

Supplementary Information

Improving the differentiation potential of pluripotent stem cells by optimizing culture conditions

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Supplementary Fig. 1 (Fig. S1)

Gene expression profiles of H9 cells cultured with Es8 on VTN-N (Es8/VTN) for 5 days just before transferring to Es6 medium, and cultured with Es6 on VTN-N (Es6/VTN) for 5 days.

Target Name	Category	Es8 / VTN day5	Es6 / VTN day5
CDH9	Ectoderm	0.51	22.53
COL2A1	Ectoderm	2.50	0.95
DMBX1	Ectoderm	0.30	2.11
DRD4	Ectoderm	0.14	0.29
EN1	Ectoderm	0.02	0.47
LMX1A	Ectoderm	0.01	12.33
MAP2	Ectoderm	5.74	8.84
MYO3B	Ectoderm	0.45	1.48
NOS2	Ectoderm	0.87	0.51
NR2F1/NR2F2	Ectoderm	0.00	0.04
NR2F2	Ectoderm	0.03	2.55
OLFM3	Ectoderm	0.35	1.07
PAPLN	Ectoderm	0.53	0.52
PAX3	Ectoderm	0.05	1.93
PAX6	Ectoderm	0.05	20.44
POU4F1	Ectoderm	0.48	0.63
PRKCA	Ectoderm	0.87	0.35
SDC2	Ectoderm	14.60	18.24
SOX1	Ectoderm	0.79	0.25
TRPM8	Ectoderm	0.73	11.44
WNT1	Ectoderm	0.37	8.40
ZBTB16	Ectoderm	0.35	0.35

Target Name	Category	Es8 / VTN day5	Es6 / VTN day5
ABCA4	Mesoderm	2.20	1.97
ALOX15	Mesoderm	0.35	0.40
BMP10	Mesoderm	0.07	0.35
CDH5	Mesoderm	3.95	1.35
CDX2	Mesoderm	0.28	2.37
COLEC10	Mesoderm	2.34	0.72
ESM1	Mesoderm	0.02	0.31
FCN3	Mesoderm	3.72	0.17
FOXF1	Mesoderm	0.50	0.70
HAND1	Mesoderm	0.03	0.86
HAND2	Mesoderm	0.19	0.36
HEY1	Mesoderm	0.91	1.31
HOPX	Mesoderm	0.07	1.09
IL6ST	Mesoderm	0.91	0.46
NKX2-5	Mesoderm	0.31	0.56
ODAM	Mesoderm	0.51	0.76
PDGFRA	Mesoderm	0.17	0.87
PLVAP	Mesoderm	1.62	1.28
RGS4	Mesoderm	0.20	0.85
SNAI2	Mesoderm	0.95	0.09
TBX3	Mesoderm	0.02	0.70
TM4SF1	Mesoderm	0.33	3.99
FGF4	Mesendoderm	0.11	0.58
GDF3	Mesendoderm	1.18	0.68
NPPB	Mesendoderm	0.02	0.00
NR5A2	Mesendoderm	45.72	8.50
PTHLH	Mesendoderm	1.08	1.16
T	Mesendoderm	0.00	0.00

Target Name	Category	Es8 / VTN day5	Es6 / VTN day5
AFP	Endoderm	0.00	0.00
CABP7	Endoderm	0.06	0.62
CDH20	Endoderm	0.22	0.97
CLDN1	Endoderm	1.46	0.41
CPLX2	Endoderm	0.15	0.35
ELAVL3	Endoderm	0.56	0.25
EOMES	Endoderm	0.03	0.07
FOXA1	Endoderm	0.05	0.14
FOXA2	Endoderm	0.08	0.00
FOXP2	Endoderm	0.28	0.47
GATA4	Endoderm	0.01	0.00
GATA6	Endoderm	0.00	0.01
HHEX	Endoderm	0.07	0.90
HMP19	Endoderm	2.59	0.51
HNF1B	Endoderm	0.05	0.00
HNF4A	Endoderm	0.00	0.02
KLF5	Endoderm	0.48	2.40
LEFTY1	Endoderm	0.13	0.01
LEFTY2	Endoderm	0.03	0.02
NODAL	Endoderm	0.03	0.00
PHOX2B	Endoderm	0.03	0.02
POU3F3	Endoderm	0.06	0.08
PRDM1	Endoderm	0.20	1.58
RXRG	Endoderm	0.02	0.74
SOX17	Endoderm	0.01	0.01
SST	Endoderm	0.48	0.18

Target Name	Category	Es8 / VTN day5	Es6 / VTN day5
CXCL5	Self-renewal	23.89	0.82
DNMT3B	Self-renewal	0.30	0.34
HESX1	Self-renewal	0.16	5.90
IDO1	Self-renewal	0.82	0.94
LCK	Self-renewal	2.35	0.46
NANOG	Self-renewal	2.40	0.38
POU5F1	Self-renewal	0.58	0.28
SOX2	Self-renewal	0.96	2.04
TRIM22	Self-renewal	17.56	3.64

Fold change legend

fc > 100	Upregulated
10 < fc <= 100	
2 < fc <= 10	
0.5 <= fc <= 2	Comparable
0.1 <= fc < 0.5	
0.01 <= fc < 0.1	
fc < 0.01	Downregulated
omitted	

Expressions of 96 genes categorized as ectoderm, mesoderm, mesendoderm, endoderm and self-renewal by TaqMan hPSC Scorecard assay are shown in the Table.

Supplementary Fig. 2 (Fig. S2)

Gene expression profiles of H9 cells transduced with mock, mCHD7 or siCHD7 were cultured with Es 8/VTN-N

Target Name	Category	mock	mCHD7	siCHD7
CDH9	Ectoderm	0.34	1.69	0.06
COL2A1	Ectoderm	0.46	0.74	0.27
DMBX1	Ectoderm	0.04	0.52	0.03
DRD4	Ectoderm	0.02	0.51	0.01
EN1	Ectoderm	0.88	1.14	0.33
LMX1A	Ectoderm	0.07	0.22	0.10
MAP2	Ectoderm	4.23	14.15	3.68
MYO3B	Ectoderm	0.27	0.24	0.88
NOS2	Ectoderm	0.71	0.22	0.66
NR2F1/NR2F2	Ectoderm	0.02	5.28	0.05
NR2F2	Ectoderm	0.08	1.00	0.07
OLFM3	Ectoderm	0.81	1.93	0.32
PAPLN	Ectoderm	0.19	0.35	0.53
PAX3	Ectoderm	0.18	13.22	0.03
PAX6	Ectoderm	0.03	3.48	0.03
POU4F1	Ectoderm	0.69	13.62	0.22
PRKCA	Ectoderm	0.94	1.05	0.77
SDC2	Ectoderm	5.55	23.56	4.59
SOX1	Ectoderm	0.14	0.12	0.14
TRPM8	Ectoderm	0.61	0.48	0.34
WNT1	Ectoderm	0.04	2.19	0.03
ZBTB16	Ectoderm	0.06	0.07	0.08

Target Name	Category	mock	mCHD7	siCHD7
ABCA4	Mesoderm	0.50	0.25	0.25
ALOX15	Mesoderm	0.42	0.39	0.49
BMP10	Mesoderm	0.36	0.70	0.04
CDH5	Mesoderm	5.76	22.87	2.13
CDX2	Mesoderm	0.14	0.30	0.05
COLEC10	Mesoderm	0.61	1.20	0.59
ESM1	Mesoderm	0.36	1.09	0.01
FCN3	Mesoderm	0.74	0.23	0.56
FOXF1	Mesoderm	0.62	0.97	0.24
HAND1	Mesoderm	0.00	0.13	0.03
HAND2	Mesoderm	1.21	11.57	0.29
HEY1	Mesoderm	0.76	3.03	0.75
HOPX	Mesoderm	0.25	4.80	0.23
IL6ST	Mesoderm	0.76	0.94	0.53
NKX2-5	Mesoderm	0.89	2.41	0.23
ODAM	Mesoderm	2.02	8.55	0.67
PDGFRA	Mesoderm	0.16	0.59	0.09
PLVAP	Mesoderm	0.68	0.28	0.76
RGS4	Mesoderm	0.23	0.57	0.34
SNAI2	Mesoderm	0.26	1.82	0.27
TBX3	Mesoderm	0.01	0.14	0.04
TM4SF1	Mesoderm	0.92	2.83	0.65
FGF4	Mesendoderm	0.12	0.52	0.30
GDF3	Mesendoderm	2.29	1.33	4.43
NPPB	Mesendoderm	0.02	5.98	0.03
NR5A2	Mesendoderm	79.00	99.35	97.38
PTHLH	Mesendoderm	0.34	61.61	0.43
T	Mesendoderm	0.00	0.00	0.00

Target Name	Category	mock	mCHD7	siCHD7
AFP	Endoderm	0.01	0.05	0.01
CABP7	Endoderm	0.06	0.18	0.22
CDH20	Endoderm	0.15	2.92	0.30
CLDN1	Endoderm	1.59	2.60	1.25
CPLX2	Endoderm	0.48	1.02	0.62
ELAVL3	Endoderm	0.27	0.32	0.17
EOMES	Endoderm	0.05	1.95	0.03
FOXA1	Endoderm	0.07	7.44	0.01
FOXA2	Endoderm	4.66	0.04	1.73
FOXP2	Endoderm	0.63	2.26	0.14
GATA4	Endoderm	0.01	0.14	0.00
GATA6	Endoderm	0.01	1.95	0.00
HHEX	Endoderm	0.02	1.71	0.01
HMP19	Endoderm	1.01	0.31	0.89
HNF1B	Endoderm	0.04	0.15	0.02
HNF4A	Endoderm	0.01	0.04	0.02
KLF5	Endoderm	0.34	15.86	0.36
LEFTY1	Endoderm	0.07	0.92	0.02
LEFTY2	Endoderm	0.02	1.04	0.00
NODAL	Endoderm	0.03	0.07	0.01
PHOX2B	Endoderm	0.02	0.65	0.02
POU3F3	Endoderm	0.03	0.31	0.04
PRDM1	Endoderm	0.05	0.54	0.04
RXRG	Endoderm	0.19	0.39	0.07
SOX17	Endoderm	0.00	2.88	0.00
SST	Endoderm	0.80	0.93	0.19

Target Name	Category	mock	mCHD7	siCHD7
CXCL5	Self-renewal	5.15	32.01	1.96
DNMT3B	Self-renewal	0.32	0.29	0.33
HESX1	Self-renewal	0.85	0.44	0.74
IDO1	Self-renewal	0.75	0.36	1.14
LCK	Self-renewal	1.95	0.97	1.35
NANOG	Self-renewal	0.69	1.18	0.54
POU5F1	Self-renewal	1.26	0.57	1.21
SOX2	Self-renewal	0.97	0.86	0.59
TRIM22	Self-renewal	2.61	16.80	2.05

Fold change legend

fc > 100	Upregulated
10 < fc <= 100	
2 < fc <= 10	
0.5 <= fc <= 2	Comparable
0.1 <= fc < 0.5	
0.01 <= fc < 0.1	
fc < 0.01	Downregulated
omitted	

Expressions of 96 genes categorized as ectoderm, mesoderm, mesendoderm, endoderm and self-renewal by TaqMan hPSC Scorecard assay are shown in the Table.

Supplementary Fig. 2 (Fig. S2)

Gene expression profiles of H9 cells transduced with mock, mCHD7 or siCHD7 were cultured with RFF2/VTN-N

Target Name	Category	mock	mCHD7	siCHD7
CDH9	Ectoderm	0.01	0.07	0.00
COL2A1	Ectoderm	0.13	0.17	0.05
DMBX1	Ectoderm	0.07	0.34	0.10
DRD4	Ectoderm	0.46	0.22	0.28
EN1	Ectoderm	0.29	1.08	0.30
LMX1A	Ectoderm	0.06	0.10	0.05
MAP2	Ectoderm	1.53	10.66	2.43
MYO3B	Ectoderm	0.70	0.76	0.48
NOS2	Ectoderm	0.78	0.94	0.72
NR2F1/NR2F2	Ectoderm	0.02	0.29	0.02
NR2F2	Ectoderm	0.14	0.32	0.17
OLFM3	Ectoderm	0.36	0.93	0.61
PAPLN	Ectoderm	0.34	0.14	0.16
PAX3	Ectoderm	0.15	0.47	0.16
PAX6	Ectoderm	0.06	0.35	0.11
POU4F1	Ectoderm	0.49	0.57	0.31
PRKCA	Ectoderm	0.28	0.68	0.30
SDC2	Ectoderm	16.02	27.47	17.79
SOX1	Ectoderm	0.41	0.15	0.30
TRPM8	Ectoderm	0.92	0.02	1.03
WNT1	Ectoderm	0.33	3.85	0.04
ZBTB16	Ectoderm	0.26	0.35	0.21

Target Name	Category	mock	mCHD7	siCHD7
ABCA4	Mesoderm	0.49	2.23	0.29
ALOX15	Mesoderm	2.43	1.10	2.63
BMP10	Mesoderm	0.04	2.34	1.43
CDH5	Mesoderm	0.37	18.20	0.73
CDX2	Mesoderm	0.14	1.40	0.08
COLEC10	Mesoderm	0.96	1.07	0.57
ESM1	Mesoderm	0.73	0.70	0.79
FCN3	Mesoderm	0.84	0.57	0.56
FOXF1	Mesoderm	0.63	2.44	0.69
HAND1	Mesoderm	0.02	5.63	0.06
HAND2	Mesoderm	0.22	4.44	0.34
HEY1	Mesoderm	0.81	0.65	0.96
HOPX	Mesoderm	3.91	1.55	0.31
IL6ST	Mesoderm	0.59	4.08	0.82
NKX2-5	Mesoderm	1.73	8.98	1.52
ODAM	Mesoderm	0.04	6.38	0.35
PDGFRA	Mesoderm	1.35	0.58	1.10
PLVAP	Mesoderm	1.15	0.35	0.77
RGS4	Mesoderm	0.14	1.65	0.10
SNAI2	Mesoderm	0.22	0.68	0.46
TBX3	Mesoderm	0.21	4.93	0.39
TM4SF1	Mesoderm	0.11	0.73	0.22
FGF4	Mesendoderm	14.27	3.24	3.79
GDF3	Mesendoderm	13.73	14.41	11.49
NPPB	Mesendoderm	0.45	13.67	1.13
NR5A2	Mesendoderm	96.08	159.46	105.36
PTHLH	Mesendoderm	0.46	22.26	1.06
T	Mesendoderm	0.00	0.00	0.00

Target Name	Category	mock	mCHD7	siCHD7
AFP	Endoderm	0.00	0.06	0.02
CABP7	Endoderm	1.05	0.71	1.02
CDH20	Endoderm	0.38	1.45	0.49
CLDN1	Endoderm	1.86	5.01	2.19
CPLX2	Endoderm	1.72	0.55	2.05
ELAVL3	Endoderm	2.93	1.09	1.26
EOMES	Endoderm	0.02	0.23	0.01
FOXA1	Endoderm	0.04	0.39	0.08
FOXA2	Endoderm	0.01	0.04	0.03
FOXP2	Endoderm	0.17	0.78	0.15
GATA4	Endoderm	0.02	0.11	0.02
GATA6	Endoderm	0.03	1.96	0.04
HHEX	Endoderm	0.24	0.40	0.07
HMP19	Endoderm	1.48	0.65	1.07
HNFB1	Endoderm	0.01	0.08	0.01
HNFB4	Endoderm	0.02	0.56	0.00
KLF5	Endoderm	1.37	9.88	2.04
LEFTY1	Endoderm	0.75	0.93	0.54
LEFTY2	Endoderm	0.21	0.59	0.26
NODAL	Endoderm	0.98	1.29	1.26
PHOX2B	Endoderm	0.02	0.03	0.02
POU3F3	Endoderm	0.03	0.12	0.03
PRDM1	Endoderm	0.15	0.85	0.16
RXRG	Endoderm	0.02	2.05	0.01
SOX17	Endoderm	0.05	0.47	0.00
SST	Endoderm	4.63	11.65	5.67

Target Name	Category	mock	mCHD7	siCHD7
CXCL5	Self-renewal	9.06	17.59	14.54
DNMT3B	Self-renewal	1.51	0.54	1.29
HESX1	Self-renewal	0.24	0.16	0.27
IDO1	Self-renewal	1.09	0.17	0.65
LCK	Self-renewal	1.13	0.74	0.88
NANOG	Self-renewal	2.77	2.12	2.03
POU5F1	Self-renewal	2.83	1.11	1.59
SOX2	Self-renewal	0.00	0.05	0.42
TRIM22	Self-renewal	10.81	30.29	8.67

Fold change legend

fc > 100	Upregulated
10 < fc <= 100	
2 < fc <= 10	
0.5 <= fc <= 2	Comparable
0.1 <= fc < 0.5	
0.01 <= fc < 0.1	
fc < 0.01	Downregulated
omitted	

Expressions of 96 genes categorized as ectoderm, mesoderm, mesendoderm, endoderm and self-renewal by TaqMan hPSC Scorecard assay are shown in the Table.