

Supplementary Information

Differentiation potential of Pluripotent Stem Cells correlates to the level of CHD7

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KhES-1 Score card 96 Genes

| Target Name | Category | undiff Es8 | EBs Es8 | undiff RFF2 | EBs RFF2 | undiff Es8 2nd | EBs Es8 2nd |
|-------------|----------|------------|---------|-------------|----------|----------------|-------------|
| CDH9 | Ectoderm | 0.06 | 0.76 | 0.02 | 0.04 | 0.23 | 0.92 |
| COL2A1 | Ectoderm | 1.17 | 2.46 | 0.31 | 0.29 | 3.31 | 2.15 |
| DMB1 | Ectoderm | 0.14 | 17.14 | 0.16 | 0.07 | 0.05 | 2.25 |
| DRD4 | Ectoderm | 0.08 | 0.18 | 0.29 | 0.28 | 0.04 | 1.01 |
| EN1 | Ectoderm | 0.45 | 14.79 | 0.60 | 0.97 | 0.21 | 7.75 |
| LMNA | Ectoderm | 0.00 | 254.88 | 0.07 | 0.05 | 0.00 | 125.81 |
| MPF2 | Ectoderm | 3.90 | 58.08 | 3.14 | 0.23 | 2.27 | 21.90 |
| MYO3B | Ectoderm | 0.58 | 0.03 | 0.67 | 0.44 | 0.12 | 0.19 |
| NOS2 | Ectoderm | 0.97 | 2.06 | 0.50 | 0.34 | 0.51 | 9.09 |
| NR2F1/NR2F2 | Ectoderm | 0.03 | 543.33 | 0.00 | 0.00 | 0.00 | 777.76 |
| NR2F2 | Ectoderm | 0.04 | 25.37 | 0.43 | 0.17 | 0.07 | 18.38 |
| OLFBP3 | Ectoderm | 0.70 | 94.61 | 0.31 | 0.11 | 0.28 | 180.28 |
| PARLN | Ectoderm | 0.82 | 0.02 | 0.23 | 0.27 | 0.63 | 0.00 |
| PAK3 | Ectoderm | 0.05 | 54.90 | 0.10 | 0.07 | 0.07 | 25.41 |
| PAW3 | Ectoderm | 0.05 | 313.64 | 0.12 | 0.03 | 0.06 | 199.72 |
| POU4F1 | Ectoderm | 1.75 | 122.25 | 0.22 | 0.60 | 0.30 | 180.48 |
| PRKCA | Ectoderm | 0.70 | 2.61 | 0.55 | 0.43 | 0.37 | 1.33 |
| SDC2 | Ectoderm | 0.49 | 13.23 | 18.16 | 3.24 | 0.69 | 8.50 |
| SOX1 | Ectoderm | 0.05 | 0.46 | 0.07 | 0.11 | 0.02 | 0.92 |
| TRPM8 | Ectoderm | 0.20 | 49.15 | 0.49 | 0.72 | 1.03 | 18.82 |
| WNT1 | Ectoderm | 0.30 | 1.21.13 | 1.23 | 1.08 | 0.03 | 656.31 |
| ZBTB16 | Ectoderm | 0.20 | 150.04 | 0.20 | 0.25 | 0.08 | 224.99 |

| Target Name | Category | undiff Es8 | EBs Es8 | undiff RFF2 | EBs RFF2 | undiff Es8 2nd | EBs Es8 2nd |
|-------------|--------------|------------|---------|-------------|----------|----------------|-------------|
| CXCL5 | Self-renewal | 7.04 | 0.01 | 8.39 | 4.17 | 6.59 | 0.02 |
| DNMT3B | Self-renewal | 0.83 | 0.02 | 1.18 | 1.24 | 0.55 | 0.03 |
| HESX1 | Self-renewal | 0.41 | 0.01 | 0.24 | 0.43 | 0.77 | 0.01 |
| IDO1 | Self-renewal | 0.88 | 0.00 | 0.67 | 1.83 | 0.77 | 0.00 |
| LCK | Self-renewal | 2.29 | 0.00 | 1.30 | 0.70 | 1.22 | 0.00 |
| NANOG | Self-renewal | 1.02 | 0.02 | 2.93 | 4.06 | 1.41 | 0.00 |
| POU5F1 | Self-renewal | 0.87 | 0.03 | 0.87 | 1.01 | 0.68 | 0.01 |
| SOX2 | Self-renewal | 0.75 | 0.21 | 0.73 | 0.95 | 1.15 | 0.28 |
| TRIM2 | Self-renewal | 3.62 | 0.20 | 12.91 | 4.46 | 2.44 | 0.25 |

| Target Name | Category | undiff Es8 | EBs Es8 | undiff RFF2 | EBs RFF2 | undiff Es8 2nd | EBs Es8 2nd |
|-------------|----------|------------|---------|-------------|----------|----------------|-------------|
| ABC44 | Mesoderm | 0.67 | 79.03 | 1.10 | 0.81 | 0.62 | 15.88 |
| ALOX15 | Mesoderm | 1.28 | 2.30 | 2.29 | 0.76 | 0.58 | 2.80 |
| BMP10 | Mesoderm | 1.20 | 0.10 | 1.89 | 0.32 | 0.92 | 0.10 |
| CDH5 | Mesoderm | 2.40 | 6.17 | 1.34 | 0.39 | 0.77 | 6.47 |
| CDX2 | Mesoderm | 0.20 | 0.69 | 0.37 | 0.73 | 0.05 | 0.04 |
| COLLEC10 | Mesoderm | 0.85 | 7.65 | 0.72 | 1.66 | 0.25 | 12.33 |
| ESM1 | Mesoderm | 0.84 | 1139.26 | 1.92 | 2.09 | 0.67 | 946.39 |
| FGN3 | Mesoderm | 1.20 | 0.01 | 1.27 | 0.47 | 0.32 | 0.55 |
| FOXP1 | Mesoderm | 0.21 | 3.00 | 0.65 | 0.39 | 0.08 | 3.23 |
| HAND1 | Mesoderm | 0.03 | 0.02 | 0.08 | 0.03 | 0.00 | 0.20 |
| HAND2 | Mesoderm | 0.92 | 0.66 | 0.20 | 0.25 | 0.68 | 2.17 |
| HEY1 | Mesoderm | 1.38 | 27.06 | 0.50 | 0.35 | 1.33 | 53.95 |
| HOPX | Mesoderm | 0.28 | 2.40 | 2.50 | 2.62 | 1.70 | 2.24 |
| IL6ST | Mesoderm | 1.04 | 12.52 | 0.89 | 0.77 | 0.81 | 9.77 |
| NKX2.5 | Mesoderm | 0.45 | 0.44 | 1.97 | 3.29 | 0.43 | 1.56 |
| ODAM | Mesoderm | 0.96 | 4.62 | 1.31 | 0.17 | 0.25 | 3.93 |
| PDGFRA | Mesoderm | 0.20 | 0.81 | 0.81 | 0.42 | 0.59 | 0.16 |
| PLUP | Mesoderm | 0.53 | 3.42 | 1.30 | 0.54 | 0.29 | 1.12 |
| RGSA | Mesoderm | 0.14 | 22.32 | 0.35 | 0.21 | 0.10 | 11.72 |
| SNAP2 | Mesoderm | 0.32 | 19.84 | 0.50 | 0.16 | 0.25 | 13.91 |
| TBQ3 | Mesoderm | 0.92 | 6.54 | 1.03 | 0.09 | 0.01 | 11.16 |
| TMSF1 | Mesoderm | 0.92 | 10.64 | 0.34 | 0.14 | 0.97 | 14.80 |
| FGF4 | Mesoderm | 0.15 | 0.00 | 13.69 | 17.75 | 0.34 | 0.00 |
| GDF3 | Mesoderm | 4.79 | 0.00 | 29.48 | 38.14 | 4.50 | 0.00 |
| NFPB | Mesoderm | 0.02 | 0.01 | 2.09 | 0.16 | 0.16 | 0.02 |
| NRS2A2 | Mesoderm | 181.40 | 113.46 | 134.06 | 70.15 | 171.16 | 84.35 |
| PTHLH | Mesoderm | 0.20 | 26.07 | 0.74 | 0.68 | 2.72 | 4.67 |
| T | Mesoderm | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| Target Name | Category | undiff Es8 | EBs Es8 | undiff RFF2 | EBs RFF2 | undiff Es8 2nd | EBs Es8 2nd |
|-------------|----------|------------|---------|-------------|----------|----------------|-------------|
| 4F7 | Endoderm | 0.01 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 |
| CAEP7 | Endoderm | 0.15 | 5.89 | 2.44 | 8.69 | 0.18 | 1.87 |
| CDH20 | Endoderm | 0.17 | 50.55 | 0.29 | 0.20 | 0.13 | 159.58 |
| CLDN1 | Endoderm | 1.26 | 105.08 | 1.59 | 0.97 | 0.63 | 73.47 |
| CPLX2 | Endoderm | 0.72 | 17.27 | 1.63 | 2.75 | 0.54 | 21.62 |
| ELAM3 | Endoderm | 0.49 | 46.43 | 1.40 | 2.07 | 0.37 | 64.46 |
| ECMES | Endoderm | 0.04 | 1.36 | 0.03 | 0.03 | 0.02 | 1.75 |
| FOXA1 | Endoderm | 0.06 | 0.14 | 0.12 | 0.01 | 0.05 | 0.10 |
| FOXA2 | Endoderm | 2.42 | 0.01 | 0.01 | 0.07 | 2.54 | 0.01 |
| FOXP2 | Endoderm | 0.27 | 12.97 | 0.05 | 0.18 | 0.13 | 16.40 |
| GATA4 | Endoderm | 0.01 | 0.00 | 0.01 | 0.03 | 0.00 | 0.00 |
| GATA6 | Endoderm | 0.00 | 0.00 | 0.05 | 0.62 | 0.01 | 0.01 |
| HHEX | Endoderm | 0.17 | 0.09 | 0.16 | 0.35 | 0.06 | 0.01 |
| HMP19 | Endoderm | 0.66 | 255.83 | 0.58 | 0.55 | 0.54 | 121.00 |
| HNF1B | Endoderm | 0.17 | 0.00 | 0.01 | 0.01 | 0.02 | 0.06 |
| HNF4A | Endoderm | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.07 |
| KLF5 | Endoderm | 0.40 | 2.88 | 3.02 | 1.44 | 0.76 | 1.42 |
| LEFTY1 | Endoderm | 0.01 | 0.00 | 0.31 | 0.26 | 0.28 | 0.00 |
| LEFTY2 | Endoderm | 0.01 | 0.00 | 0.15 | 0.20 | 0.69 | 0.00 |
| NODAL | Endoderm | 0.04 | 0.01 | 0.70 | 1.10 | 0.23 | 0.01 |
| PHOX2B | Endoderm | 0.03 | 3.01 | 0.06 | 0.02 | 0.02 | 1.17 |
| POU3F3 | Endoderm | 0.05 | 18.13 | 0.07 | 0.04 | 0.03 | 26.48 |
| PRDM1 | Endoderm | 0.12 | 0.18 | 0.10 | 0.19 | 0.04 | 0.36 |
| RXRG | Endoderm | 0.19 | 321.89 | 0.12 | 0.17 | 0.00 | 316.58 |
| SOX17 | Endoderm | 0.01 | 0.01 | 0.05 | 0.03 | 0.01 | 0.01 |
| SST | Endoderm | 0.71 | 423.43 | 10.12 | 3.16 | 1.27 | 1,809.87 |

| 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 | |

Figure S1. Gene expression of a subset of genes in ESC in the undifferentiated state and in EBs determined by qRT-PCR.

The expression of 96 genes in KhES-1 categorized as Self-renewal, Ectoderm-, Mesoderm-, Mesendoderm- or Endoderm-lineage before or after EB formation were determined by the qRT-PCR scorecard panel. Notably, genes related to self-renewal were not downregulated nor were those related to differentiation lineages upregulated in EBs generated from KhES-1 cultured with RFF2. undiff Es8: undifferentiated KhES-1 culture with Es8. EBs Es8: EBs from KhES-1 cultured with Es8. undiff RFF2: undifferentiated KhES-1 cultured with RFF2. EBs RFF2: EBs from KhES-1 cultured with RFF2. undiff Es8 2nd: undifferentiated KhES-1 culture with Es8 again after transferred from RFF2 medium. EBs Es8 2nd: EBs from KhES-1 cultured with Es8 again after transferred from RFF2 medium.

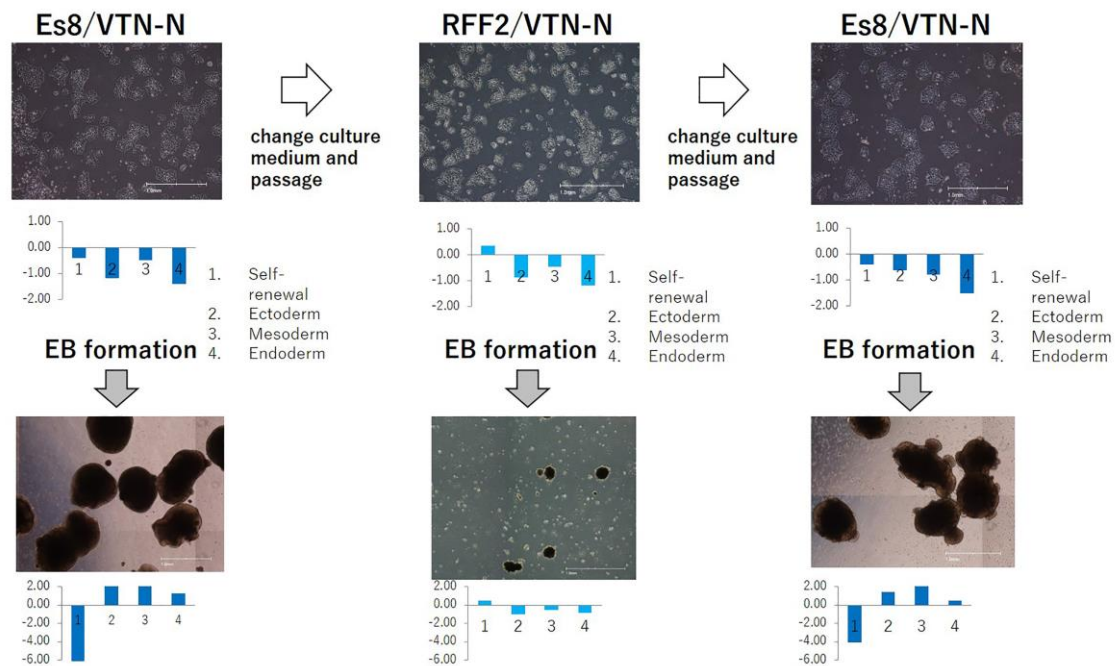


Figure S2. The differentiation potential of iPSCs was altered by culture conditions. The potential for EB formation by iPSCs can be altered reversibly by the culture conditions. iPSCs (PFX#9) in single-cell suspensions were seeded on VNT-N-coated dishes and cultured with Es8 medium (left, upper photograph) for 5 passages. The cells were then collected for EB formation assays (left, lower photograph) or transferred to Repro FF2 medium (RFF2, middle upper photograph). PFX#9 cells were cultured for 5 passages and collected for EB formation assays (middle, lower photo) or transferred to Es8 medium again (right upper photograph). PFX#9 cells were cultured for 5 passages, followed by EB formation assays (right, lower photograph). Photographs of PFX#9 cultures with Es8 or RFF2 (upper panels) at day 1 of culture and EBs on day 14 in EB formation assays (lower panels). Gene expression profiles in cells in the indicated culture conditions were determined using a qRT-PCR scorecard panel and appended below the relevant photograph. Scale bar: 1.0 mm.

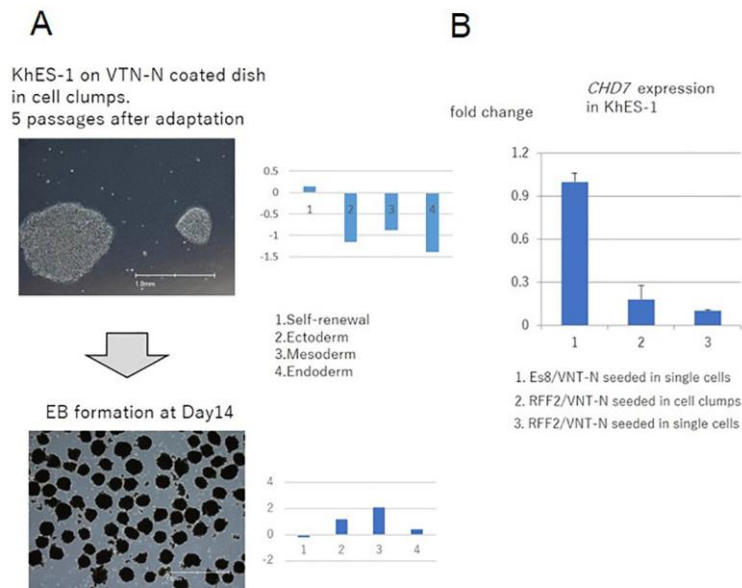
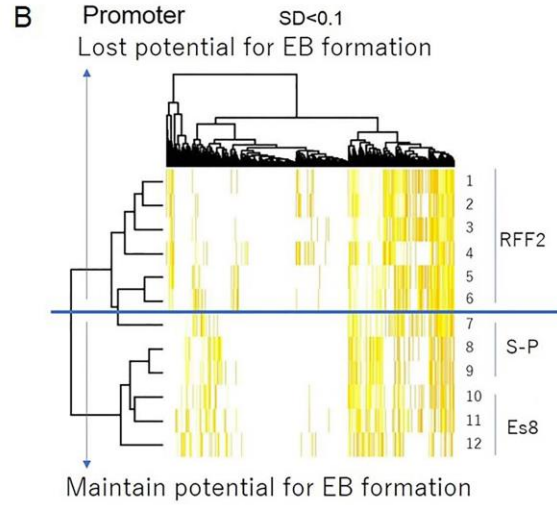
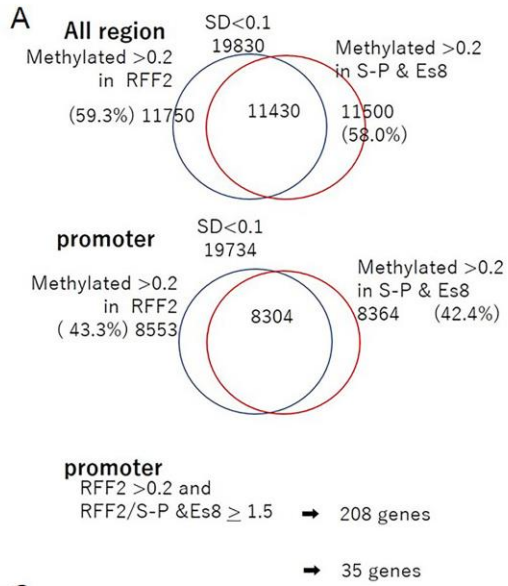


Figure S3. Culture condition alters the differentiation potential of ESC. KhES-1 cells cultured on feeder were transferred to vitronectin-N (VNT-N)-coated dish in cell clumps and cultured for 5 passages for adaptation. The differentiation potential of cells was verified by EB formation assay. A: photos of KhES-1 cells in clumps before EB formation assay and its gene expression profile determined by qRT-PCR scorecard panel are appended in the right (Upper panels). Photos of Day 14-EBs and its genetic profile by scorecard panel (left panels). B. Expression of *CHD7* determined by qRT-PCR in designated culture conditions while maintained in the undifferentiated state (n=3 analytical replicates).



C

self-renewal

| GENE_SYMBOL | RFF2 | S-P&Es8 | Ratio |
|-------------|------|---------|-------|
| KLF4 | 0.02 | 0.02 | 1.02 |
| KLF2 | 0.21 | 0.19 | 1.12 |
| TFCP2L1 | 0.06 | 0.06 | 0.97 |
| SOX2 | 0.02 | 0.02 | 0.94 |
| EP300(p300) | 0.04 | 0.04 | 0.85 |
| ZFP42(REX1) | 0.03 | 0.07 | 0.43 |
| NANOG | 0.36 | 0.23 | 1.58 |
| POU5F1 | 0.26 | 0.36 | 0.71 |
| HESX1 | 0.64 | 0.59 | 1.08 |
| LCK | 0.73 | 0.69 | 1.07 |
| DNMT3B | 0.69 | 0.71 | 0.97 |
| TRIM22 | 0.88 | 0.89 | 0.98 |
| IDO1 | 0.92 | 0.93 | 0.99 |

ectoderm

| GENE_SYMBOL | RFF2 | S-P&Es8 | Ratio |
|-------------|------|---------|-------|
| COL2A1 | 0.04 | 0.04 | 0.98 |
| DRD4 | 0.04 | 0.06 | 0.78 |
| EN1 | 0.07 | 0.05 | 1.39 |
| LMX1A | 0.03 | 0.03 | 1.09 |
| NR2F1 | 0.04 | 0.03 | 1.29 |
| NR2F2 | 0.08 | 0.06 | 1.32 |
| OLFM3 | 0.08 | 0.07 | 1.14 |
| PAPLN | 0.10 | 0.11 | 0.91 |
| PAX3 | 0.05 | 0.04 | 1.45 |
| PAX6 | 0.03 | 0.03 | 1.00 |
| POU4F1 | 0.03 | 0.03 | 1.08 |
| PRKCA | 0.03 | 0.03 | 0.97 |
| SDC2 | 0.04 | 0.03 | 1.17 |
| SOX1 | 0.06 | 0.04 | 1.47 |
| WNT1 | 0.11 | 0.09 | 1.27 |
| ZBTB16 | 0.06 | 0.05 | 1.22 |
| CDH9 | 0.35 | 0.25 | 1.42 |
| LMX1A | 0.84 | 0.88 | 1.01 |
| DMBX1 | 0.82 | 0.83 | 0.99 |
| TRPM8 | 0.89 | 0.88 | 1.01 |
| NOS2 | 0.89 | 0.90 | 1.00 |
| MYO3B | 0.92 | 0.93 | 0.99 |

mesoderm

| GENE_SYMBOL | RFF2 | S-P&Es8 | Ratio |
|-------------|------|---------|-------|
| ALOX15 | 0.12 | 0.11 | 1.10 |
| CDX2 | 0.03 | 0.02 | 1.14 |
| FOXF1 | 0.04 | 0.03 | 1.28 |
| HAND1 | 0.03 | 0.03 | 1.06 |
| HAND2 | 0.05 | 0.03 | 1.56 |
| HEY1 | 0.01 | 0.02 | 0.86 |
| IL6ST | 0.04 | 0.04 | 1.02 |
| NKX2-5 | 0.10 | 0.04 | 2.75 |
| PDGFRA | 0.04 | 0.03 | 1.09 |
| SNAI2 | 0.05 | 0.03 | 1.35 |
| TBX3 | 0.03 | 0.03 | 0.98 |
| RGS4 | 0.33 | 0.19 | 1.71 |
| HOPX | 0.22 | 0.22 | 1.02 |
| ESM1 | 0.79 | 0.82 | 0.97 |
| CDH5 | 0.85 | 0.84 | 1.02 |
| TMS6F1 | 0.85 | 0.86 | 0.99 |
| PLVAP | 0.87 | 0.90 | 0.98 |
| ABCA4 | 0.89 | 0.90 | 0.98 |
| FCN3 | 0.90 | 0.91 | 0.98 |
| BMP10 | 0.92 | 0.92 | 1.00 |
| COLEC10 | 0.94 | 0.94 | 1.00 |

mesendoderm

| GENE_SYMBOL | RFF2 | S-P&Es8 | Ratio |
|-------------|------|---------|-------|
| FGF4 | 0.05 | 0.05 | 0.89 |
| NPPB | 0.14 | 0.09 | 1.44 |
| PTHLH | 0.03 | 0.03 | 1.18 |
| T | 0.03 | 0.03 | 1.18 |
| GDF3 | 0.80 | 0.83 | 0.97 |
| NR5A2 | 0.91 | 0.92 | 0.99 |

endoderm

| GENE_SYMBOL | RFF2 | S-P&Es8 | Ratio |
|-------------|------|---------|-------|
| CABP7 | 0.07 | 0.08 | 0.85 |
| CLDN1 | 0.03 | 0.03 | 1.01 |
| CPLX2 | 0.13 | 0.10 | 1.29 |
| EOMES | 0.07 | 0.04 | 1.66 |
| FOXA1 | 0.03 | 0.03 | 1.06 |
| FOXA2 | 0.04 | 0.04 | 0.98 |
| GATA4 | 0.04 | 0.03 | 1.13 |
| GATA6 | 0.02 | 0.02 | 0.91 |
| HHEX | 0.03 | 0.04 | 0.86 |
| HMP19 | 0.09 | 0.07 | 1.38 |
| HNF1B | 0.03 | 0.03 | 1.22 |
| KLF5 | 0.03 | 0.03 | 1.04 |
| NODAL | 0.06 | 0.07 | 0.83 |
| PHOX2B | 0.09 | 0.05 | 1.75 |
| POU3F3 | 0.03 | 0.03 | 1.04 |
| PRDM1 | 0.03 | 0.03 | 0.94 |
| SOX17 | 0.04 | 0.03 | 1.35 |
| ELAVL3 | 0.25 | 0.21 | 1.16 |
| SST | 0.42 | 0.40 | 1.05 |
| LEFTY1 | 0.70 | 0.74 | 0.96 |
| FOXP2 | 0.83 | 0.78 | 1.06 |
| LEFTY2 | 0.84 | 0.81 | 1.03 |
| HNF4A | 0.91 | 0.90 | 1.01 |
| CDH20 | 0.91 | 0.93 | 0.98 |
| AFP | 0.92 | 0.94 | 0.98 |

Figure S4. Methylation comparison study of PSCs cultured under various conditions

- A. The methylation status of the cells was determined with an Illumina Human Methylation Bead Chip. Average methylation scores from 6 PSC samples cultured with RFF2 (3 iPSC [PFX#9] samples and 3 ESC [KhES-1] samples). Scores were compared with the average of 6 PSC samples in S-P or Es8 medium (1 sample of PFX#9 with S-P; 1 sample of KhES-1 with S-P; 1 sample of H9 with S-P; 1 sample of PFX#9 with Es8; 2 samples of H9 with Es8). The number of gene promoter regions or all regions whose methylation scores exceeded 0.2 by culturing with RFF2 or S-P and Es8 is displayed in a Venn diagram.
- B. Clustering of methylation patterns in the promoter region of PSCs cultured with RFF2, S-P, or Es8 medium. Lanes #1–3: iPSCs (PFX#9) cultured with RFF2 medium. Lanes #4–6: ESCs (KhES-1) cultured with RFF2 from 6 independent experiments. Lane #7: PFX#9 cells with S-P. Lane #8: KhES-1 cells with S-P. Lane #9: H9 ESCs with S-P. Lane #10: PFX#9 cells with Es8. Lanes #11, 12: H9 cells with Es8 from 6 independent experiments.
- C. Table of methylation status of promoters of representative genes for self-renewal, ectoderm, mesoderm, mesendoderm, and endoderm lineage in PSCs cultured with RFF2, S-P, or Es8 medium. The average methylation status in 6 PSCs (3 iPSCs plus 3 ESCs) cultured with RFF2 or 6 PSCs (1 iPSC sample and 2 ESC samples with S-P and 1 iPSC sample and 2 ESCs with Es8) cultured with S-P or Es8 (S-P & Es8) are shown in the table. All cells were maintained in the undifferentiated state. Methylation status defined as follow: hypomethylated, < 0.2 ; moderate, 0.2 to < 0.5 ; hypermethylated, > 0.5 . Shown in blue, yellow, and orange columns, respectively.

| Target Name | Category | Day 4 | | | Day 5 | | | Day 14 | | | Target Name | Category | Day 4 | | | Day 5 | | | Day 14 | | |
|-------------|----------|-------------------------|--------|-------|-------------------------|--------|----------|-------------------------|--------|--------|--------------|--------------|-------------------------|--------|-------|-------------------------|--------|------|-------------------------|--------|----------|
| | | Non-transfected control | siCHD7 | mock | Non-transfected control | siCHD7 | mock | Non-transfected control | siCHD7 | mock | | | Non-transfected control | siCHD7 | mock | Non-transfected control | siCHD7 | mock | Non-transfected control | siCHD7 | mock |
| CD49 | Ectoderm | 21.84 | 6.58 | 24.33 | 12.24 | 14.17 | 15.60 | 0.75 | 9.90 | 0.92 | APP | Endoderm | 0.00 | 0.06 | 0.02 | 0.01 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 |
| COL2A1 | Ectoderm | 0.27 | 0.30 | 0.43 | 21.39 | 1.75 | 4.02 | 2.46 | 16.59 | 2.15 | CABP7 | Endoderm | 14.04 | 10.74 | 11.74 | 8.53 | 6.08 | 5.30 | 5.89 | 0.65 | 1.87 |
| DMB1 | Ectoderm | 59.16 | 27.86 | 84.61 | 98.25 | 76.12 | 86.97 | 17.14 | 47.83 | 2.25 | CDH20 | Endoderm | 4.53 | 0.83 | 2.71 | 8.97 | 2.30 | 4.72 | 50.55 | 8.42 | 158.58 |
| DRD4 | Ectoderm | 1.87 | 0.94 | 1.23 | 0.86 | 1.59 | 1.54 | 0.18 | 7.08 | 1.01 | GLI1H1 | Endoderm | 0.55 | 1.91 | 1.40 | 1.40 | 0.51 | 1.02 | 165.08 | 3.26 | 73.47 |
| EN1 | Ectoderm | 11.68 | 14.37 | 28.77 | 273.00 | 10.30 | 23.02 | 14.78 | 156.84 | 7.75 | GPIX2 | Endoderm | 2.31 | 2.48 | 1.02 | 1.66 | 1.01 | 1.23 | 17.27 | 0.48 | 23.62 |
| LMX1A | Ectoderm | 15.69 | 3.63 | 15.28 | 27.40 | 31.49 | 38.62 | 254.88 | 25.25 | 126.91 | ELAVL3 | Endoderm | 0.92 | 0.79 | 0.74 | 1.15 | 0.48 | 0.62 | 46.43 | 0.83 | 64.46 |
| MAP2 | Ectoderm | 5.59 | 3.46 | 4.10 | 12.01 | 11.69 | 13.90 | 56.08 | 18.50 | 21.90 | ECMES | Endoderm | 0.08 | 0.17 | 0.22 | 2.35 | 0.11 | 0.30 | 1.36 | 0.01 | 1.75 |
| MYO3B | Ectoderm | 9.04 | 2.22 | 8.67 | 9.81 | 8.11 | 11.68 | 30.83 | 99.81 | 0.19 | FOXA1 | Endoderm | 0.74 | 0.52 | 1.95 | 1.41 | 0.73 | 1.11 | 0.14 | 2.93 | 0.10 |
| NOS2 | Ectoderm | 0.16 | 0.32 | 0.07 | 4.86 | 2.49 | 10.81 | 2.06 | 149.85 | 9.09 | FOXA2 | Endoderm | 0.08 | 0.20 | 0.16 | 0.45 | 0.08 | 0.11 | 0.01 | 0.02 | 0.01 |
| NR2F1NR2F2 | Ectoderm | 0.10 | 0.05 | 0.08 | 67.72 | 3.59 | 14.40 | 543.33 | 91.65 | 777.76 | FOXP2 | Endoderm | 1.39 | 1.42 | 3.52 | 3.15 | 0.66 | 0.67 | 12.97 | 1.37 | 16.40 |
| NR2F2 | Ectoderm | 15.79 | 2.66 | 9.75 | 9.54 | 13.92 | 30.12 | 25.37 | 157.89 | 16.38 | GATA4 | Endoderm | 0.04 | 0.09 | 0.09 | 1.05 | 0.17 | 0.17 | 0.00 | 0.00 | 0.00 |
| OLFM3 | Ectoderm | 11.37 | 6.63 | 23.46 | 48.67 | 29.47 | 36.91 | 94.01 | 49.54 | 165.29 | GATA6 | Endoderm | 0.01 | 0.03 | 0.02 | 0.15 | 0.01 | 0.02 | 0.00 | 0.03 | 0.01 |
| PAPLN | Ectoderm | 2.47 | 1.71 | 1.47 | 1.34 | 1.45 | 0.97 | 0.02 | 1.98 | 0.00 | HHEX | Endoderm | 1.04 | 0.51 | 0.72 | 0.23 | 0.43 | 0.25 | 0.09 | 0.04 | 0.01 |
| PAX3 | Ectoderm | 31.41 | 2.36 | 34.57 | 219.52 | 159.73 | 349.60 | 54.90 | 211.02 | 25.41 | HMP19 | Endoderm | 0.20 | 3.07 | 3.76 | 2.83 | 0.18 | 0.22 | 255.83 | 2.28 | 121.00 |
| PAX6 | Ectoderm | 0.50 | 0.50 | 2.63 | 7.50 | 15.46 | 21.43 | 313.84 | 307.88 | 199.72 | HNF1B | Endoderm | 0.07 | 0.01 | 0.13 | 0.15 | 0.01 | 0.02 | 0.00 | 0.01 | 0.06 |
| POU4F1 | Ectoderm | 3.12 | 8.97 | 10.40 | 22.88 | 0.48 | 7.71 | 122.25 | 123.41 | 189.46 | HNF4A | Endoderm | 0.06 | 0.01 | 0.23 | 0.25 | 0.07 | 0.12 | 0.02 | 0.11 | 0.07 |
| PRKCA | Ectoderm | 0.54 | 0.43 | 0.33 | 0.87 | 0.47 | 0.49 | 2.61 | 0.31 | 1.33 | KLIF5 | Endoderm | 0.91 | 2.36 | 1.79 | 1.60 | 1.40 | 1.81 | 2.88 | 0.44 | 1.42 |
| SDC2 | Ectoderm | 11.85 | 10.25 | 14.39 | 17.14 | 19.91 | 18.58 | 13.23 | 21.92 | 8.50 | LEFTY1 | Endoderm | 0.01 | 0.06 | 0.01 | 0.06 | 0.01 | 0.02 | 0.00 | 0.01 | 0.00 |
| SOX1 | Ectoderm | 0.03 | 0.47 | 0.17 | 0.57 | 0.14 | 0.35 | 0.46 | 2.33 | 0.92 | LEFTY2 | Endoderm | 0.02 | 0.11 | 0.04 | 0.23 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 |
| TRPM8 | Ectoderm | 27.67 | 3.50 | 13.04 | 10.55 | 8.69 | 15.14 | 49.15 | 25.94 | 16.82 | NODAL | Endoderm | 0.06 | 0.45 | 0.16 | 0.60 | 0.16 | 0.25 | 0.01 | 0.01 | 0.01 |
| WN11 | Ectoderm | 86.76 | 15.67 | 87.34 | 1,534.88 | 539.36 | 689.73 | 1,171.13 | 868.42 | 656.31 | PHOX2B | Endoderm | 0.10 | 0.33 | 0.64 | 0.28 | 0.49 | 0.17 | 3.01 | 0.02 | 1.17 |
| ZBTB16 | Ectoderm | 0.54 | 2.57 | 1.62 | 31.61 | 8.27 | 27.43 | 150.04 | 189.18 | 224.99 | POU3F3 | Endoderm | 0.19 | 0.28 | 0.73 | 1.75 | 0.19 | 0.47 | 18.13 | 1.28 | 26.48 |
| ABC4 | Mesoderm | 5.12 | 3.45 | 6.40 | 8.25 | 3.44 | 7.95 | 79.03 | 24.07 | 15.98 | PRDM1 | Endoderm | 1.48 | 1.82 | 2.95 | 1.34 | 2.10 | 1.72 | 0.18 | 0.45 | 0.36 |
| ALOX15 | Mesoderm | 1.34 | 0.88 | 0.90 | 0.82 | 1.60 | 0.78 | 2.30 | 0.45 | 2.80 | RXRG | Endoderm | 1.68 | 1.29 | 5.33 | 7.87 | 3.31 | 3.96 | 391.89 | 3.87 | 316.34 |
| BMP10 | Mesoderm | 0.21 | 6.83 | 8.38 | 0.58 | 5.36 | 0.36 | 0.10 | 0.26 | 0.10 | SOX17 | Endoderm | 0.03 | 0.11 | 0.20 | 1.15 | 0.07 | 0.03 | 0.01 | 0.04 | 0.01 |
| CDH5 | Mesoderm | 1.60 | 2.48 | 0.43 | 5.15 | 3.98 | 3.61 | 6.17 | 1.00 | 6.47 | SST | Endoderm | 1.40 | 0.93 | 5.57 | 33.25 | 0.50 | 5.64 | 423.45 | 5.49 | 1,809.87 |
| CDX2 | Mesoderm | 1.10 | 4.32 | 4.26 | 5.02 | 0.45 | 0.99 | 9.88 | 1.97 | 3.04 | CXCL5 | Self-renewal | 1.72 | 2.16 | 1.19 | 0.79 | 0.62 | 0.55 | 0.01 | 0.03 | 0.02 |
| COLEC10 | Mesoderm | 0.52 | 0.48 | 0.53 | 6.55 | 8.69 | 2.96 | 7.65 | 1.08 | 12.33 | DNM1B | Self-renewal | 0.67 | 0.58 | 0.58 | 0.43 | 0.44 | 0.32 | 0.02 | 0.08 | 0.03 |
| ESM1 | Mesoderm | 0.06 | 3.06 | 0.36 | 0.16 | 1.14 | 1,138.26 | 1.59 | 845.79 | HESX1 | Self-renewal | 1.09 | 0.27 | 0.67 | 0.31 | 0.76 | 0.57 | 0.01 | 0.33 | 0.01 | |
| FCM3 | Mesoderm | 0.52 | 0.72 | 0.10 | 2.17 | 4.75 | 1.39 | 0.01 | 0.10 | 0.55 | IDO1 | Self-renewal | 0.49 | 0.42 | 0.24 | 0.14 | 0.17 | 0.10 | 0.00 | 0.00 | 0.00 |
| FOXF1 | Mesoderm | 2.56 | 8.53 | 7.71 | 12.62 | 2.56 | 3.22 | 3.00 | 0.98 | 3.23 | LCK | Self-renewal | 0.27 | 0.22 | 0.14 | 0.15 | 0.12 | 0.11 | 0.00 | 0.01 | 0.00 |
| HAND1 | Mesoderm | 2.99 | 2.51 | 3.30 | 3.02 | 1.96 | 1.74 | 0.02 | 0.15 | 0.20 | NANGG | Self-renewal | 0.38 | 0.79 | 0.49 | 0.57 | 0.37 | 0.22 | 0.02 | 0.01 | 0.00 |
| HAND2 | Mesoderm | 2.29 | 1.67 | 3.03 | 5.00 | 0.29 | 3.75 | 0.66 | 3.32 | 2.17 | POU5F1 | Self-renewal | 0.45 | 0.68 | 0.36 | 0.26 | 0.16 | 0.11 | 0.03 | 0.01 | 0.01 |
| HEY1 | Mesoderm | 2.27 | 2.34 | 1.78 | 7.01 | 3.00 | 3.56 | 27.06 | 4.60 | 53.95 | SOX2 | Self-renewal | 1.55 | 1.08 | 1.75 | 0.85 | 1.48 | 1.16 | 0.21 | 1.89 | 0.28 |
| HOPX | Mesoderm | 27.35 | 11.35 | 52.63 | 28.58 | 23.10 | 38.80 | 2.40 | 5.10 | 2.24 | TRIM22 | Self-renewal | 4.42 | 7.18 | 5.03 | 2.96 | 3.81 | 2.94 | 0.20 | 0.41 | 0.25 |
| IL6ST | Mesoderm | 0.81 | 1.22 | 1.11 | 0.82 | 0.73 | 0.66 | 12.52 | 1.18 | 9.77 | | | | | | | | | | | |
| NR02-5 | Mesoderm | 2.34 | 3.23 | 16.27 | 3.98 | 5.04 | 3.20 | 0.44 | 0.51 | 1.56 | | | | | | | | | | | |
| ODAM | Mesoderm | 0.19 | 0.61 | 1.18 | 0.52 | 0.90 | 1.77 | 4.62 | 2.26 | 3.93 | | | | | | | | | | | |
| PDGFRA | Mesoderm | 0.97 | 0.69 | 0.66 | 1.18 | 0.94 | 0.66 | 0.91 | 0.37 | 0.16 | | | | | | | | | | | |
| PLVAP | Mesoderm | 0.59 | 0.29 | 0.11 | 2.53 | 0.80 | 0.66 | 3.42 | 1.11 | 1.12 | | | | | | | | | | | |
| RG54 | Mesoderm | 0.40 | 0.29 | 0.19 | 0.98 | 0.52 | 0.62 | 22.32 | 0.40 | 11.72 | | | | | | | | | | | |
| SN2 | Mesoderm | 0.66 | 0.68 | 1.54 | 34.49 | 5.58 | 13.15 | 19.64 | 11.63 | 13.91 | | | | | | | | | | | |
| TBK3 | Mesoderm | 0.27 | 0.62 | 0.71 | 1.77 | 0.28 | 0.57 | 6.64 | 0.51 | 11.16 | | | | | | | | | | | |
| TMSF1 | Mesoderm | 1.96 | 1.15 | 1.89 | 0.88 | 1.51 | 1.25 | 10.64 | 0.22 | 14.80 | | | | | | | | | | | |
| FGF4 | Mesoderm | 2.32 | 1.64 | 1.62 | 2.04 | 1.99 | 1.15 | 0.00 | 0.18 | 0.00 | | | | | | | | | | | |
| GDF3 | Mesoderm | 1.55 | 3.71 | 1.28 | 5.33 | 1.45 | 1.21 | 0.00 | 0.02 | 0.00 | | | | | | | | | | | |
| NPPB | Mesoderm | 0.08 | 0.53 | 0.18 | 0.06 | 0.11 | 0.06 | 0.01 | 0.00 | 0.02 | | | | | | | | | | | |
| NR5A2 | Mesoderm | 70.03 | 69.60 | 69.59 | 120.59 | 49.58 | 64.03 | 113.46 | 7.25 | 84.35 | | | | | | | | | | | |
| PTH1H | Mesoderm | 6.99 | 54.08 | 20.75 | 9.29 | 12.33 | 10.77 | 26.07 | 0.19 | 4.67 | | | | | | | | | | | |
| T | Mesoderm | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | |

| 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 | |

Figure S5. Table for 96 genes expression of *mock*- or *siCHD7*-transfected KhES-1 in EB formation assay day 4, 5 or 14 determined by qRT-PCR scorecard panel. This is a raw data before normalization for bar graphs in Figure 2D.

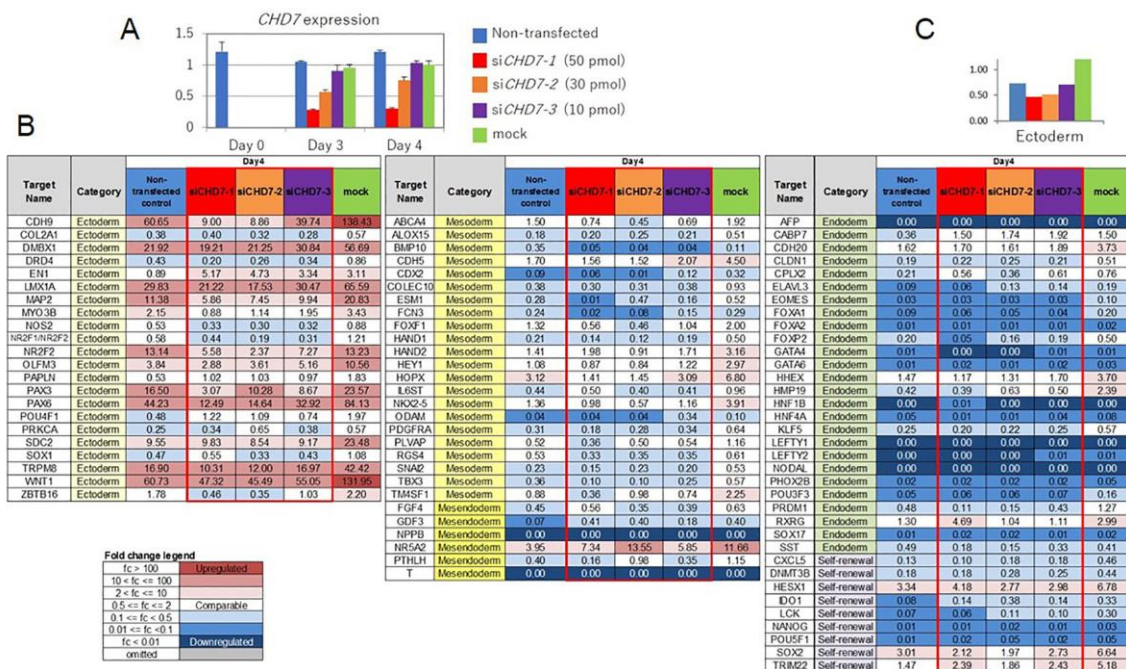


Figure S6. Differentiation potential of KhES-1 correlates to the expression level of *CHD7*. *CHD7* in KhES-1 was downregulated by introducing either 10, 30 or 50 pmol of si*CHD7* into cells (A) subsequently, differentiation potential was determined by qRT-PCR of 96 genes with a scorecard panel (B). Ectoderm differentiation potential after normalized was as bar graph (C).

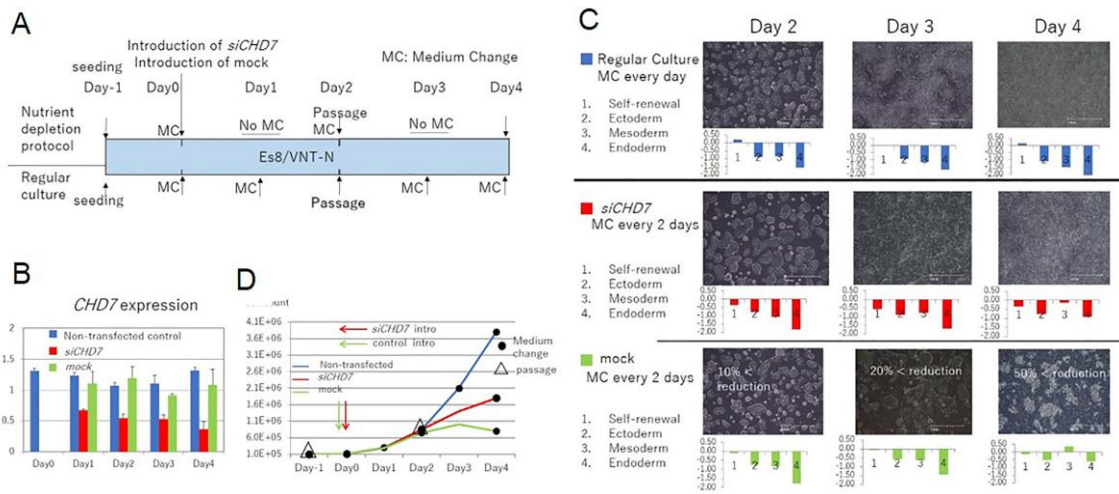


Figure S7. Downregulation of *CHD7* supported the survival of ESCs cultured with nutrient-depleted media.

- A. Protocol for induction of differentiation by nutrient-depleted Es8 medium and transfection with *siCHD7*. *siCHD7* or control siRNA (mock) was transfected into cells after changing the medium (Es8) on day 0. The medium was then changed every 2 days (days 2 and 4). Cells were harvested 42 h (day 2), 72 h, and 96 h after transfection with siRNA for cell counting and determination of gene expression by qRT-PCR. ESCs were normally cultured in Es8 medium with daily medium changes and passaged every 3 days to maintain an undifferentiated state.
- B. *CHD7* gene expression was determined by qRT-PCR in KhES-1 cells transfected with *siCHD7* or control siRNA (mock) or in non-transfected cells on days 0, 1, 2, 3 and 4. *CHD7* gene expression was standardized according to the average *CHD7* expression in KhES-1 cells cultured with Es8 medium measured independently 3 times.
- C. Photomicrographs of non-transfected regular cultures of KhES-1 cells (upper panels, daily medium changes), *siCHD7*-transfected KhES-1 cells (middle panels, medium changes every 2 days), and control siRNA-transfected (mock) KhES-1 cells (lower panels, medium changes every 2 days) on days 2, 3, and 4 are shown. Gene expression profiles of KhES-1 cells, as determined by qRT-PCR scorecard panel, are shown below the corresponding photograph. Scale bar: 1 mm.
- D. Numbers of non-transfected KhES-1 cells (blue line), *siCHD7*-transfected cells (red line), or mock-transfected cells (green line) on the designated cultured day are plotted as a linear graph. Representative data sets from 3 independent experiments are shown.

| Target Name | Category | Day1 | | | Day2 | | | Day3 | | | Target Name | Category | Day1 | | | Day2 | | | Day3 | | |
|-------------|----------|-------------------------|--------|-------|-------------------------|--------|--------|-------------------------|--------|--------|-------------|--------------|-------------------------|-------|-------|-------------------------|-------|-------|-------------------------|-------|-------|
| | | Non-transfected control | mCHD7 | mock | Non-transfected control | mCHD7 | mock | Non-transfected control | mCHD7 | mock | | | Non-transfected control | mCHD7 | mock | Non-transfected control | mCHD7 | mock | Non-transfected control | mCHD7 | mock |
| CDH9 | Ectoderm | 0.01 | 0.36 | 0.02 | 0.04 | 0.08 | 0.01 | 0.01 | 0.05 | 0.03 | AFP | Endoderm | 0.04 | 0.32 | 0.03 | 0.00 | 0.10 | 0.01 | 0.00 | 0.06 | 0.00 |
| COL2A1 | Ectoderm | 0.21 | 1.75 | 0.19 | 0.77 | 3.09 | 0.43 | 0.35 | 1.03 | 0.33 | CABP7 | Endoderm | 2.02 | 5.18 | 2.05 | 2.70 | 3.55 | 2.48 | 3.66 | 8.92 | 5.14 |
| DMBX1 | Ectoderm | 0.42 | 0.40 | 0.34 | 0.16 | 0.51 | 0.22 | 0.13 | 0.38 | 0.10 | CDH20 | Endoderm | 0.75 | 3.28 | 0.39 | 0.38 | 3.05 | 1.01 | 0.34 | 1.26 | 0.45 |
| DRD4 | Ectoderm | 0.22 | 0.50 | 0.24 | 0.32 | 0.46 | 0.22 | 0.33 | 0.56 | 0.33 | QLD11 | Endoderm | 1.84 | 3.95 | 2.30 | 2.79 | 4.35 | 2.95 | 1.78 | 2.40 | 2.16 |
| EN1 | Ectoderm | 0.65 | 69.19 | 1.23 | 0.87 | 8.41 | 2.96 | 0.44 | 2.43 | 0.36 | CPLX2 | Endoderm | 2.34 | 4.33 | 2.17 | 1.64 | 2.91 | 2.27 | 2.45 | 3.19 | 2.99 |
| LMNA | Ectoderm | 0.00 | 2.64 | 0.08 | 0.01 | 2.46 | 0.29 | 0.00 | 0.48 | 0.02 | ELAVL3 | Endoderm | 0.81 | 3.33 | 0.88 | 3.67 | 2.33 | 2.07 | 2.45 | 0.85 | 1.70 |
| MAP2 | Ectoderm | 7.24 | 10.18 | 11.63 | 14.79 | 14.96 | 16.94 | 2.66 | 5.35 | 3.88 | EOMES | Endoderm | 0.02 | 13.59 | 0.07 | 0.05 | 5.22 | 0.05 | 0.03 | 1.94 | 0.03 |
| MPO3B | Ectoderm | 0.43 | 0.35 | 0.29 | 0.79 | 0.32 | 2.36 | 0.54 | 0.95 | 0.81 | FOXA1 | Endoderm | 0.10 | 21.37 | 0.07 | 0.07 | 14.07 | 0.19 | 0.02 | 4.44 | 0.05 |
| NOS2 | Ectoderm | 0.82 | 1.31 | 0.75 | 0.70 | 1.68 | 1.08 | 0.77 | 0.68 | 0.90 | FOXA2 | Endoderm | 0.01 | 0.11 | 0.03 | 0.04 | 0.27 | 0.05 | 0.04 | 0.29 | 0.05 |
| NR2F1-NR2F2 | Ectoderm | 0.00 | 21.43 | 0.03 | 0.01 | 28.47 | 0.31 | 0.01 | 7.33 | 0.06 | FOXP2 | Endoderm | 0.24 | 3.96 | 0.12 | 0.22 | 7.37 | 0.32 | 0.12 | 2.35 | 0.16 |
| NR2F2 | Ectoderm | 0.30 | 3.44 | 0.23 | 0.63 | 1.56 | 1.10 | 0.32 | 1.38 | 0.41 | GATA4 | Endoderm | 0.02 | 4.26 | 0.09 | 0.03 | 3.65 | 0.10 | 0.03 | 1.21 | 0.06 |
| OLIG3 | Ectoderm | 0.68 | 2.00 | 0.57 | 0.39 | 1.23 | 0.44 | 0.27 | 0.77 | 0.46 | GATA6 | Endoderm | 0.10 | 2.67 | 0.27 | 0.68 | 2.40 | 0.48 | 0.03 | 1.28 | 0.25 |
| PAPLN | Ectoderm | 0.19 | 0.41 | 0.21 | 0.44 | 0.43 | 0.34 | 0.36 | 0.35 | 0.39 | HHEX | Endoderm | 0.10 | 4.36 | 0.11 | 0.16 | 2.62 | 0.12 | 0.13 | 1.01 | 0.21 |
| PAX3 | Ectoderm | 0.58 | 59.94 | 0.33 | 1.94 | 12.68 | 0.50 | 0.07 | 5.65 | 0.10 | HMP19 | Endoderm | 0.44 | 0.52 | 0.52 | 0.80 | 1.31 | 0.72 | 1.33 | 0.75 | 0.76 |
| PAX6 | Ectoderm | 0.13 | 16.25 | 0.18 | 0.11 | 10.65 | 0.13 | 0.04 | 2.26 | 0.11 | HNF1B | Endoderm | 0.02 | 0.36 | 0.06 | 0.03 | 0.32 | 0.05 | 0.01 | 0.22 | 0.05 |
| POU4F1 | Ectoderm | 0.40 | 42.21 | 0.04 | 0.04 | 33.30 | 0.10 | 0.49 | 7.77 | 0.32 | HNF4A | Endoderm | 0.03 | 0.67 | 0.12 | 0.62 | 0.99 | 0.28 | 0.02 | 0.31 | 0.14 |
| PRKCA | Ectoderm | 0.77 | 0.57 | 0.75 | 0.79 | 0.84 | 0.72 | 0.65 | 0.78 | 0.72 | KLF5 | Endoderm | 4.02 | 19.30 | 0.91 | 4.14 | 11.28 | 5.08 | 1.97 | 6.25 | 2.96 |
| SDC2 | Ectoderm | 29.34 | 20.10 | 19.66 | 26.51 | 18.81 | 18.98 | 17.11 | 15.36 | 16.78 | LEFTY1 | Endoderm | 0.44 | 0.31 | 0.36 | 0.35 | 0.30 | 0.43 | 0.26 | 0.23 | 0.28 |
| SOX1 | Ectoderm | 0.65 | 0.22 | 0.67 | 0.11 | 0.19 | 0.62 | 0.19 | 0.14 | 0.10 | LEFTY2 | Endoderm | 0.26 | 0.14 | 0.23 | 0.11 | 0.23 | 0.16 | 0.05 | 0.14 | 0.09 |
| TRPM8 | Ectoderm | 0.57 | 0.37 | 0.67 | 0.28 | 0.63 | 0.78 | 1.59 | 1.53 | 0.93 | NODAL | Endoderm | 1.11 | 0.65 | 1.06 | 1.19 | 1.40 | 1.57 | 0.71 | 1.15 | 0.70 |
| WNT1 | Ectoderm | 1.73 | 3.56 | 4.36 | 1.45 | 4.66 | 1.49 | 2.25 | 10.01 | 1.82 | PHOX2B | Endoderm | 0.03 | 2.55 | 0.03 | 0.03 | 1.73 | 0.03 | 0.03 | 0.40 | 0.04 |
| ZBTB16 | Ectoderm | 0.24 | 0.58 | 0.27 | 0.27 | 0.74 | 0.36 | 0.47 | 0.62 | 0.27 | POLJF3 | Endoderm | 0.05 | 3.46 | 0.08 | 0.05 | 1.53 | 0.06 | 0.08 | 0.44 | 0.07 |
| ABCA4 | Mesoderm | 0.59 | 0.87 | 0.75 | 0.99 | 2.17 | 0.92 | 1.17 | 2.41 | 1.80 | PRDM1 | Endoderm | 0.21 | 1.74 | 0.17 | 0.31 | 3.99 | 0.49 | 0.34 | 1.70 | 0.44 |
| ALOX15 | Mesoderm | 2.62 | 2.67 | 2.32 | 3.03 | 2.18 | 2.74 | 3.13 | 2.35 | 3.47 | PRDM6 | Endoderm | 0.06 | 2.07 | 0.58 | 0.01 | 6.17 | 0.93 | 0.02 | 2.02 | 0.35 |
| BMP10 | Mesoderm | 1.29 | 3.39 | 1.24 | 3.85 | 3.14 | 2.46 | 0.32 | 0.06 | 1.29 | SOX17 | Endoderm | 0.07 | 54.39 | 0.07 | 0.12 | 4.12 | 0.11 | 0.01 | 1.76 | 0.25 |
| CDH6 | Mesoderm | 6.21 | 3.01 | 4.44 | 4.59 | 7.23 | 11.32 | 1.09 | 8.08 | 3.76 | SST | Endoderm | 12.92 | 12.19 | 10.67 | 10.84 | 17.65 | 14.97 | 19.21 | 23.81 | 16.72 |
| CDJC2 | Mesoderm | 0.15 | 8.22 | 0.16 | 0.65 | 10.09 | 0.32 | 0.27 | 6.01 | 0.69 | CCN1L5 | Self-renewal | 12.60 | 7.18 | 10.05 | 8.51 | 5.12 | 6.78 | 6.31 | 4.87 | 6.68 |
| COL1C10 | Mesoderm | 0.49 | 0.72 | 1.09 | 1.65 | 2.39 | 2.72 | 1.95 | 1.41 | 2.17 | DNMT3B | Self-renewal | 1.23 | 0.62 | 1.01 | 1.17 | 0.84 | 1.45 | 1.80 | 1.39 | 1.64 |
| ESM1 | Mesoderm | 1.83 | 1.74 | 1.58 | 0.63 | 0.51 | 0.56 | 2.82 | 3.67 | 2.68 | HEX1 | Self-renewal | 0.41 | 0.24 | 0.28 | 0.26 | 0.21 | 0.21 | 0.37 | 0.32 | 0.39 |
| FCN3 | Mesoderm | 0.69 | 0.66 | 0.58 | 1.46 | 0.95 | 1.02 | 1.76 | 0.47 | 1.17 | IDO1 | Self-renewal | 0.55 | 0.38 | 0.31 | 0.76 | 0.41 | 0.48 | 0.90 | 0.68 | 0.83 |
| FOXP1 | Mesoderm | 0.30 | 13.98 | 0.51 | 0.69 | 17.82 | 1.58 | 0.18 | 6.37 | 0.92 | LOX | Self-renewal | 1.28 | 1.07 | 0.99 | 1.70 | 0.92 | 1.54 | 2.01 | 1.10 | 1.76 |
| HAND1 | Mesoderm | 0.18 | 1.57 | 0.26 | 0.14 | 1.63 | 0.70 | 0.10 | 5.67 | 0.89 | NRNG1 | Self-renewal | 3.09 | 2.59 | 2.94 | 4.67 | 2.42 | 3.17 | 3.92 | 2.62 | 3.24 |
| HAND2 | Mesoderm | 0.37 | 273.66 | 0.63 | 0.62 | 317.00 | 1.04 | 0.24 | 60.26 | 0.72 | POU5F1 | Self-renewal | 0.98 | 0.55 | 0.64 | 1.80 | 1.35 | 1.30 | 1.63 | 0.77 | 1.30 |
| HEY1 | Mesoderm | 0.74 | 1.33 | 0.65 | 0.59 | 1.01 | 0.77 | 0.44 | 0.72 | 0.51 | SOX2 | Self-renewal | 0.76 | 0.65 | 0.56 | 0.71 | 0.71 | 0.61 | 0.74 | 0.78 | 0.67 |
| HOPX | Mesoderm | 1.90 | 15.67 | 0.40 | 2.09 | 2.38 | 0.50 | 0.28 | 1.46 | 0.44 | TRIM22 | Self-renewal | 21.76 | 34.22 | 19.36 | 28.64 | 17.95 | 22.26 | 17.20 | 14.86 | 21.28 |
| IL15T | Mesoderm | 1.15 | 1.51 | 1.05 | 1.65 | 3.67 | 2.05 | 0.81 | 2.49 | 1.36 | | | | | | | | | | | |
| NOX2-5 | Mesoderm | 2.60 | 33.32 | 2.56 | 2.65 | 25.85 | 3.16 | 2.11 | 6.08 | 2.34 | | | | | | | | | | | |
| ODAM | Mesoderm | 0.96 | 15.69 | 1.71 | 1.30 | 4.54 | 1.48 | 0.53 | 1.87 | 1.40 | | | | | | | | | | | |
| PDGFRA | Mesoderm | 1.25 | 0.57 | 0.84 | 0.70 | 0.39 | 0.84 | 0.51 | 0.73 | 0.81 | | | | | | | | | | | |
| PRVAP | Mesoderm | 1.23 | 1.32 | 0.78 | 1.70 | 1.85 | 1.32 | 2.12 | 1.19 | 1.53 | | | | | | | | | | | |
| RGS4 | Mesoderm | 0.77 | 1.53 | 0.32 | 0.30 | 3.24 | 0.37 | 1.27 | 2.02 | 1.48 | | | | | | | | | | | |
| SNAP2 | Mesoderm | 0.60 | 0.73 | 0.72 | 0.39 | 0.75 | 0.59 | 0.32 | 0.97 | 0.46 | | | | | | | | | | | |
| TBC1 | Mesoderm | 1.42 | 3.02 | 1.83 | 0.59 | 3.39 | 1.24 | 0.85 | 2.82 | 1.92 | | | | | | | | | | | |
| TMSF1 | Mesoderm | 0.36 | 0.46 | 0.22 | 0.37 | 0.46 | 0.39 | 0.39 | 0.99 | 0.42 | | | | | | | | | | | |
| FGF4 | Mesoderm | 10.49 | 5.04 | 5.56 | 12.41 | 5.41 | 11.18 | 12.77 | 19.31 | 16.21 | | | | | | | | | | | |
| GDF3 | Mesoderm | 23.80 | 9.35 | 15.71 | 26.97 | 12.60 | 22.59 | 42.30 | 26.71 | 37.12 | | | | | | | | | | | |
| NPRB | Mesoderm | 3.15 | 12.79 | 7.14 | 2.16 | 16.30 | 4.78 | 0.79 | 7.36 | 2.09 | | | | | | | | | | | |
| NPS2 | Mesoderm | 29.66 | 29.54 | 22.06 | 16.83 | 315.07 | 161.89 | 194.43 | 141.36 | 178.16 | | | | | | | | | | | |
| PTH1L | Mesoderm | 1.02 | 45.00 | 1.24 | 0.52 | 32.69 | 1.38 | 0.28 | 9.52 | 0.73 | | | | | | | | | | | |
| T | Mesoderm | 0.00 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.02 | 0.00 | | | | | | | | | | | |

| Fold change legend | |
|----------------------|---------------|
| $fc > 100$ | Upregulated |
| $10 < fc \leq 100$ | |
| $2 < fc \leq 10$ | |
| $0.5 \leq fc \leq 2$ | Comparable |
| $0.1 \leq fc < 0.5$ | |
| $0.01 \leq fc < 0.1$ | |
| $fc < 0.01$ | Downregulated |
| omitted | |

Figure S8. Gene expression data from ESC after introduction of *mCHD7* determined by the qRT-PCR.

mRNA encoding *CHD7* isoform 2 (*mCHD7*) or *mock* RNA (*mock*) was transfected into KhES-1 cells cultured with RFF2 medium, and the expression levels of 96 genes in KhES-1 at 1, 2, and 3 days after transfection were determined by the qRT-PCR scorecard panel. Notably, three germ layer differentiation was observed simultaneously with upregulation of *CHD7* isoform 2 mRNA.

| Day 0 | | | | | | Day 3 | | | | | | | | | |
|-------------|----------|-------------------------|-------------------------|----------|----------|--------------|-------|-------------|----------|-------------------------|-------------------------|----------|----------|--------------|------|
| Target Name | Category | Non-transfected control | Non-transfected control | CHD1 DN1 | CHD7 DN2 | CHD7 DN1+DN2 | mock | Target Name | Category | Non-transfected control | Non-transfected control | CHD1 DN1 | CHD7 DN2 | CHD7 DN1+DN2 | mock |
| CDH9 | Ectoderm | 0.06 | 33.24 | 36.69 | 21.58 | 25.70 | 33.72 | ABCC4 | Mesoderm | 0.27 | 0.11 | 0.10 | 0.41 | 0.10 | 0.74 |
| COL2A1 | Ectoderm | 0.21 | 0.28 | 0.26 | 0.25 | 0.43 | 0.63 | ALOX15 | Mesoderm | 1.04 | 0.30 | 0.33 | 0.37 | 0.47 | 0.30 |
| DMB1 | Ectoderm | 0.46 | 12.58 | 10.76 | 10.26 | 7.39 | 14.48 | BMP10 | Mesoderm | 0.70 | 0.71 | 0.76 | 0.66 | 0.66 | 0.66 |
| DRG1 | Ectoderm | 0.00 | 0.29 | 0.13 | 0.16 | 0.23 | 0.20 | CH5 | Mesoderm | 3.87 | 1.29 | 1.08 | 0.94 | 0.79 | 1.96 |
| ENT | Ectoderm | 0.43 | 2.16 | 0.45 | 0.62 | 0.40 | 1.47 | CDX2 | Mesoderm | 0.02 | 0.25 | 0.04 | 0.14 | 0.00 | 0.15 |
| LIM1A | Ectoderm | 0.20 | 11.85 | 7.56 | 4.39 | 4.52 | 7.21 | COLEC10 | Mesoderm | 0.26 | 1.09 | 0.56 | 0.69 | 0.49 | 2.24 |
| MAP2 | Ectoderm | 1.33 | 9.79 | 8.59 | 7.47 | 6.56 | 10.03 | ESR1 | Mesoderm | 0.08 | 0.62 | 0.66 | 0.66 | 0.62 | 0.48 |
| MYO3B | Ectoderm | 0.68 | 2.04 | 0.92 | 0.48 | 0.45 | 2.19 | FCN3 | Mesoderm | 0.60 | 0.17 | 0.12 | 0.15 | 0.29 | 0.35 |
| NOS2 | Ectoderm | 0.77 | 0.30 | 0.30 | 0.30 | 0.31 | 0.34 | FOXP1 | Mesoderm | 0.25 | 0.53 | 0.58 | 0.58 | 0.68 | 0.75 |
| NR2F1 | Ectoderm | 0.01 | 0.84 | 0.81 | 0.81 | 0.84 | 0.16 | HAND1 | Mesoderm | 0.33 | 0.34 | 0.19 | 0.27 | 0.25 | 0.37 |
| NR2F2 | Ectoderm | 0.01 | 0.44 | 0.31 | 0.22 | 0.21 | 0.38 | HAND2 | Mesoderm | 0.17 | 0.02 | 0.12 | 0.28 | 0.66 | 0.46 |
| OLFML3 | Ectoderm | 0.97 | 1.86 | 1.70 | 1.01 | 1.40 | 1.88 | HEY1 | Mesoderm | 1.54 | 1.11 | 1.00 | 1.18 | 1.07 | 2.12 |
| PAPLN | Ectoderm | 0.12 | 1.47 | 1.41 | 1.18 | 1.00 | 1.80 | HOPX | Mesoderm | 0.86 | 2.00 | 2.09 | 3.88 | 4.35 | 2.82 |
| PAX3 | Ectoderm | 0.04 | 1.50 | 0.13 | 0.06 | 0.05 | 1.45 | IL6ST | Mesoderm | 0.91 | 0.50 | 0.49 | 0.34 | 0.34 | 0.40 |
| PAX6 | Ectoderm | 0.04 | 2.89 | 1.36 | 0.33 | 0.38 | 1.92 | NKX2-5 | Mesoderm | 0.02 | 0.59 | 0.32 | 0.13 | 0.29 | 0.33 |
| POU4F1 | Ectoderm | 0.54 | 1.50 | 0.81 | 0.56 | 1.31 | 1.29 | COX4I | Mesoderm | 0.52 | 0.65 | 0.66 | 0.67 | 0.78 | 0.86 |
| PRKCA | Ectoderm | 0.63 | 0.65 | 0.77 | 0.81 | 1.05 | 0.67 | PDGFRA | Mesoderm | 0.35 | 0.78 | 0.68 | 0.35 | 0.41 | 0.80 |
| SDC2 | Ectoderm | 3.19 | 16.09 | 13.11 | 9.03 | 8.23 | 15.14 | PLVAP | Mesoderm | 0.66 | 0.65 | 0.67 | 0.70 | 0.63 | 0.75 |
| SOX1 | Ectoderm | 0.03 | 0.64 | 0.43 | 0.48 | 0.53 | 0.58 | RGS4 | Mesoderm | 0.13 | 0.14 | 0.12 | 0.27 | 1.14 | 0.12 |
| TRPM8 | Ectoderm | 0.38 | 3.21 | 2.00 | 0.55 | 0.61 | 2.10 | SNAI2 | Mesoderm | 0.30 | 0.03 | 0.06 | 0.08 | 0.07 | 0.05 |
| WNT1 | Ectoderm | 0.05 | 12.66 | 4.94 | 2.17 | 1.60 | 9.55 | TBX3 | Mesoderm | 0.01 | 0.17 | 0.04 | 0.07 | 0.05 | 0.12 |
| ZBTB16 | Ectoderm | 0.11 | 0.16 | 0.11 | 0.10 | 0.08 | 0.08 | TNFRSF1 | Mesoderm | 0.19 | 4.27 | 4.43 | 2.56 | 2.79 | 4.25 |
| | | | | | | | | FGF4 | Mesoderm | 0.14 | 0.97 | 0.23 | 0.18 | 0.23 | 0.83 |
| | | | | | | | | GDF3 | Mesoderm | 3.63 | 0.51 | 0.71 | 0.64 | 0.60 | 0.47 |
| | | | | | | | | HNF1B | Mesoderm | 0.01 | 0.03 | 0.01 | 0.01 | 0.02 | 0.01 |
| | | | | | | | | NRS4A2 | Mesoderm | 63.62 | 6.73 | 10.20 | 12.81 | 16.46 | 8.78 |
| | | | | | | | | PTH1LH | Mesoderm | 0.41 | 1.63 | 2.46 | 2.17 | 3.07 | 1.64 |
| | | | | | | | | T | Mesoderm | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| Day 0 | | | | | | Day 3 | | | | | |
|-------------|--------------|-------------------------|-------------------------|----------|----------|--------------|------|--|--|--|--|
| Target Name | Category | Non-transfected control | Non-transfected control | CHD1 DN1 | CHD7 DN2 | CHD7 DN1+DN2 | mock | | | | |
| CXCL5 | Self-renewal | 2.66 | 1.08 | 1.36 | 2.29 | 1.63 | 1.51 | | | | |
| DNMT3B | Self-renewal | 0.78 | 0.49 | 0.55 | 0.56 | 0.57 | 0.66 | | | | |
| HES1 | Self-renewal | 0.66 | 2.97 | 2.50 | 1.85 | 1.77 | 2.24 | | | | |
| IDO1 | Self-renewal | 0.90 | 1.26 | 1.46 | 1.06 | 1.36 | 1.31 | | | | |
| LCK | Self-renewal | 1.31 | 0.26 | 0.29 | 0.20 | 0.20 | 0.27 | | | | |
| NANOG | Self-renewal | 1.07 | 0.10 | 0.18 | 0.13 | 0.16 | 0.15 | | | | |
| POU5F1 | Self-renewal | 1.05 | 0.09 | 0.21 | 0.14 | 0.19 | 0.14 | | | | |
| SOX2 | Self-renewal | 0.64 | 2.20 | 2.48 | 1.52 | 1.81 | 2.04 | | | | |
| TRIM22 | Self-renewal | 1.68 | 2.27 | 3.30 | 1.84 | 1.69 | 2.16 | | | | |

| 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 | |

Figure S9. Table depicting the expression of a 96-gene panel from *mock*, *DN1*-, *DN2*- or *DN1+DN2*-transfected KhES-1 in an EB formation assay at day 0 or 3 determined by a qRT-PCR scorecard panel. This is a raw data before normalization for bar graphs in Figure 4C.

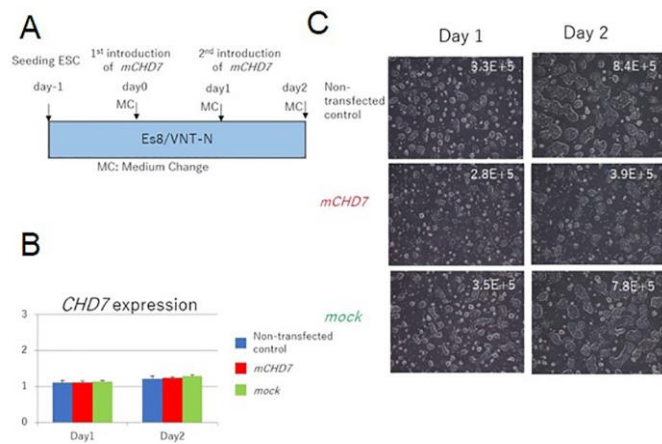


Figure S10. Introduction of *mCHD7* failed to generate *CHD7*-overexpressing ESCs in culture with Es8 medium.

- A. Protocol for transfection with mRNA for *CHD7* isoform 2 (*mCHD7*) and cell culture.
- B. Evaluation of *mCHD7* expression by qRT-PCR on days 1 and 2. The 3' PCR primer sets to detect all of *CHD7* isoforms (isoform 1, 2 and X4) was used. *CHD7* gene expression was standardized according to the average *CHD7* expression in KhES-1 cells cultured with Es8 medium and measured independently 3 times. A representative result from 3 biological replicates is shown. (n=3 analytical replicates).
- C. Cell counting and cell morphology of *CHD7*- or mock-transfected KhES-1 cells on days 1 and 2. The cell numbers in one well of a 6-well plate is appended in the upper right corner of the relevant photograph.

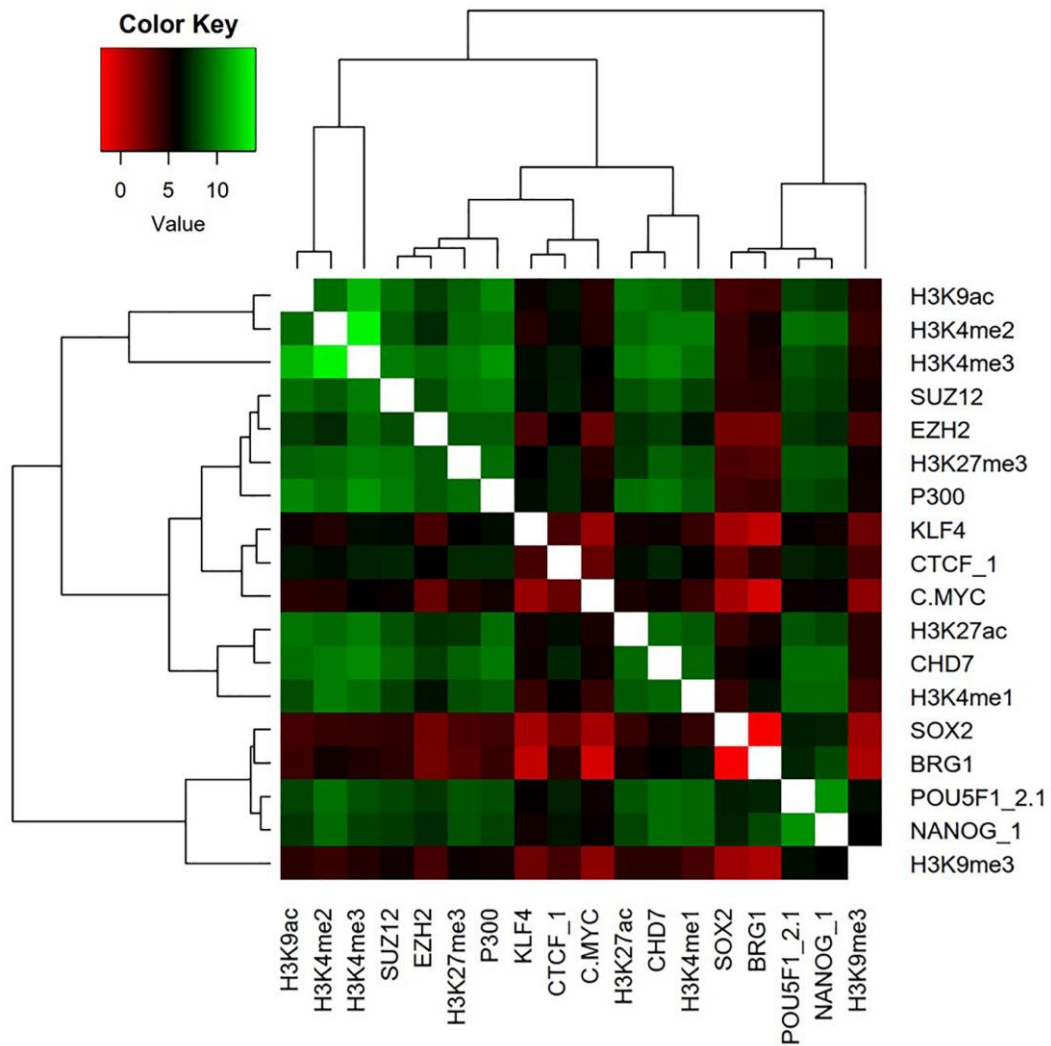


Figure S11. ChIP-seq 18 factors (H1) and BRG1 (H9). Heat map were obtained from a public ESC ChIP-seq database. The odds ratio representing the correlation between binding sites for each pair of factors was calculated. Green indicates high homology between factors and red indicates no high homology between factors.

Human ESC H1 or H9

| Gene | Accession No. |
|----------|---------------|
| CHD7 | GSM1003473 |
| P300 | GSM1003513 |
| SUZ12 | GSM1003573 |
| EZH2 | GSM1003524 |
| POU5F1 | GSM803438 |
| NANOG | GSM803437 |
| CTCF | GSM803419 |
| KLF4 | GSM447584 |
| C-MYC | GSM447585 |
| SOX2 | GSM456570 |
| BRG1 | GSM602297 |
| H3K4me1 | GSM733782 |
| H3K4me2 | GSM733670 |
| H3K4me3 | GSM733657 |
| H3K9me3 | GSM1003585 |
| H3K27me3 | GSM733748 |
| H3K27ac | GSM733718 |
| H3K9ac | GSM733773 |

Figure S12. List of accession numbers from the GEO public database
GSM accession numbers in the GEO database used for odds ratios mapping and gene
expression profiles.

qPCR Primers

| Gene | F/R | seq (5'-3') |
|-------------------|-----|------------------------------------|
| POU5F1 | F | GAA ACC CAC ACT GCA GCA GA |
| POU5F1 | R | TCG CTT GCC CTT CTG GCG |
| SOX2 | F | GGG AAA TGG GAG GGG TGC AAA AGA GG |
| SOX2 | R | TTG CGT GAG TGT GGA TGG GAT TGG TG |
| NANOG | F | CTC AGC TAC AAA CAG GTG AAG AC |
| NANOG | R | TCC CTG GTG GTA GGA AGA GTA AA |
| p300 | F | TTG AAT GTA CAG AGT GCG GAA GA |
| p300 | R | AAC AGC CAT CAC AGA CGA ATC C |
| CHD7 | F | GGT TCC CAC ACT CGT GCA TA |
| CHD7 | R | TGC GCC TCG GGA CAG A |
| CHD7 isoform 2 | F | CCC ATG AAA GCA ATG AGT AAT CC |
| CHD7 isoform 2 | R | TCC ATT GGT ATC CCA GCA CTT C |
| CHD7 Chromodomain | F | TGATGGACTTGGAACACAAAGTG |
| CHD7 Chromodomain | R | TGAAGGGAAGCGACTTGGTT |
| CHD7 sant slide | F | CAAACATGGCTATGAGAAGTACAACCTC |
| CHD7 sant slide | R | CCGACTCGTTCCAGAAAGCA |
| GAPDH | F | CCA CTC CTC CAC CTT TGA CG |
| GAPDH | R | ATG AGG TCC ACC ACC CTG TT |

ChIP Primers

| Refseq No. | Gene | Primer |
|-------------------------------|--------|------------------|
| NM_203289.3 | POU5F1 | GPH1024786(-)01A |
| NM_024865.2 | NANOG | GPH1002937(-)01A |
| NM_001429.3 | p300 | GPH1008986(-)01A |
| NM_001128844.1 NM_003072.3 | BRG1 | GPH1006548(-)01A |
| NM_015355.2 | SUZ12 | GPH1005657(-)01A |
| NM_152998.1 NM_004456.3 | EZH2 | GPH1025912(-)01A |

Taq man PCR primer set 1

| Refseq No. | Gene | Taqman probe |
|---|------|---------------|
| NM_017780 XM_011517553.2 XM_011517554.2 XM_011517555.2 XM_017013612.1 XM_017013613.1 XM_011517560 | CHD7 | Hs00215010_m1 |

Taq man PCR primer set 2

| Refseq No. | Gene | P/F/R | seq (5'-3') |
|--------------|------|-------|--------------------------------|
| NM_001316690 | CHD7 | P | TATGACTCAGAAACCGAAACAGAAACGACA |
| | CHD7 | F | GCCCTTTCTAGAGAAACCAAGTG |
| | CHD7 | R | AGGCACCCTTTCTTCTCCTG |

Taq man PCR primer set 3

| Refseq No. | Gene | P/F/R | seq (5'-3') |
|---|------|-------|------------------------------|
| NM_017780 XM_011517553.2 XM_011517554.2 XM_011517555.2 XM_017013612.1 XM_017013613.1 | CHD7 | P | CACGGACGCTATAAACGCCAACTCACTG |
| | CHD7 | F | GAATCTGCTTGTCTATGTTGGG |
| | CHD7 | R | AGGATGTTCTGCAGATGGT |

Figure S13. List of primer sets used for qRT-PCR.