	1.5	1.4
Transformation related protein 53	Trp53	-1.3	1.3	-1.0	1.1
Inflammation					
Chemokine (C-C motif) ligand 21b	Ccl21b	-5.0	-16.6	-2.3	-5.8
Chemokine (C-C motif) ligand 3	Ccl3	5.4	30.7	6.3	3.4
Chemokine (C-C motif) ligand 4	Ccl4	3.4	21.5	5.4	2.1
Chemokine (C-X-C motif) ligand 10	Cxcl10	2.4	10.8	2.3	1.5
Interleukin 18	Il18	-3.0	-2.1	-1.5	-1.1
Interleukin 1 alpha	Il1a	-2.9	1.0	-1.2	1.2
Interleukin 1 beta	Il1b	1.2	3.5	1.5	2.0
Interleukin 6	Il6	1.9	7.3	1.0	-1.1
Lymphotoxin A	Lta	-1.2	1.1	1.0	-1.0
Macrophage migration inhibitory factor	Mif	-1.5	-1.3	-1.9	-2.1
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	Nfkb1	-1.3	1.5	-1.0	1.2
Nitric oxide synthase 2, inducible	Nos2	6.5	24.4	1.7	1.1
Serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	50.8	57.3	4.1	-1.0
DNA Damage and Repair					
Ataxia telangiectasia mutated homolog (human)	Atm	-1.7	-2.4	-1.3	-1.0
CHK2 checkpoint homolog (S. pombe)	Chek2	-1.1	1.6	1.4	1.9
Excision repair cross-complementing rodent repair deficiency, complementation group 1	Ercc1	1.1	2.1	1.2	-1.1
Excision repair cross-complementing rodent repair deficiency, complementation group 4	Ercc4	-1.5	-2.0	-1.3	-1.2
RAD23a homolog (S. cerevisiae)	Rad23a	-2.0	-2.7	-1.9	-1.4
RAD50 homolog (S. cerevisiae)	Rad50	-1.8	-1.5	-1.1	1.0
UDP glucuronosyltransferase 1 family, polypeptide A2	Ugt1a2	-2.8	-3.1	-2.2	-1.9
Uracil DNA glycosylase	Ung	-1.7	-1.4	1.4	-1.1
X-ray repair complementing defective repair in Chinese hamster cells 1	Xrcc1	-1.3	-1.4	-1.5	-1.3
X-ray repair complementing defective repair in Chinese hamster cells 2	Xrcc2	1.4	1.1	1.1	-1.0
X-ray repair complementing defective repair in Chinese hamster cells 4	Xrcc4	-1.1	-1.0	1.1	1.1
Apoptosis Signaling					
Annexin A5	Anxa5	2.9	5.0	1.4	1.2
Bcl2-associated X protein	Bax	1.5	2.3	1.9	1.9
Bcl2-like 1	Bcl2l1	1.7	2.6	1.4	1.5
Caspase 1	Casp1	-2.4	1.7	-1.1	1.4
Caspase 8	Casp8	-4.3	-1.1	1.0	1.1

Fas ligand (TNF superfamily, member 6)	Fasl	-1.2	1.2	1.1	-1.0
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	Nfkbia	-1.3	1.7	-1.3	-1.1
Tumor necrosis factor receptor superfamily, member 1a	Tnfrsf1a	1.1	1.5	1.7	1.7
Tumor necrosis factor (ligand) superfamily, member 10	Tnfsf10	-4.3	-3.4	-1.4	-1.2
TNFRSF1A-associated via death domain	Tradd	-1.6	-1.3	-1.1	-1.0

Table S5. Primer sequences for RT-PCR

Gene	Primer sequence 5'-3'	Amplicon size expected
Oct4	F: GACAACAATGAAAATCTTCAGGAGA R: TTCTGGCGCCGGTTACAGAACCA	218 bp
Alb	F: TGCTTGAATGTGCTGATGACAGGG R: AAGGCAAGTCAGCAGGCATCTCATC	161 bp
AFP	F: TGCAGCCAAAGTGAAGAGGGAAGA R: CATAGCGAGCAGCCCAAAGAAGAA	260 bp
CK-19	F: ATGGCCGAGCAGAACCGGAA R: CCATGAGCCGCTGGTACTCC	308 bp
Vim	F: CACCTACAGCCTCTACG R: AGCGGTCATTCAGCTC	170 bp
α -SMA	F: AGTACCCGATAGAACATGG R: TTTTCTCCCGGTTGGC	153 bp
CYP1B1	F: CACCAAGGCTGAGACAGTGA R: GCCAGGTAAACTCCAAGCAC	230 bp
CYP2C9	F: GGACAGAGACGACAAGCACA R: TGGTGGGGAGAAGGTCAAT	200 bp
CYP3A4	F: TGTGCCTGAGAACACCAGAG R: GCAGAGGAGCCAAATCTACC	201 bp
CYP2E1	F: CCGCAAGCATTTTACTACA R: GCTCCTTCACCCTTTCAGAC	202 bp
CYP1A1	F: AGGCTTTTACATCCCAAGG R: GCAATGGTCTCACCGATAACA	197 bp
β -Actin	F: TCACCACCACGGCCGAGCG R: TCTCCTTCTGCATCCTGTCG	350 bp

Abbreviations: F, Forward; R, Reverse; bp, base pair; Alb, albumin; AFP, α -fetoprotein; CK-19, cytokeratin 19; Vim, vimentin; α -SMA, alpha-smooth muscle actin, CYP, cytochrome P450