

Supplemental information

**Recapitulating early human
development with 8C-like cells**

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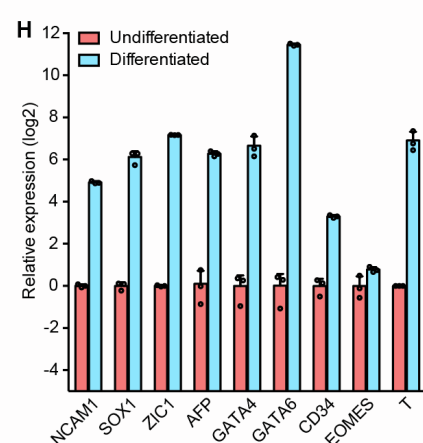
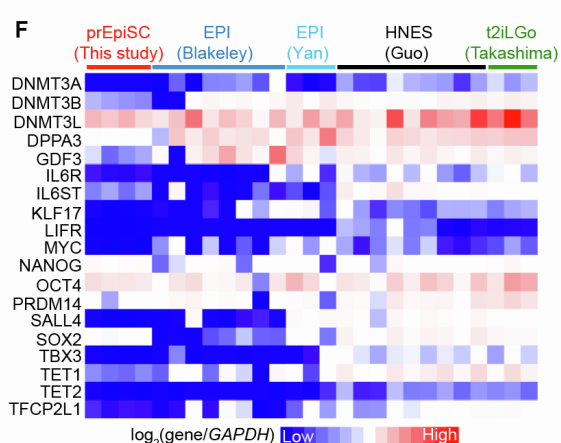
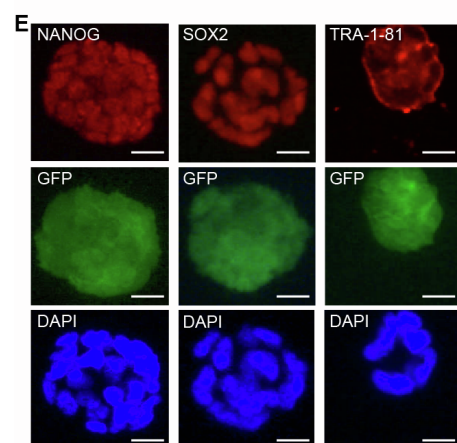
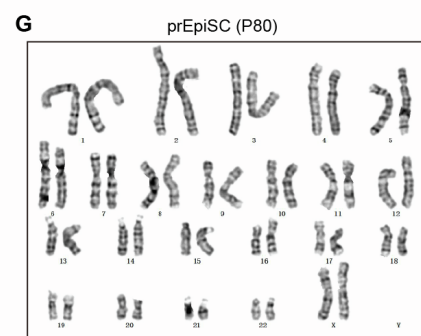
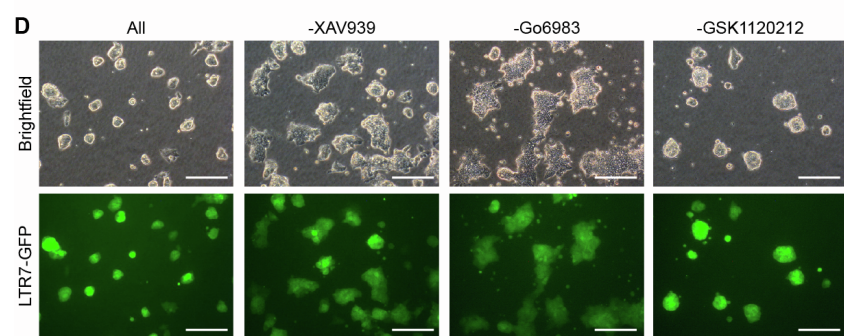
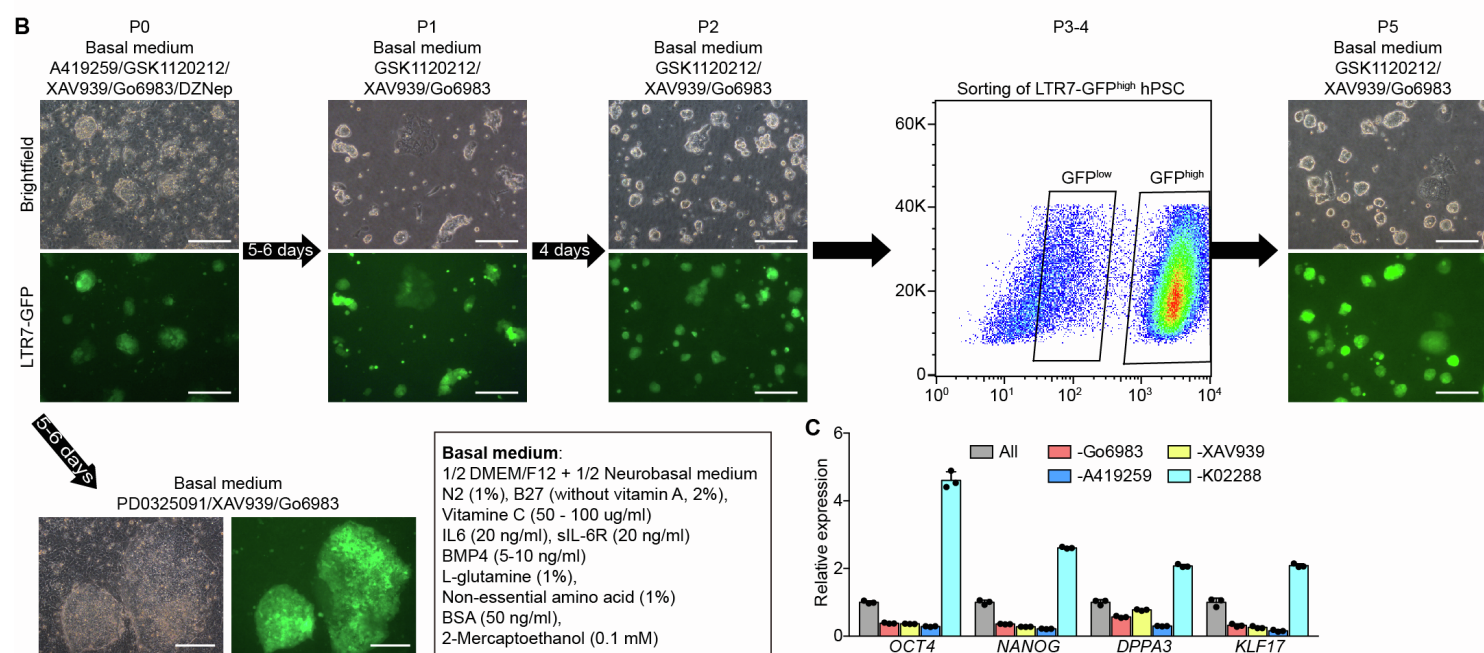
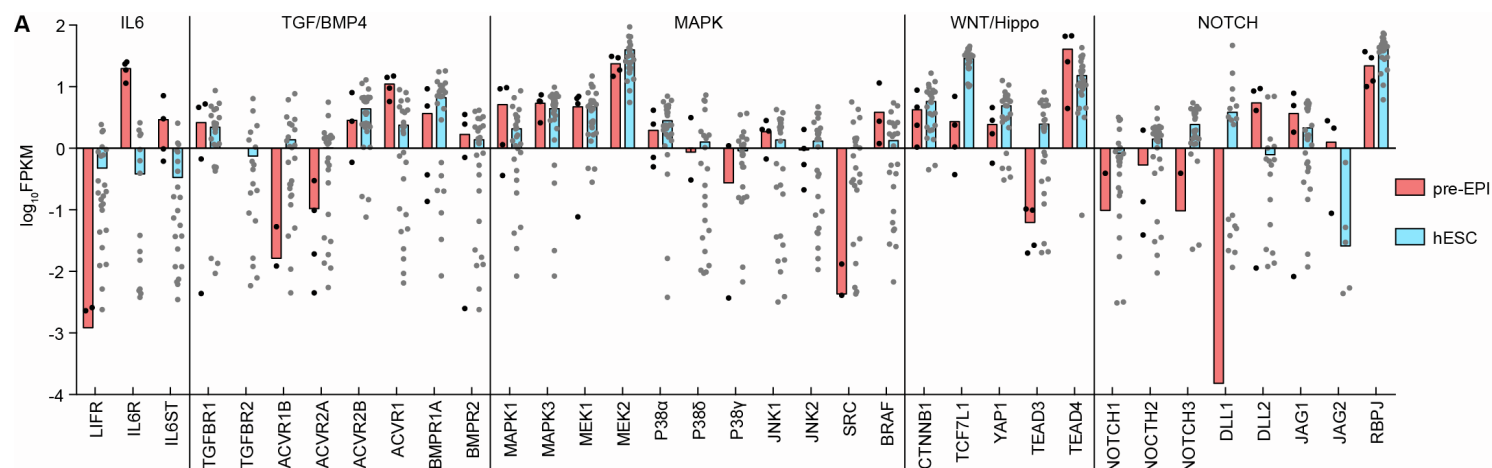


Figure S1. Derivation and characterization of prEpiSCs, related to Figure 1

(A) Comparison of multiple genes enriched for IL6, TGF/BMP4, MAPK, WNT/Hippo, and NOTCH signaling pathways between human epiblast and ESCs (Yan et al., 2013).

(B) The procedure of derivation of prEpiSCs from primed hESCs. Scale bar, 100 μ m.

(C) The expression levels of pluripotent genes during prEpiSCs induction under the indicated treatment. Data are shown as means \pm S.D. of technical replicates from two independent experiments.

(D) The representative images showing essential chemicals for prEpiSC maintenance. Scale bar, 100 μ m.

(E) The immunofluorescence analysis showing expression of NANOG, SOX2 and TRA-1-81 in prEpiSCs. Scale bar, 20 μ m.

(F) The heatmap showing the expression levels (normalized to *GAPDH*) of pluripotent genes in prEpiSCs, naïve hPSCs (HNES and t2iLGo) and human epiblast (EPI).

(G) Karyotyping analysis for prEpiSCs.

(H) The lineage marker expression levels showing the *in vitro* differentiation potential of prEpiSCs. Data are shown as means \pm S.D. (n=3 biological replicates).

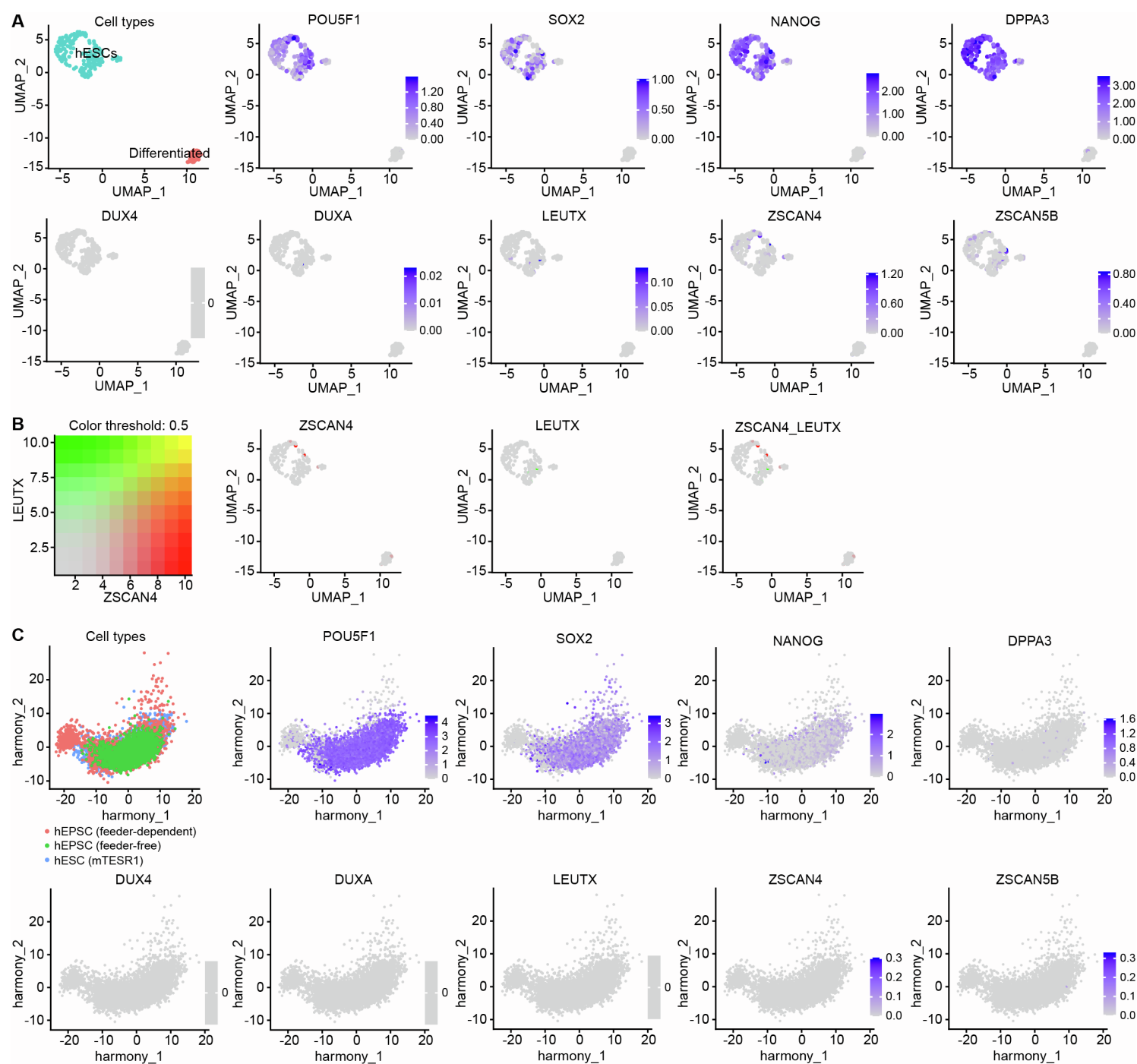


Figure S2. Expression of pluripotent and 8C-specific genes in human primed and extended pluripotent stem cells, related to Figure 2

(A) Expression levels of pluripotent and 8C-specific genes in human primed pluripotent stem cells, detected by scRNA-seq.

(B) Analysis for co-expression of *LEUTX* and *ZSCAN4* in human primed pluripotent stem cells.

(C) Expression of pluripotent and 8C-specific genes in human extended pluripotent stem cells, detected by scRNA-seq.

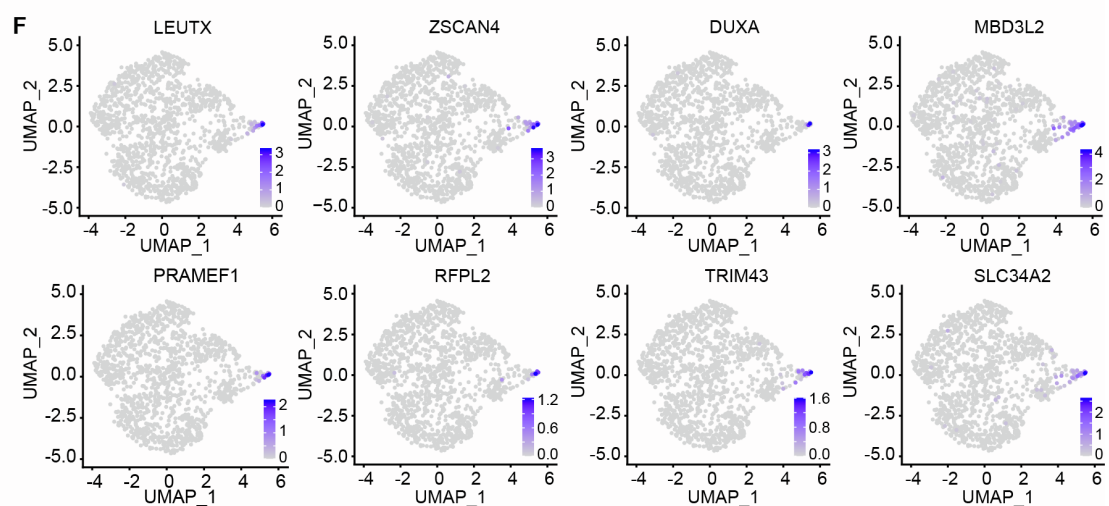
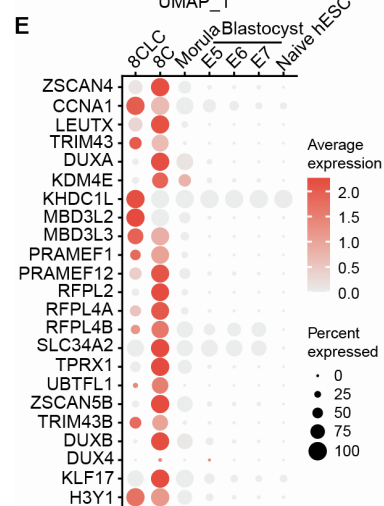
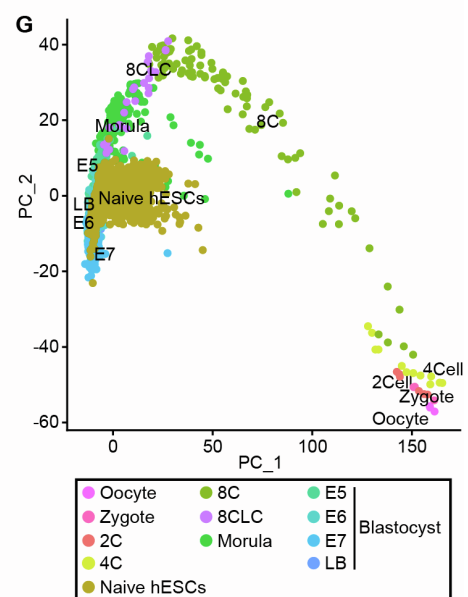
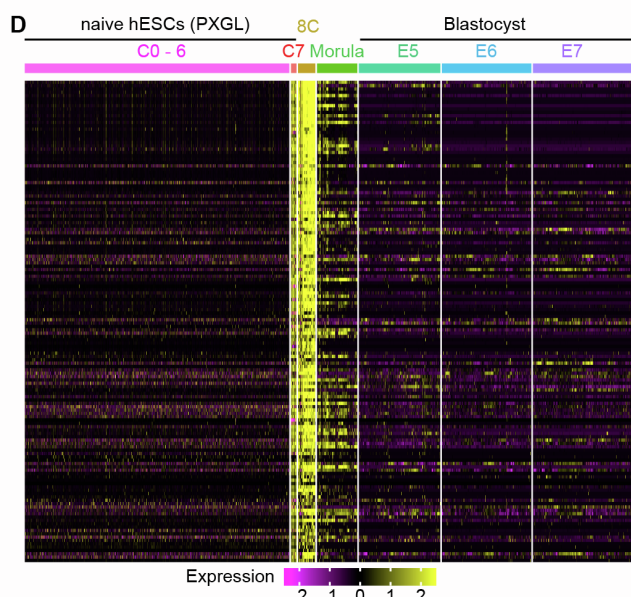
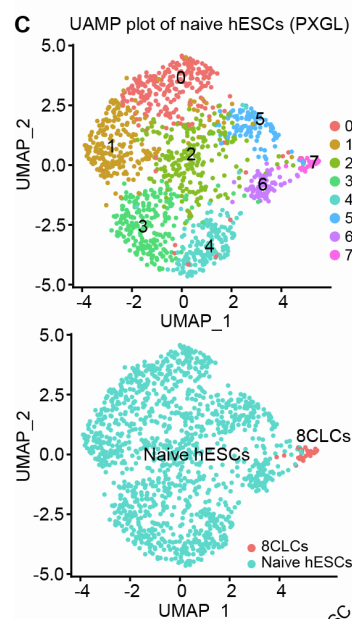
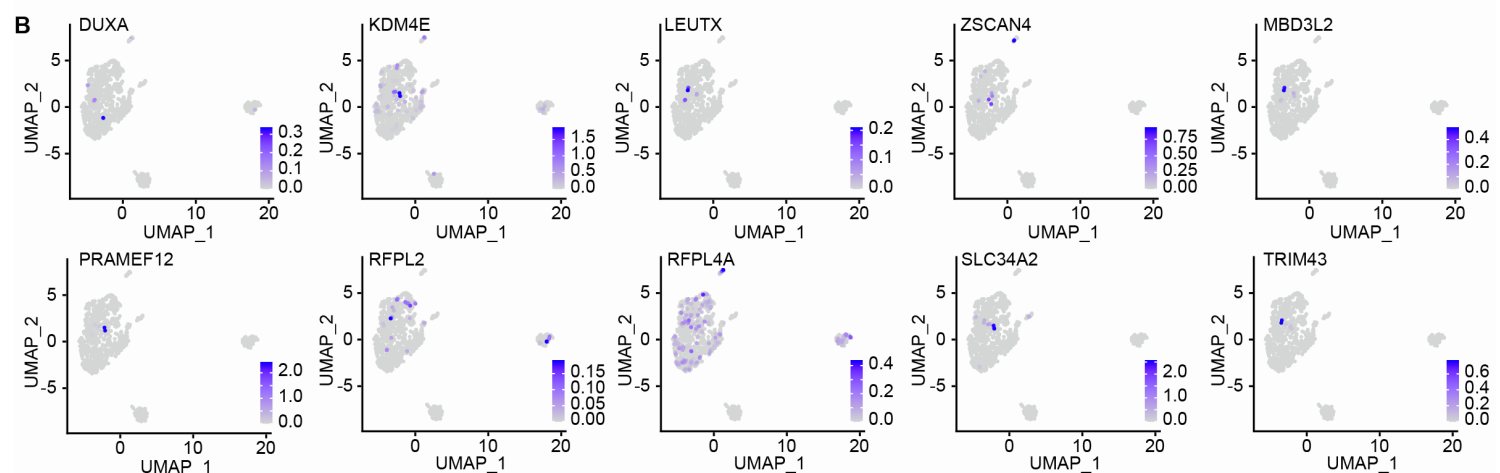
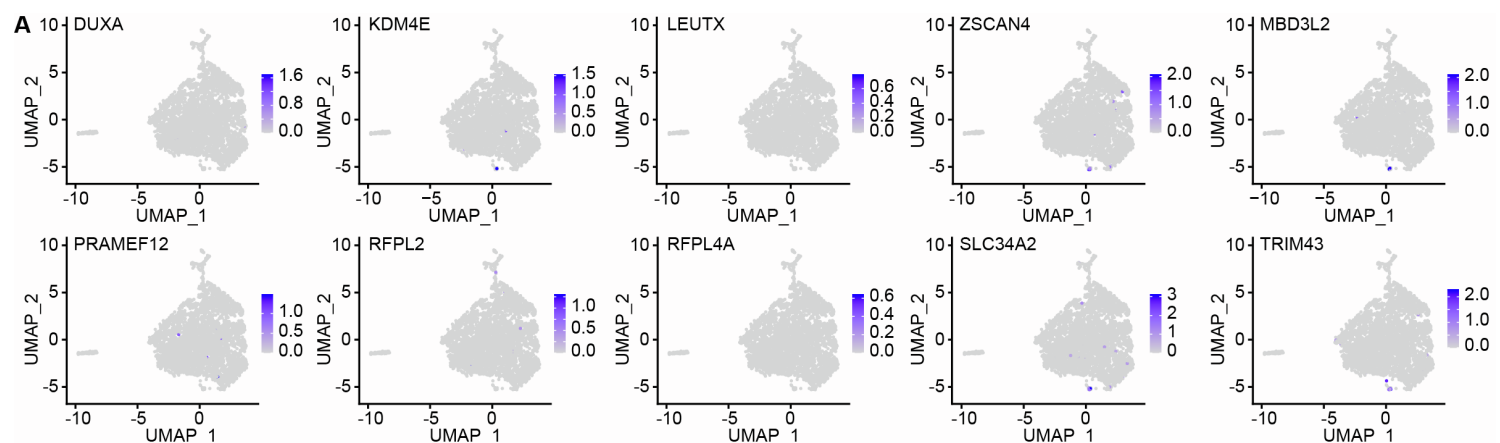


Figure S3. Identification of 8CLCs in human naïve pluripotent stem cells, related to Figure 2

(A) Expression levels of multiple 8C-specific genes in human naïve pluripotent stem cells cultured in the t2iLGo condition.

(B) Expression levels of multiple 8C-specific genes in human naïve pluripotent stem cells cultured in the 5iLA condition.

(C) UMAP plots of scRNA-seq data of human naïve pluripotent stem cells cultured in the PXGL condition.

(D) Expression levels of 8C-specific genes in human embryos and naïve pluripotent stem cells cultured in the PXGL condition.

(E) The frequency of expression and average expression levels for selected 8C-specific genes in human embryos and 8CLCs discovered from PXGL-cultured naïve pluripotent stem cells.

(F) The UMAP plot showing expression of selected 8C-specific genes in single PXGL-cultured naïve pluripotent stem cell.

(G) Principle component analysis of transcriptome of early human embryos and 8CLCs at the single-cell level.

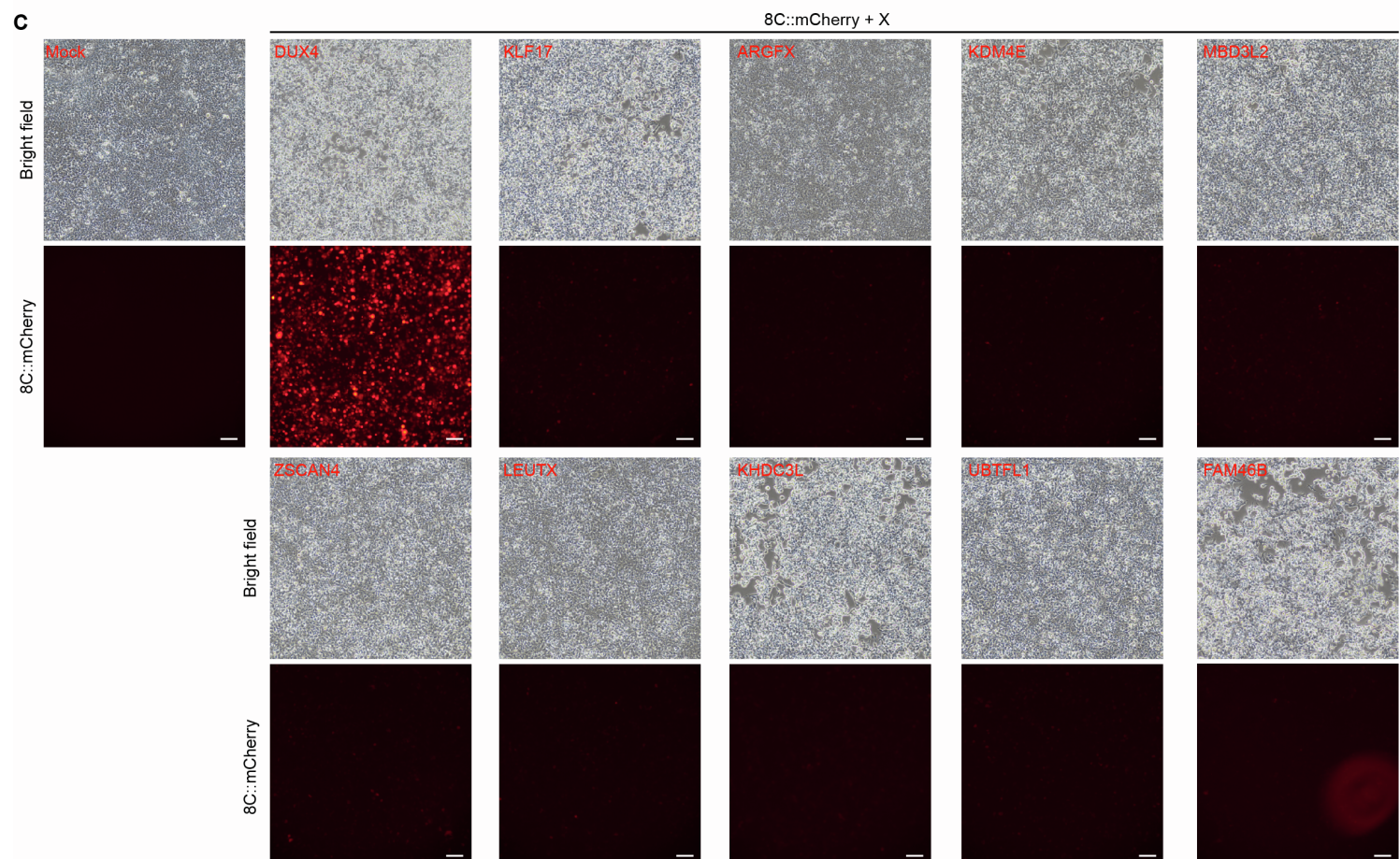
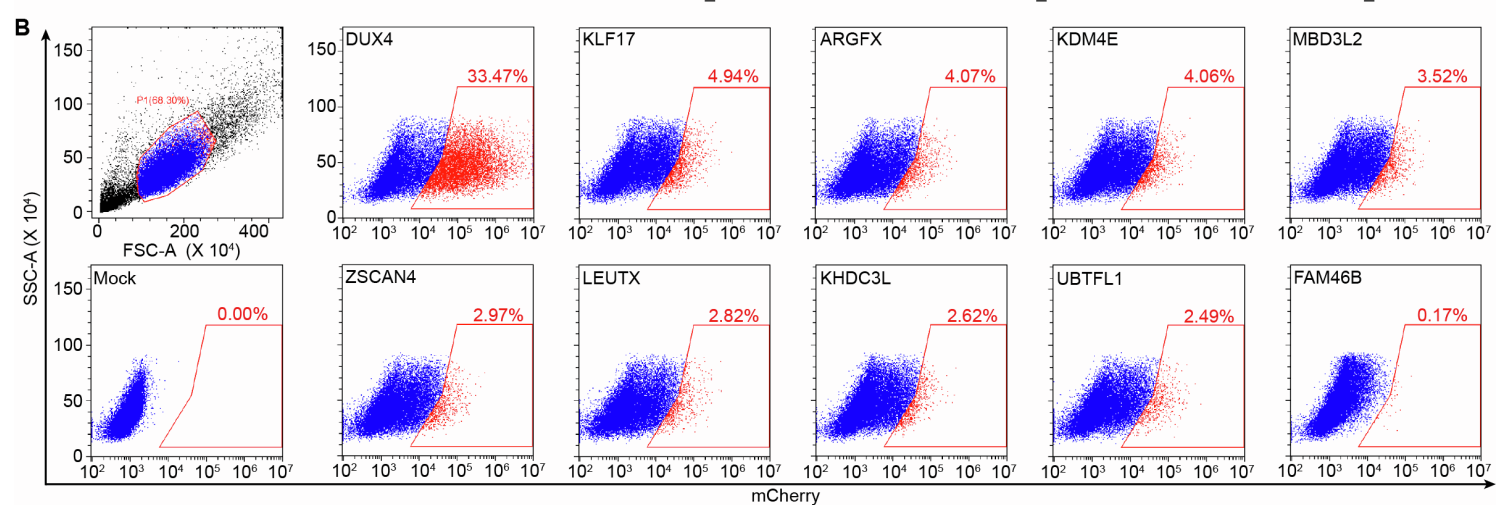
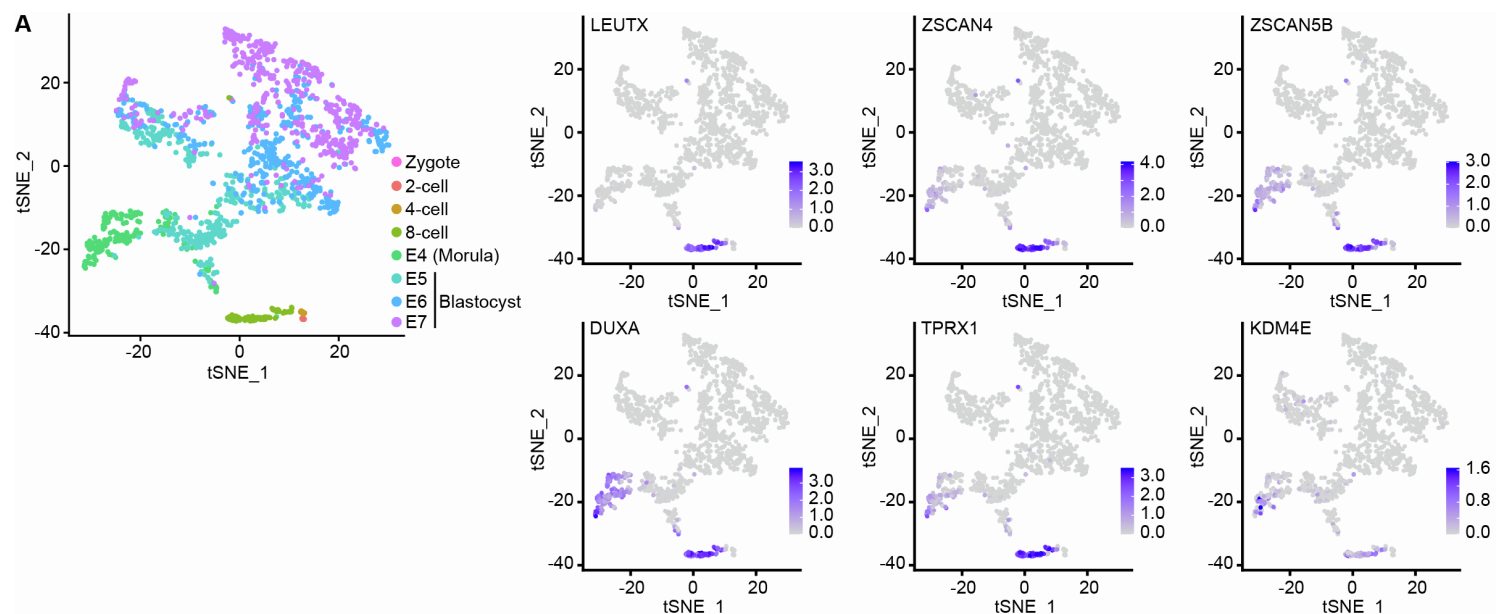
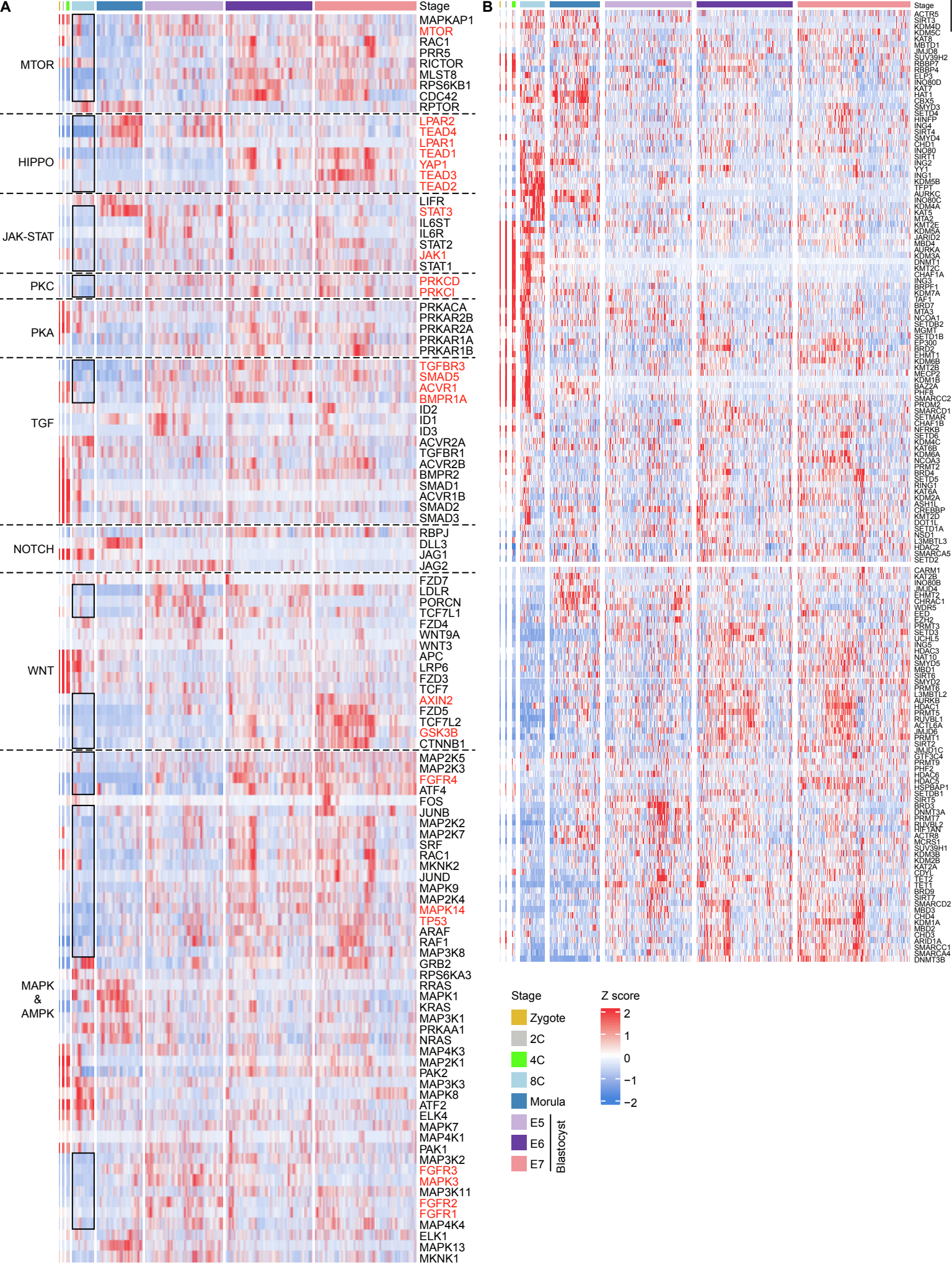


Figure S4. Development of the 8C::mCherry reporter, related to Figure 3

(A) Expression levels of selected 8C-specific transcription factors and epigenetic modifiers in early human embryos.

(B) FACS analysis of the percentage of 8C::mCherry⁺ HEK293 cells under overexpression of individual 8C-specific gene.

(C) The representative images showing differential activation of the 8C::mCherry reporter by 8C-specific transcription factors and epigenetic modifiers. Scale bar, 100 μ m.



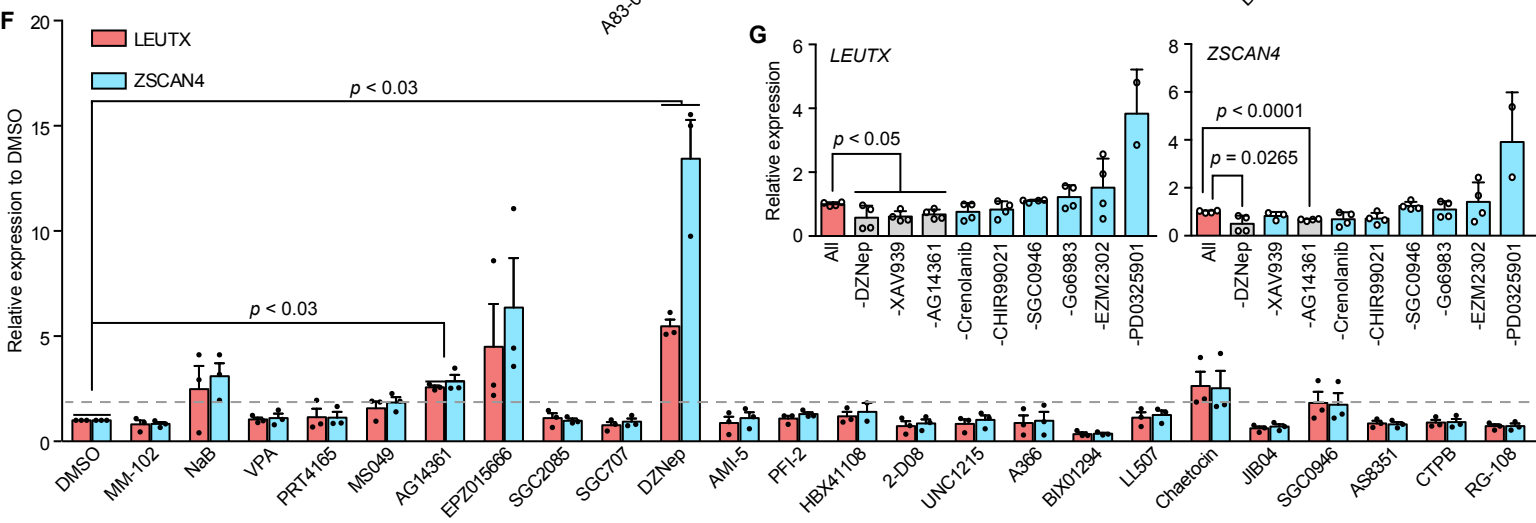
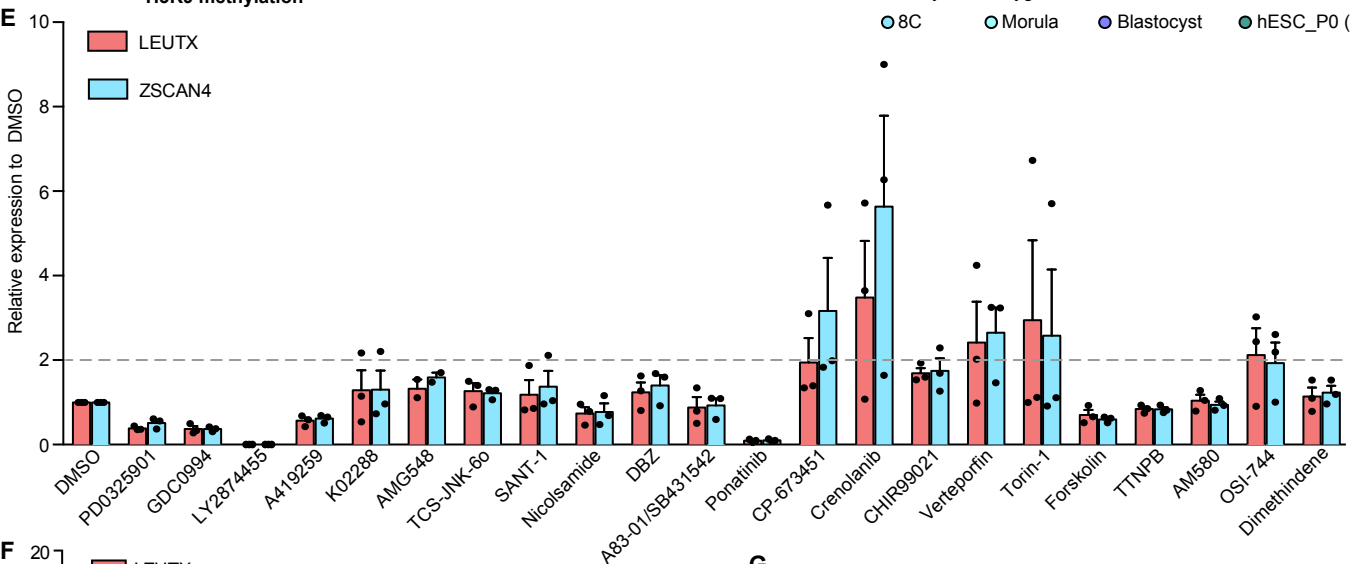
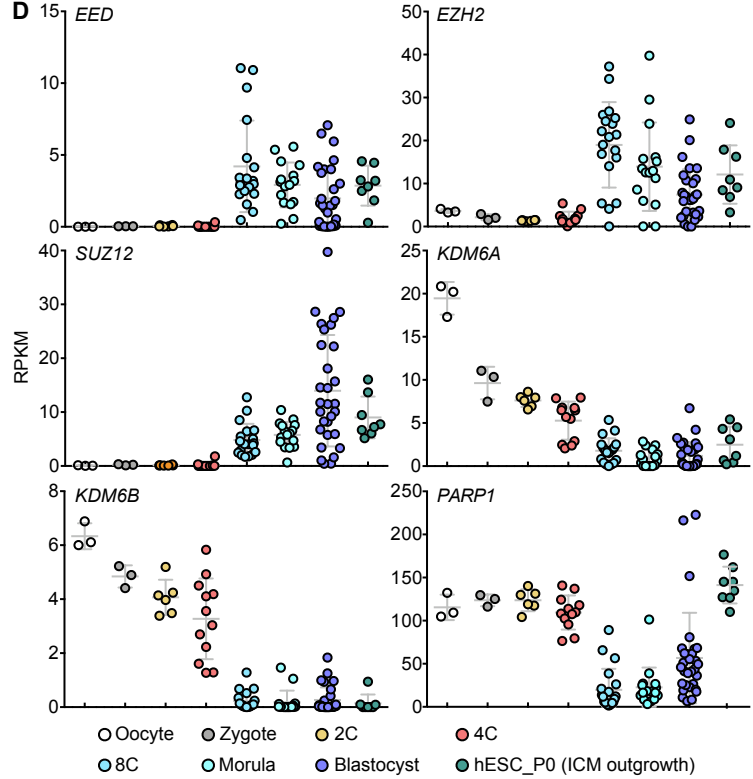
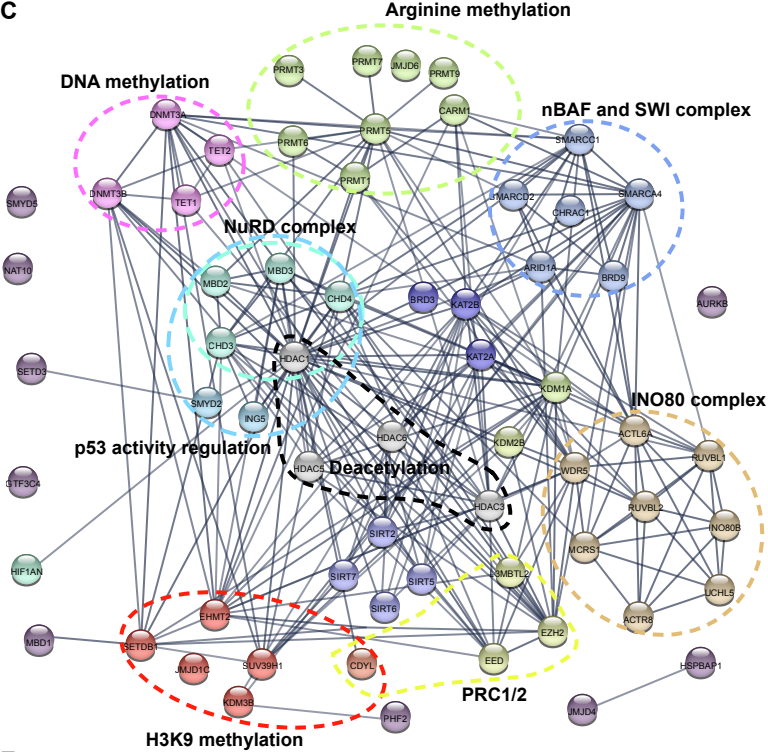


Figure S5. Optimization of culture conditions for maintenance of 8CLCs, related to Figure 4

(A) Heatmap showing the relative expression of genes associated with multiple signaling pathways in early human embryos (Petropoulos et al., 2016; Yan et al., 2013).

(B) Heatmap showing the relative expression of multiple epigenetic regulators in early human embryos (Petropoulos et al., 2016; Yan et al., 2013).

(C) The interaction of downregulated epigenetic regulators in human 8C embryos compared to blastocysts.

(D) The expression of PRC2-associated genes, demethylases of H3K27, and PARP1 in early human embryos (Yan et al., 2013).

(E) and (F) RT-qPCR analysis of expression of *LEUTX* and *ZSCAN4* in prEpiSCs under chemical treatment indicated. Data are shown as means \pm S.D. (n = 3 independent experiments).

(G) RT-qPCR analysis of expression of *LEUTX* and *ZSCAN4* in prEpiSCs under withdrawal of indicated chemical. Data are shown as means \pm S.D. (n = 2 - 4 independent experiments).