

Figure S1

**Figure S1. Verification of the pluripotency of EPS cells and the development of EPS-blastoids after implantation, Related to Figure 1**

(A) Representative images showing the clones morphology of EPS cells (derived from blastocyst or converted from ES cells) and ES cells derived from blastocyst. Scale bar, 100  $\mu\text{m}$ .

(B) Brightfield image of EPS cells-derived mice (arrow-marked) via the tetraploid complementation method, and its offspring (asterisk-marked) produced by mated with ICR females.

(C and D) Immunofluorescence staining of EPS-blastoids. Staining for NANOG (EPI), OCT4 (EPI), CDX2 (TE), PDGFR $\alpha$  (PrE), SOX17 (PrE). Scale bars, 20  $\mu\text{m}$ .

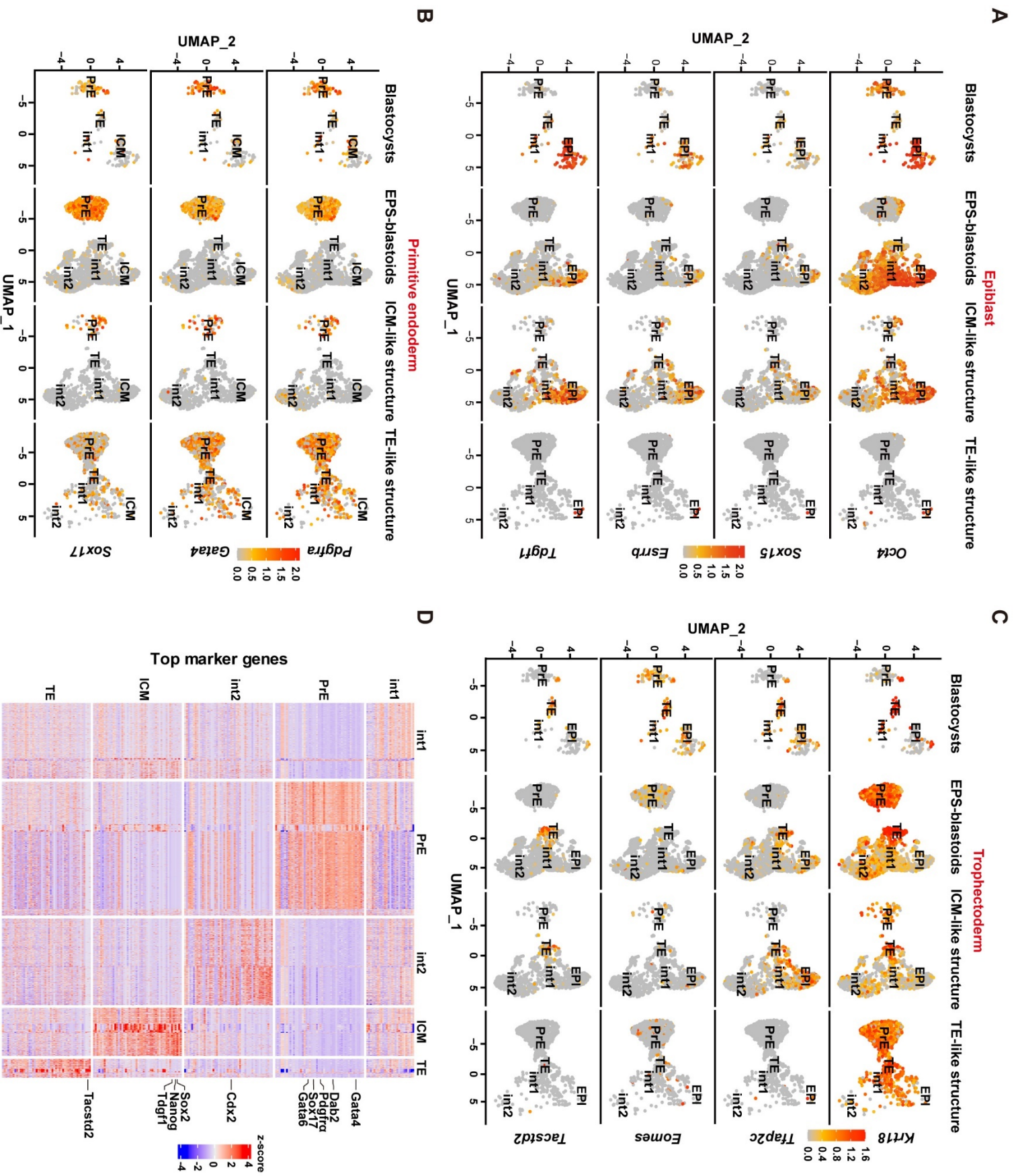
(E) The percentage of different TE-like structure categories of EPS-blastoids formed by different cell lines based on the expression of CDX2 and SOX17.

(F) The percentage of different ICM-like structure categories of EPS-blastoids formed by different cell lines based on the expression of OCT4 and CDX2. W/wo: with or without.

(G) The efficiency of induced decidua by EPS-blastoids and EBs (right). Data are represented as mean  $\pm$  SD.

(H) Brightfield (left) and fluorescent (right) images of dissected E6.5 decidua induced by EPS-blastoid (GFP-marked). Scale bar, 100  $\mu\text{m}$ .

(I) Pie charts showing the frequency of different IVC product categories of EPS-blastoids formed by different cell lines based on the expression of TFAP2C and OCT4.

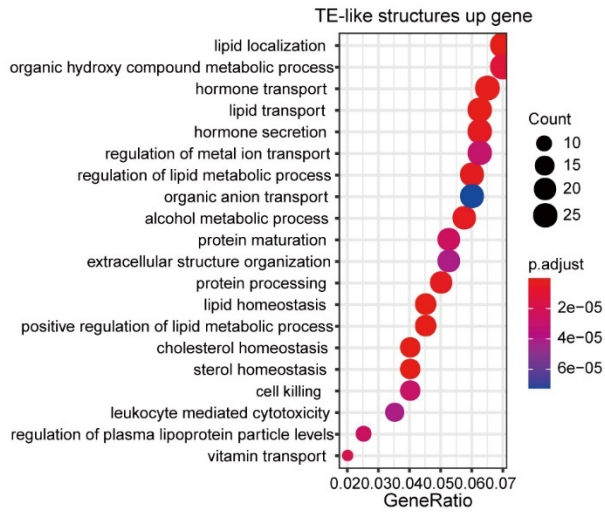


**Figure S2. Expression of lineage-specific markers from scRNA-seq data, Related to Figure 2**

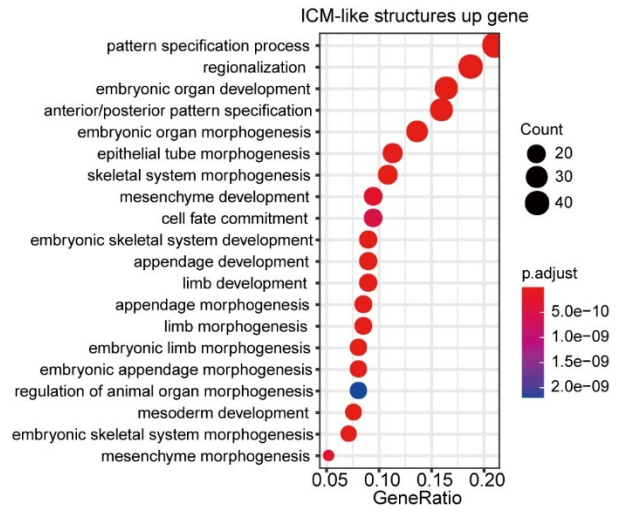
(A-C) UMAP embedding of EPS-blastoids and blastocysts. Shown is the expression of three lineage markers for Epiblast (A), Primitive endoderm (B) and Trophectoderm (C), respectively. int-1: intermediate state 1, int-2: intermediate state 2.

(D) Heat map indicating the expression of lineage-specific genes in different clusters. For visualization purposes, the top 50 marker genes were shown, and ordered by significance.

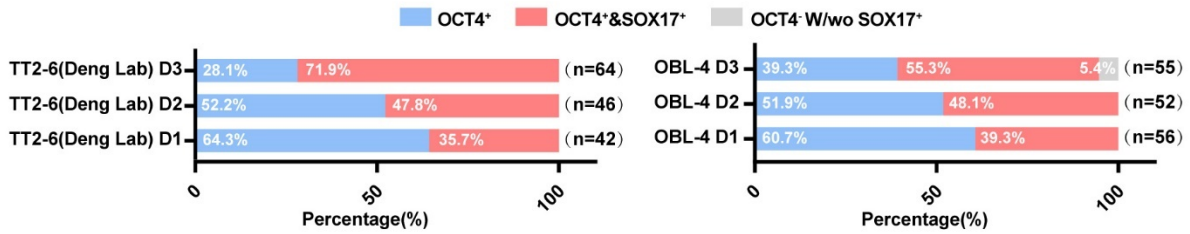
**A**



**B**



**C**



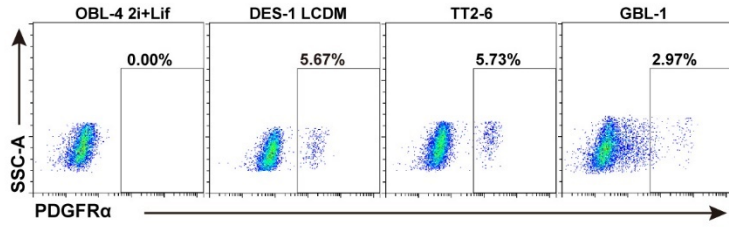
**Figure S3**

**Figure S3. Functional analysis of genes up-regulated in TE-like structure and ICM-like structure, and the cell lineage changes with the formation of blastoids. Related to Figure 3**

(A and B) GO analysis of genes up-regulated in TE-like structure (A) and ICM-like structure (B).

(C) The percentage of different aggregation product categories at different points in time (day1, day2, and day3) based on the expression of OCT4 and SOX17.

**A**



**Figure S4**

**Figure S4. FACS analysis of the PrE-like cells in EPS cell lines, Related to Figure 4**

(A) Representative FACS analysis of the percentages of PDGFR $\alpha$ <sup>+</sup> cells in ES cell line (OBL-4 2i+Lif) and EPS cell lines (DES-1 LCDM, TT2-6 and GBL-1).

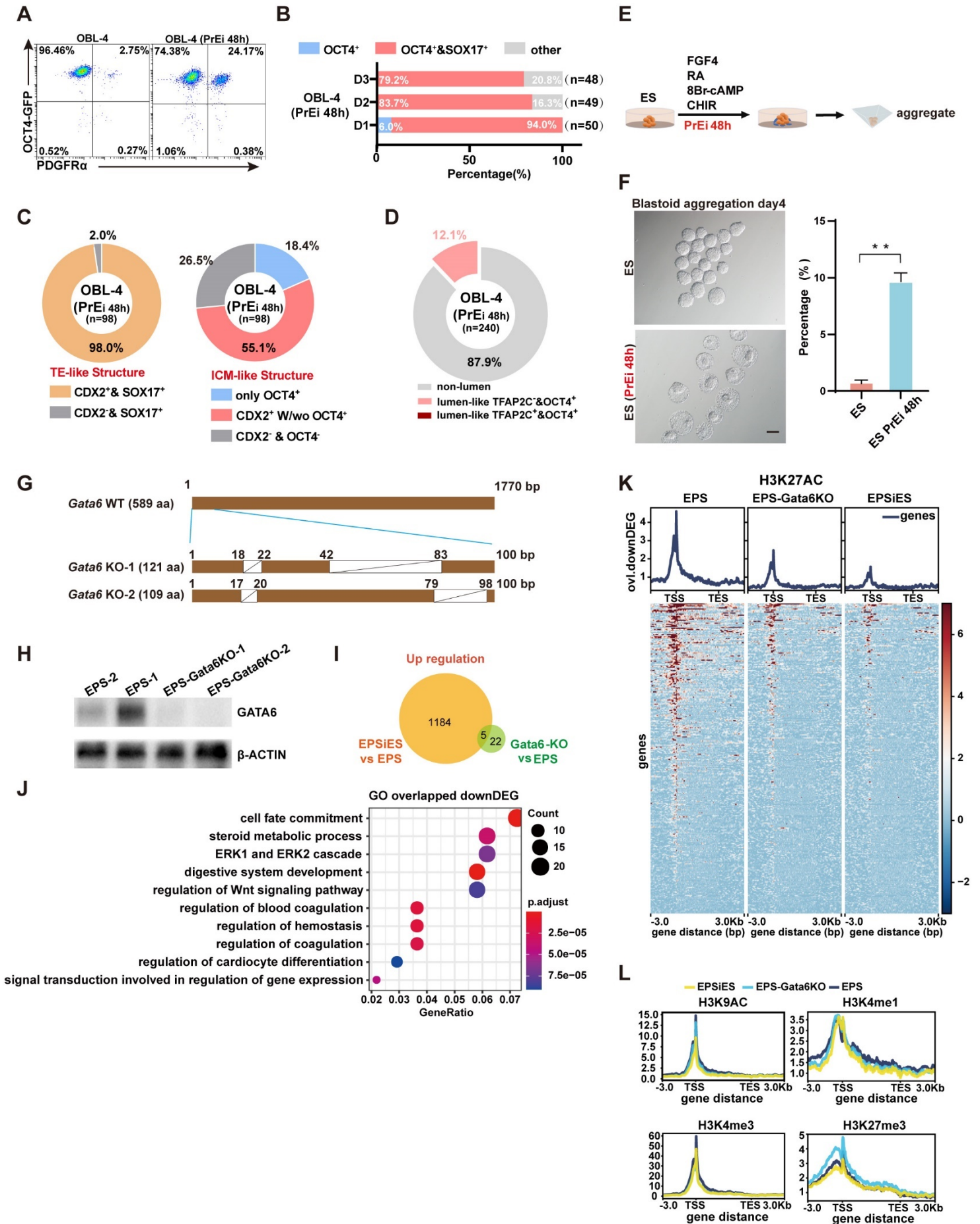


Figure S5

**Figure S5. Promotion of EPS-blastoid formation by PrE differentiation, Related to Figure 5**

(A) Representative FACS analysis of the percentages of OCT4<sup>+</sup>PDGFR $\alpha$ <sup>+</sup> cells in OBL-4 and OBL-4 PrEi (PrE induction medium treat for 48 hours).

(B) The percentage of different aggregation product categories at different points in time (day1, day2, and day3) based on the expression of OCT4 and SOX17. This aggregation product formed by OBL-4 PrEi (PrE induction medium treat for 48 hours).

(C) Pie charts showing the frequency of different TE-like structure (left) and ICM-like structure (right) categories of EPS-blastoids formed by OBL-4 PrEi (PrE induction medium treat for 48 hours) based on the expression of CDX2, SOX17 and OCT4.

(D) Pie chart showing the frequency of different IVC product categories of blastoids formed by OBL-4 PrEi (PrE induction medium treat for 48 hours) based on the expression of TFAP2C and OCT4.

(E) Schematic showed the process of blastoids aggregation of ES cells treated with PrE induction medium (N2B27 supplemented with FGF4, Retinoic acid, 8Br-cAMP and CHIR) for 48 hours.

(F) The representative morphology images of product aggregated by ES cells before and after PrE induction medium treatment (left), quantification of blastoids formation efficiency with ES cells under these two conditions (right). Data are represented as mean  $\pm$  SD; \*\*\* $P < 0.001$ , unpaired student's t test. Scale bar, 100  $\mu$ m.

(G) The genotypes of EPS-Gata6KO-1 and EPS-Gata6KO-2 cell line.

(H) Western blot analysis for the GATA6 protein levels in the EPS-2, EPS-1, EPS-Gata6KO-1 and EPS-Gata6KO-2 cells.

(I) Venn diagram for the number of shared up-regulated genes between the indicated groups.

(J) GO analysis of the shared down-regulated genes (303 genes, Shown in the figure 5N).

(K) H3K27ac signals at the TSS of shared down-regulation genes (303 genes, Shown in the figure 5N) in EPS, EPS-Gata6KO and EPSiES, represented as normalized RPKM

values.

(L) Average H3K9AC, H3K4me1, H3K4me3 and H3K27me3 signals at the TSS of shared down-regulation genes (303 genes, Shown in the figure 5N) in EPS, EPS-Gata6KO and EPSiES, represented as normalized RPKM values.

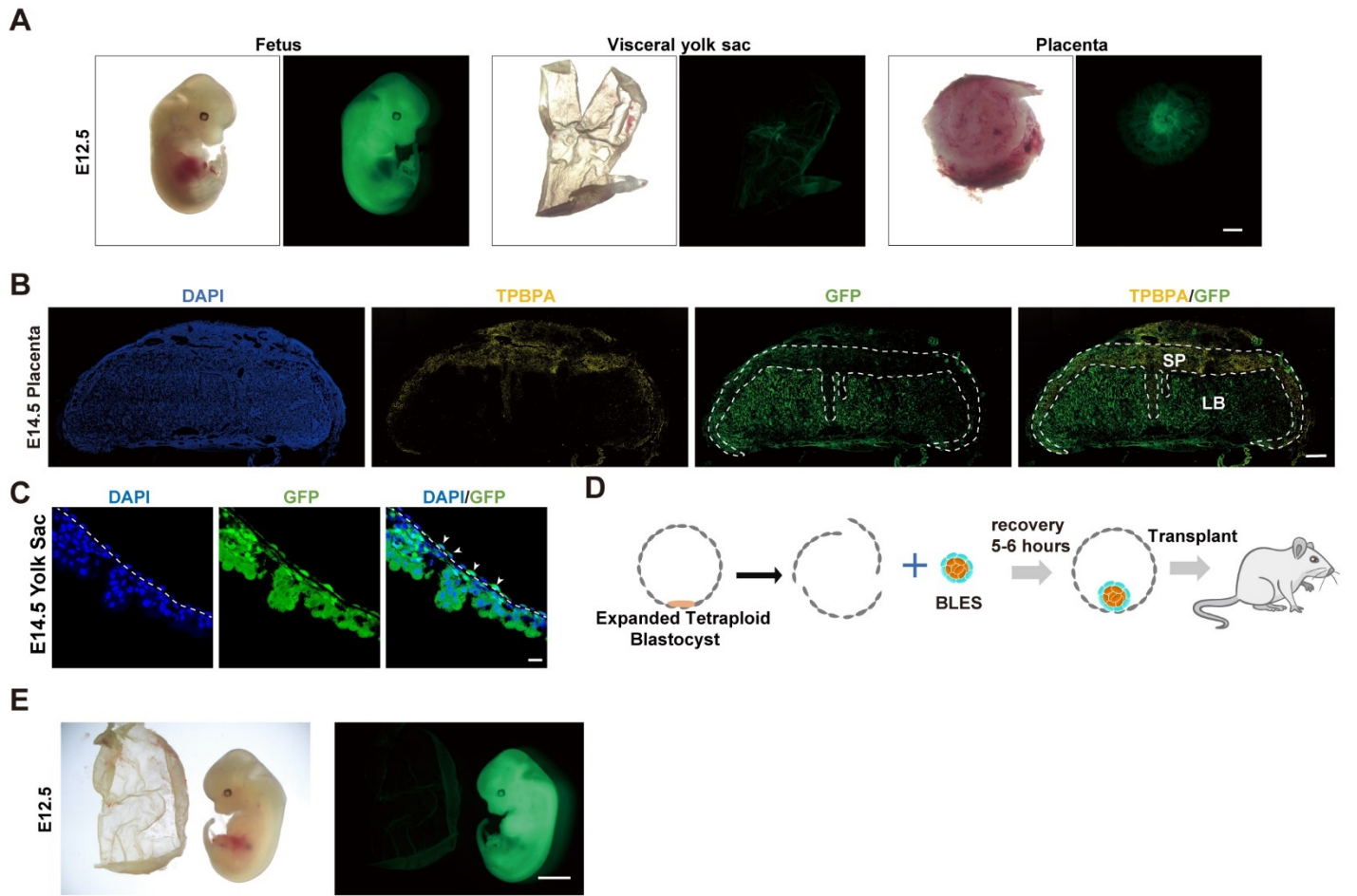


Figure S6

**Figure S6. Verification of bidirectional pluripotency of BLES, Related to Figure 6**

(A) Representative images of E12.5 chimeric embryos (containing a fetus, visceral yolk sac and placenta) produced by method in Figure 6E. Scale bar, 1000  $\mu\text{m}$ .

(B) Immunofluorescence staining of placenta of E14.5 chimeric embryos produced by method in Figure 6E. Staining for DAPI (nucleus), GFP (doner cells) and TPBPA (spongiotrophoblast layer). LB: labyrinth layer, SP: spongiotrophoblast layer. Scale bar, 500  $\mu\text{m}$ .

(C) Immunofluorescence staining of yolk sac of E14.5 chimeric embryos produced by method in Figure 6E. Staining for DAPI (nucleus) and GFP (doner cells). Scale bar, 20  $\mu\text{m}$ .

(D) Schematic showed the procedure of chimeras. Firstly, TE of tetraploid embryos at E4.5 period were digested into sheets, then aggregated with BLES (produced with marked GFP EPS cells treated by PrE induction medium for 48 hours) of day1-2. After recovering for 5-6 hours, the chimeric embryos were transplanted into the uterus of a 2.5 dpc pseudo-pregnant mouse.

(E) Representative images of E12.5 chimeric embryo produced by above method (Figure S6D). Scale bar, 2000  $\mu\text{m}$ .