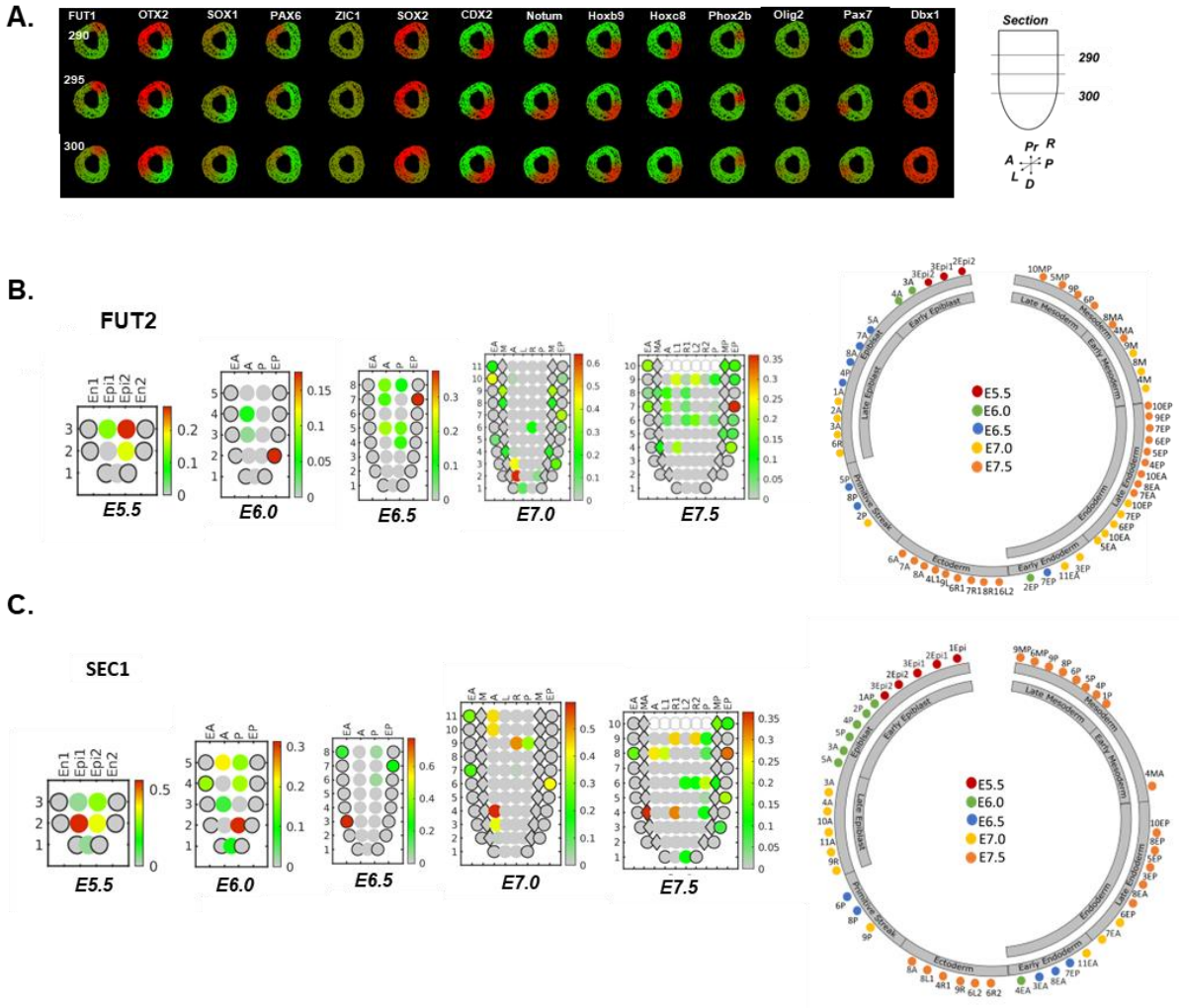
Appendix

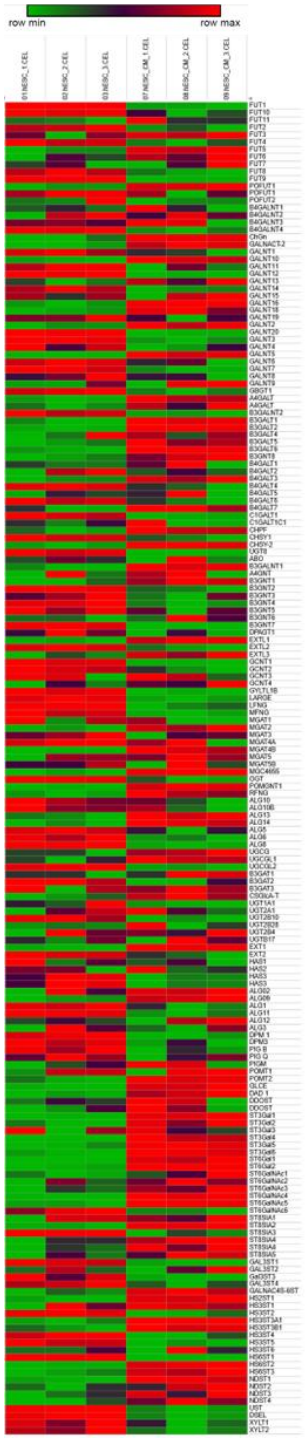


Appendix Figure S1 (related to Figure 1)

Co-expression of FUT1 with NE markers and FUT2 and SEC1 gene expression in early embryonic development

(A) Left: Illustration of FUT1 gene and NE markers in E7.5 embryos showing FUT1 gene co-expression with OTX2, PAX6, ZIC1, SOX2, SOX1, HOXC8, PHOX2B, OLIG2, PAX7, HOXB9, and DBX1 in sections 295–300, using *in silico* transcriptomic analysis in 3D and employing the database of mouse gastrulation on E5.5–E7.5. FUT1 is co-expressed with SOX2 (EPI), OTX2, and DBX1; NE markers of fore-midbrain and intermediate neural tube. Right: Schematic showing embryo sections for transcriptomic analysis. A, anterior; P, posterior; L, left; R, right; Pr, proximal; D, distal. (B) Left: 2D corn plots showing the spatiotemporal expression of the FUT2 gene in E5.5–E7.5 embryos. Right: Circle diagrams demonstrating FUT2 gene expression base on tissue classification. (C) Left: 2D corn plots showing the spatiotemporal expression of SEC1 in E5.5–E7.5 embryos. Right: Circle diagrams demonstrating SEC1 gene expression based on tissue classification. NE, neuroectoderm; En1

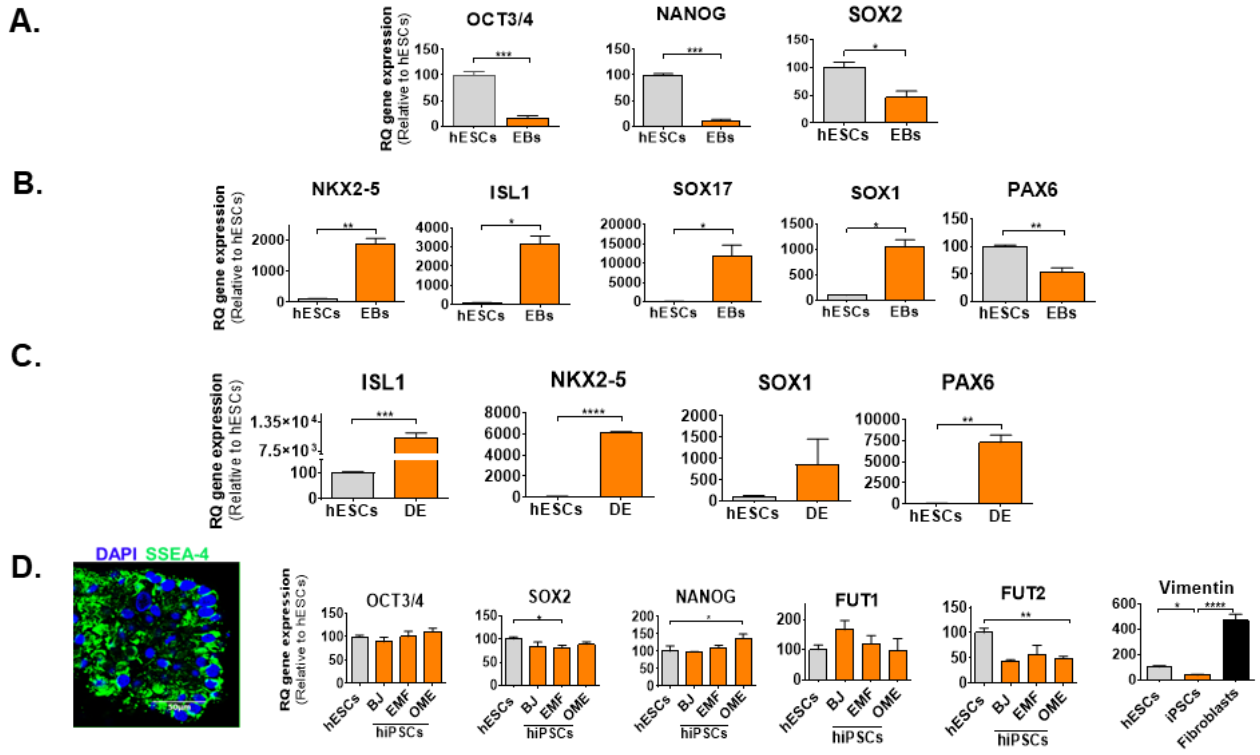
and En2, divided endoderm; Epi1 and Epi2, divided epiblast; EA, anterior endoderm; EP, posterior endoderm; A, anterior; P, posterior; M, whole mesoderm; L, left lateral; R, right lateral; L1, anterior left lateral; R1, anterior right lateral; L2, posterior left lateral; R2, posterior right lateral; MA, anterior mesoderm; MP, posterior mesoderm.



Appendix Figure S2 (related to Figure 2)

GT gene expression is changed during pluripotency and 14 d after hESC differentiation into CMs

Heatmap comparing the expression level of 181 genes involved in glycosylation of hESCs and hESC-derived CMs after 14 d of differentiation. n = 3 technical replicates. Data are means ± SEM. $p < 0.001$ (Two-tailed unpaired t test).

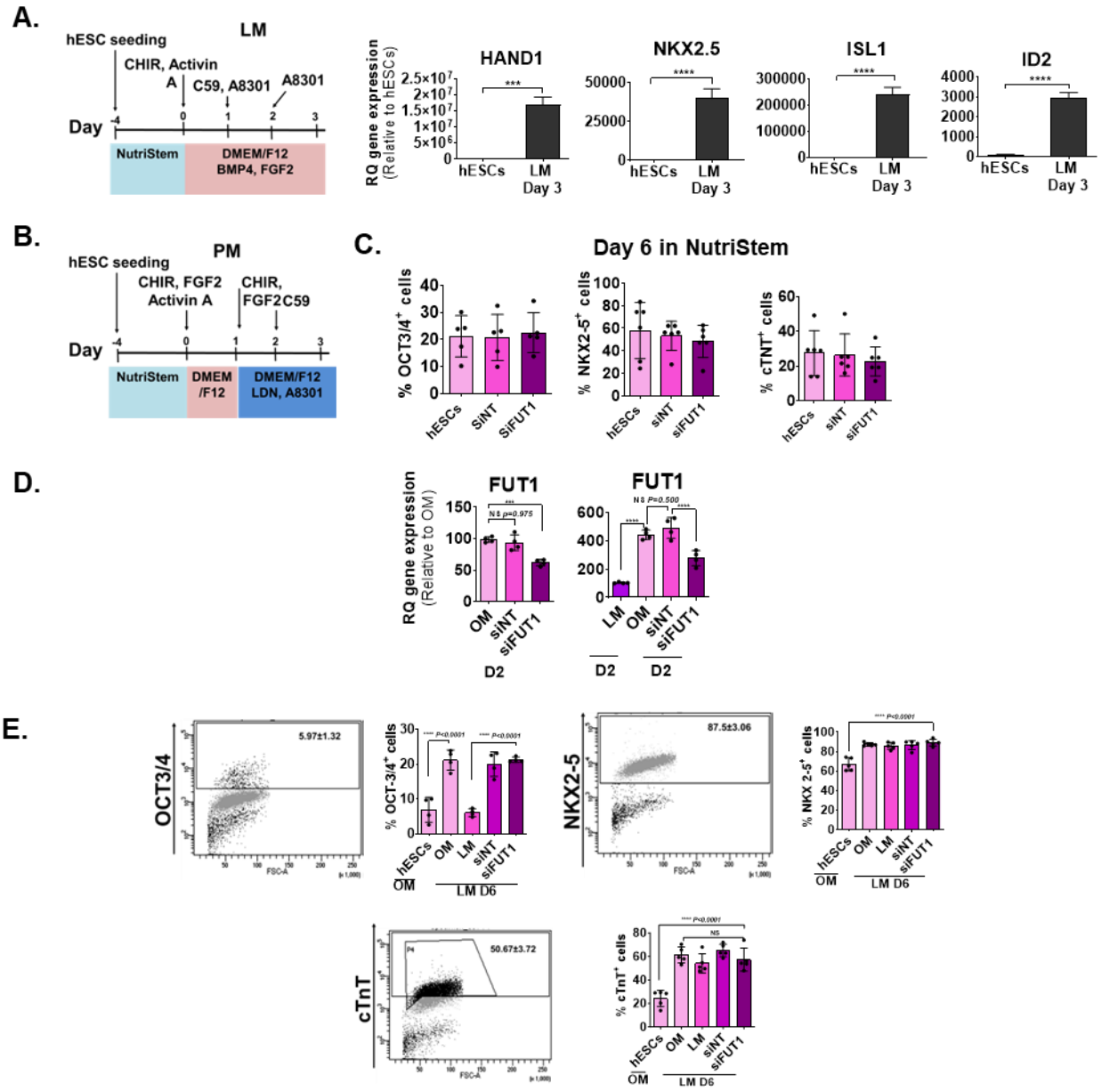


Appendix Figure S3 (related to Figure 3)

The expression of FUT1 and α-fucose in iPSCs during pluripotency and differentiation is identical to that of hESCs

(A) Quantification of the gene expression of pluripotent markers, OCT3/4, SOX2, and NANOG in hESCs and day 5 EBs, as measured by qPCR. n = 3 technical replicates. (B) Quantification of the mRNA expression of tri-germ layer markers, NKX2.5, ISL1, SOX17, SOX1, and PAX6 in WT hESCs and day 5 EBs. n = 3 technical replicates. (C) Quantification of DE gene expression in WT hESCs and day 8 DE. n = 3 technical replicates. Data are means ± SEM. NS>0.05, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ (Two-tailed unpaired t tests). (D) Left: Representative images of hiPSCs growing on Matrigel showing SSEA-4 (green) and DAPI (blue) for nucleus. Scale bars represent 50 μm. Right: Quantification of the pluripotent gene expression, OCT3/4, SOX2, and NANOG, and of FUT1 and FUT2 in hESCs in three lines of hiPSCs (BJ, EMF and OME), and Vimentin in hESCs, a pool of hiPSCs, and a pool of human fibroblasts before reprogramming. Pooled sample, n = 3 cell lines. n = 3

technical replicates. Data are means ± SEM. NS>0.05 *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (Ordinary one-way ANOVA).



Appendix Figure S4 (related to Figure 4)

Silencing FUT1 alters mesoderm gene expression

(A) Top left: Schematic showing hESC differentiation into LM using Wnt/FGF/BMP and Nodal signaling over 3 d. Top right: Quantification of the gene expression of LM, HAND1, NKX2.5, and ISL1 and ID2 in WA09 hESCs and 3 d hESC-derived LM. n=3 technical replicates. (B) Schematic showing hESC differentiation into PM using Wnt/FGF/BMP and

Nodal signaling over 3 d. (C) Quantification of the percent of positive cells expressing OCT3/4, NKX2.5, and cTnT in WT, siNT, and siFUT1, WA09 hESCs grown for 6 d in NutriStem. n=3 technical replicates. (D) Left: Quantification of FUT1 transcripts in WA09 hESCs grown in OM and in siNT and siFUT1, WA09 hESCs grown for 2 d in NutriStem. Right: Quantification of the gene expression of FUT1 in WA09 hESCs grown in OM and 2 d after differentiation into LM compared to the gene expression of FUT1 in siNT and siFUT1, WA09 hESCs 2 d after differentiation into LM. n=4 biological replicates. (E) Quantification of the percent of positive cells for pluripotent marker, OCT3/4 and for cardiac-specific markers, NKX2.5 and cTnT in WT hESCs grown in NutriStem or OM in NutriStem medium, and after differentiation into CM for 6 d in WT, siNT and siFUT1 hESCs. H9.1 hESCs were silenced with a mixture of 3 siRNA for FUT1 and 1 NT siRNA. Error bars represent ± SDs for n=3 technical replicates. The housekeeping gene GAPDH was used for normalization. Data presented are relative to the values of day 0 WT or siNT hESCs. Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ (two-tailed unpaired Student's t-tests).