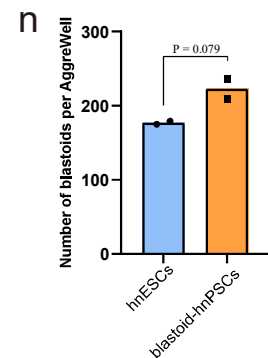
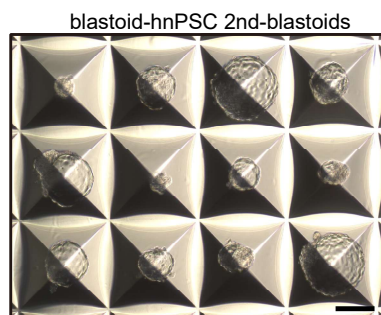
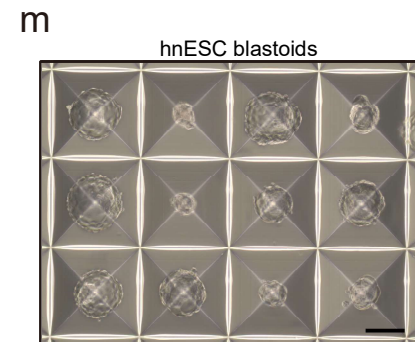
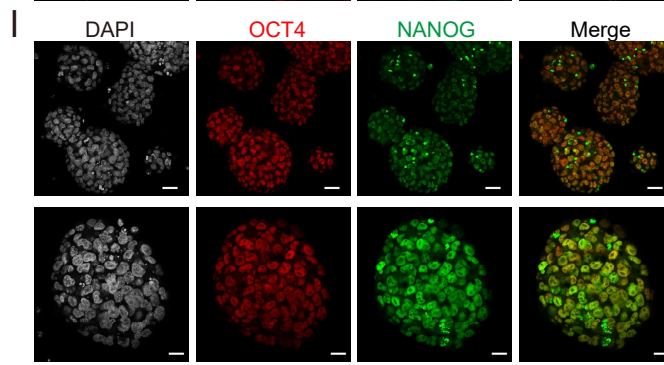
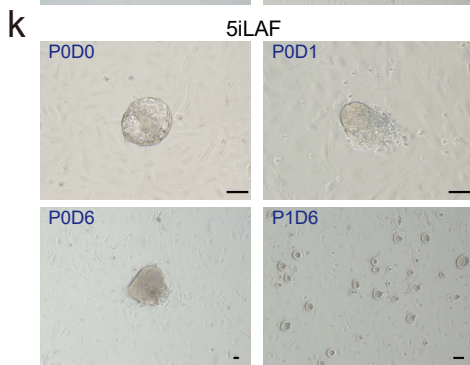
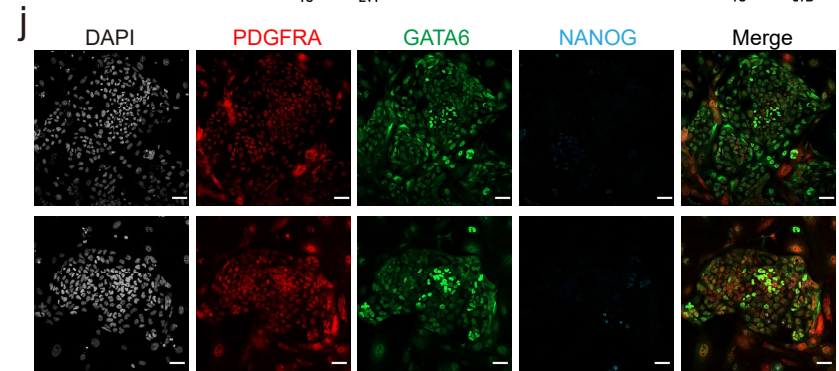
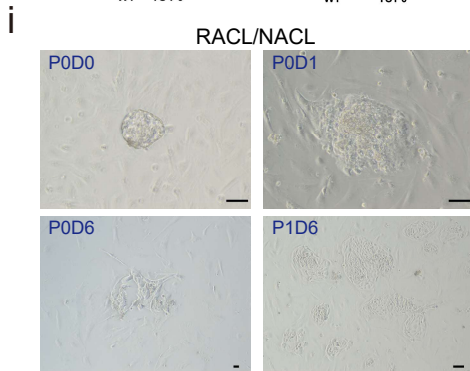
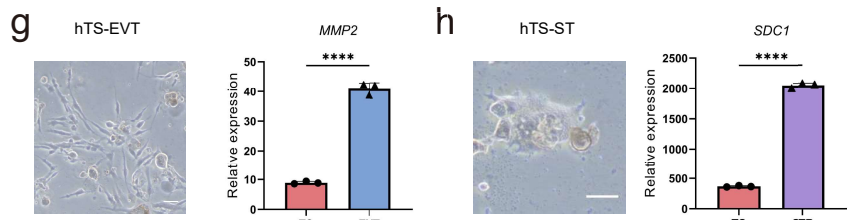
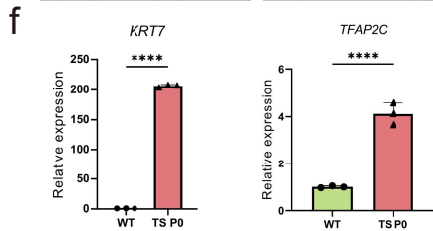
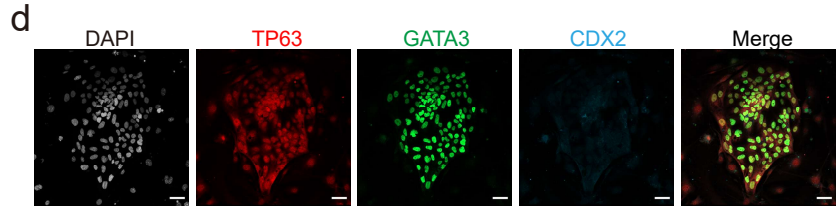
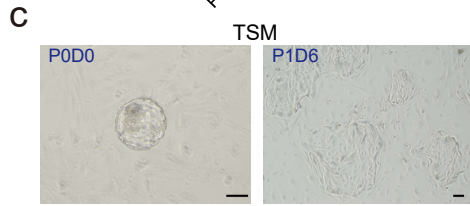
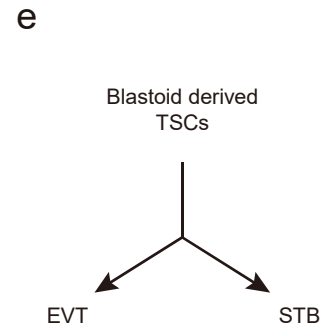
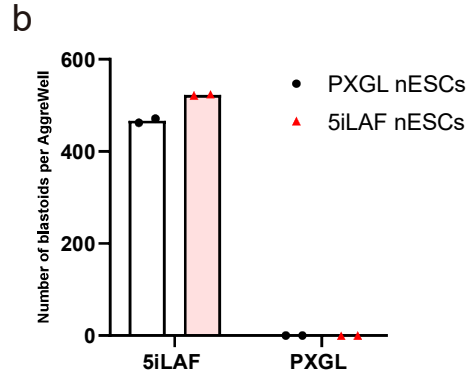
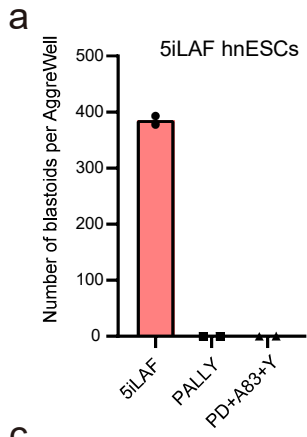
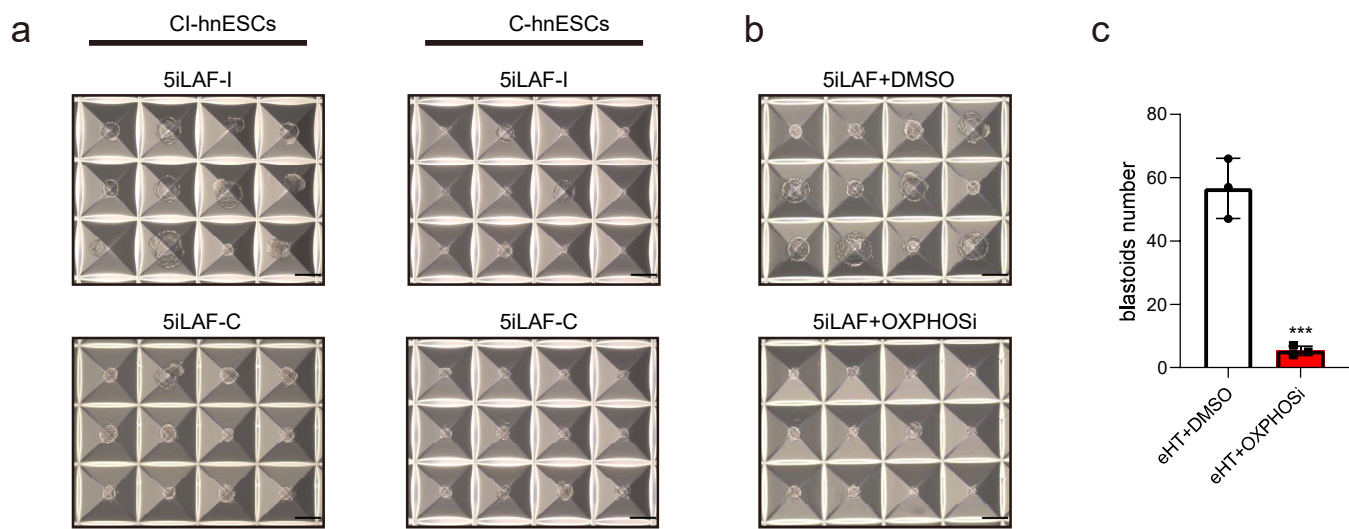


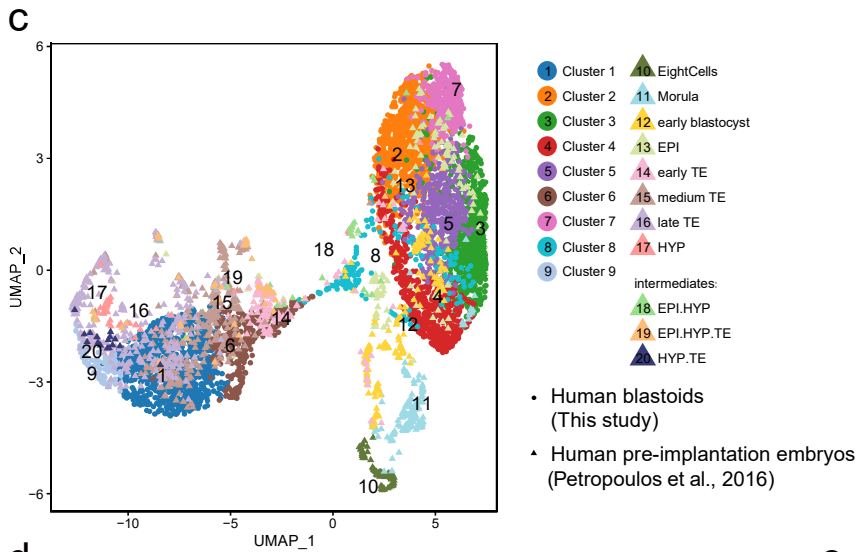
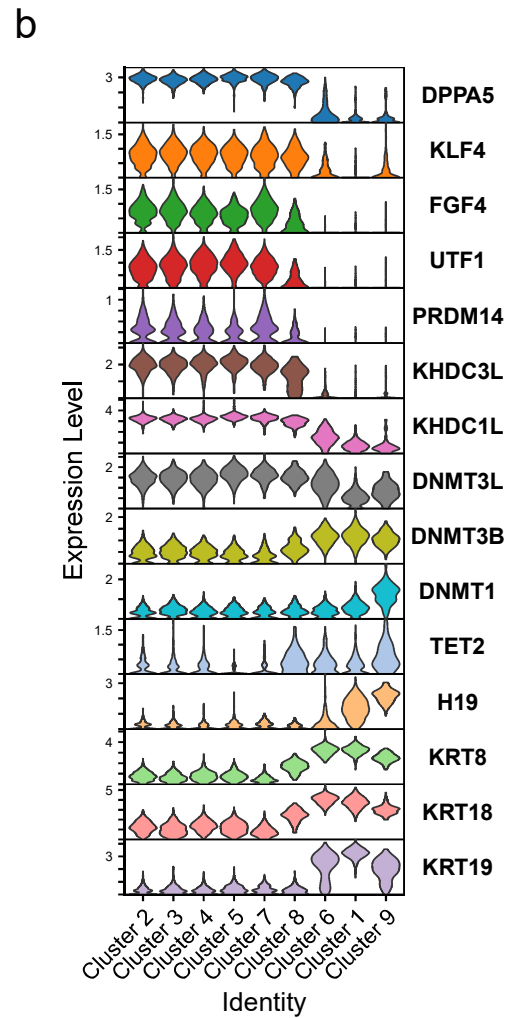
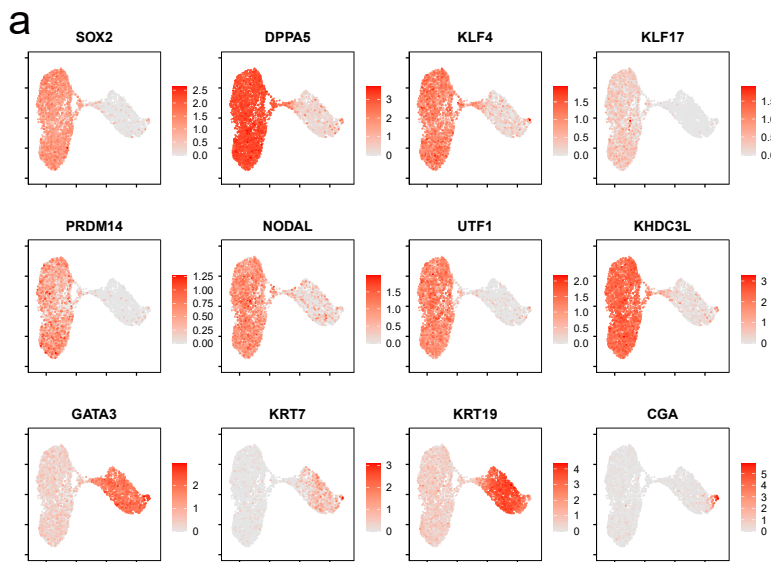
Supplementary Figure 1. **Blastocyst-like structures emerge spontaneously during hnESC culture.** **a** Representative phase-contrast images of spontaneous cysts in the hnESC cultures under different magnifications. **b** Quantification of diameter of spontaneous cysts (day2, $n = 16$, $39.13 \pm 6.95 \mu\text{m}$; day3, $n = 16$, $59.61 \pm 10.55 \mu\text{m}$). **c** Representative immunofluorescence staining images of spontaneous cysts in (a). **d** Representative phase-contrast images of blastocyst-like structures from day 6 suspended hnESC colonies. **e** Representative immunofluorescence staining images of blastocyst-like structures in (d). Scale bars, 20µm. **f** Representative immunofluorescence staining images of hnESCs, day 1 aggregates and day 6 blastoids from AggreWell. Scale bars, 20µm.



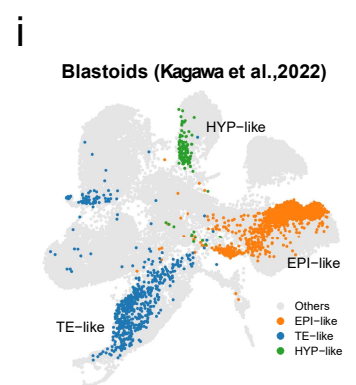
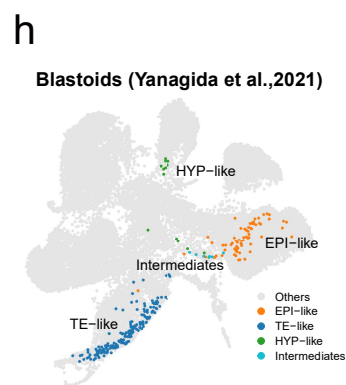
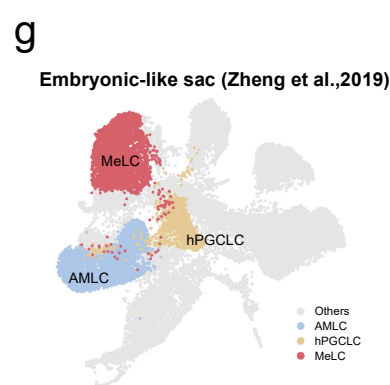
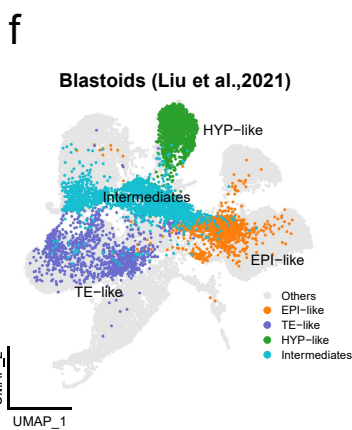
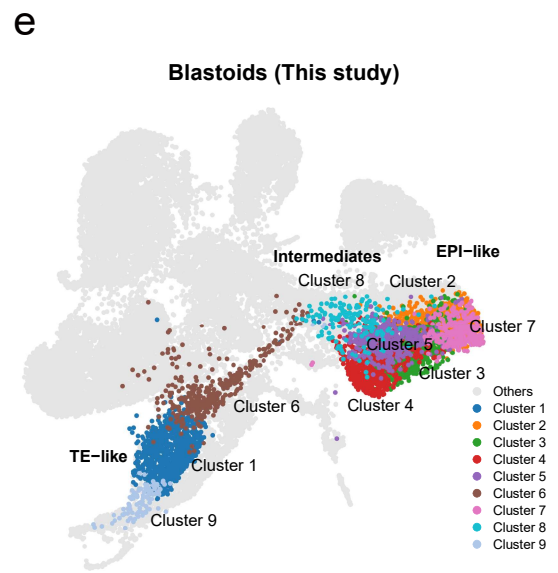
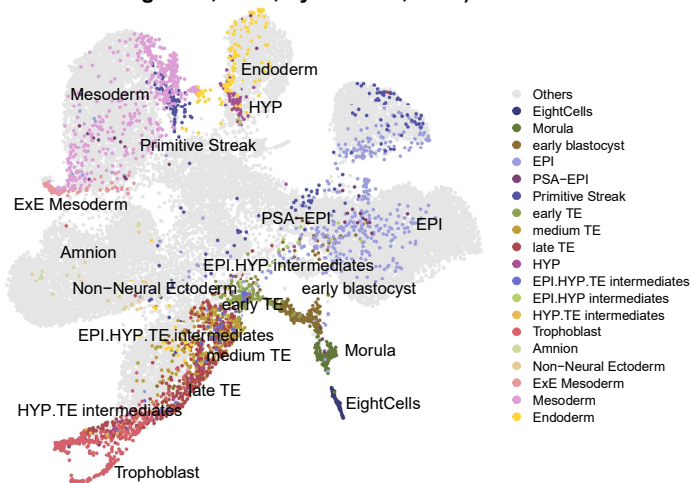
Supplementary Figure 2. **Generation of spontaneous blastoids and derivation of early embryo-like stem cell lines.** **a** Number of blastoids generated from hnESCs in AggreWells with 5iLAF medium or published protocols. PALLY; PD+A83 +Y. **b** Number of blastoids generated from PXGL and 5iLAF cultured hnESCs in AggreWells with 5iLAF or PXGL media. **c, d** Derivation (c) and representative immunofluorescence staining images (d) of TSCs from day6-blastoids. TSM, trophoderm stem cell medium. P0D0, day 0 of passage 0 and so on. Scale bars, 100 μ m. **e** Schematic of EVT and ST differentiation from blastoids-derived TSCs. **f** Quantitative gene expression of selected TS markers in P0 TSCs. **g, h** Brightfield images and representative marker expression of EVT like cells (g) and ST like cells (h) differentiated from spontaneous blastoid-derived TSCs respectively. Scale bars, 50 μ m. **i, j** Derivation (i, scale bars, 100 μ m) and representative immunofluorescence staining images (j, scale bars, 50 μ m) of nEnd cells from day6-blastoids. RAEL and NAEL, nEnd cells derivation and culture media, respectively. P1D6, day6 of passage 1 and so on. **k, l** Derivation (k, scale bars, 100 μ m) and representative immunofluorescence staining images (l, scale bars, 50 μ m) of blastoids-nPSCs from day6-blastoids. **m, n** Representative phase-contrast images (m) and quantification (n) of blastoids generated from hnESCs or blastoids-nPSCs in AggreWell with 5iLAF medium. Scale bars, 200 μ m. Unpaired two-tailed t-test. WT: hnESCs, TS P0: 1st generation of trophoblast stem cells directly derived from blastoids, TS: maintained blastoid-derived trophoblast stem cell line, EVT: extravillous trophoblast like cells, ST: syncytiotrophoblast like cells.



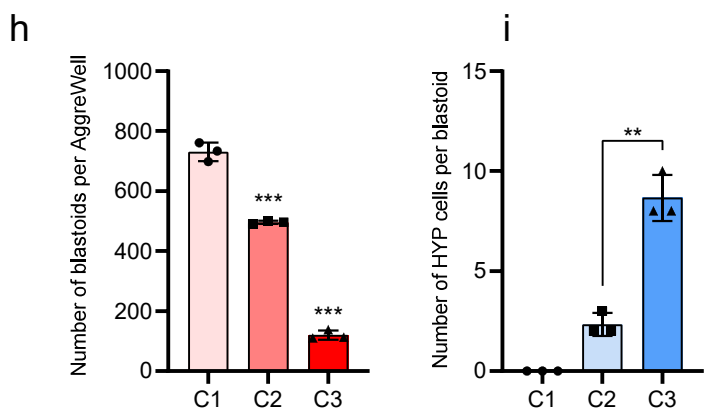
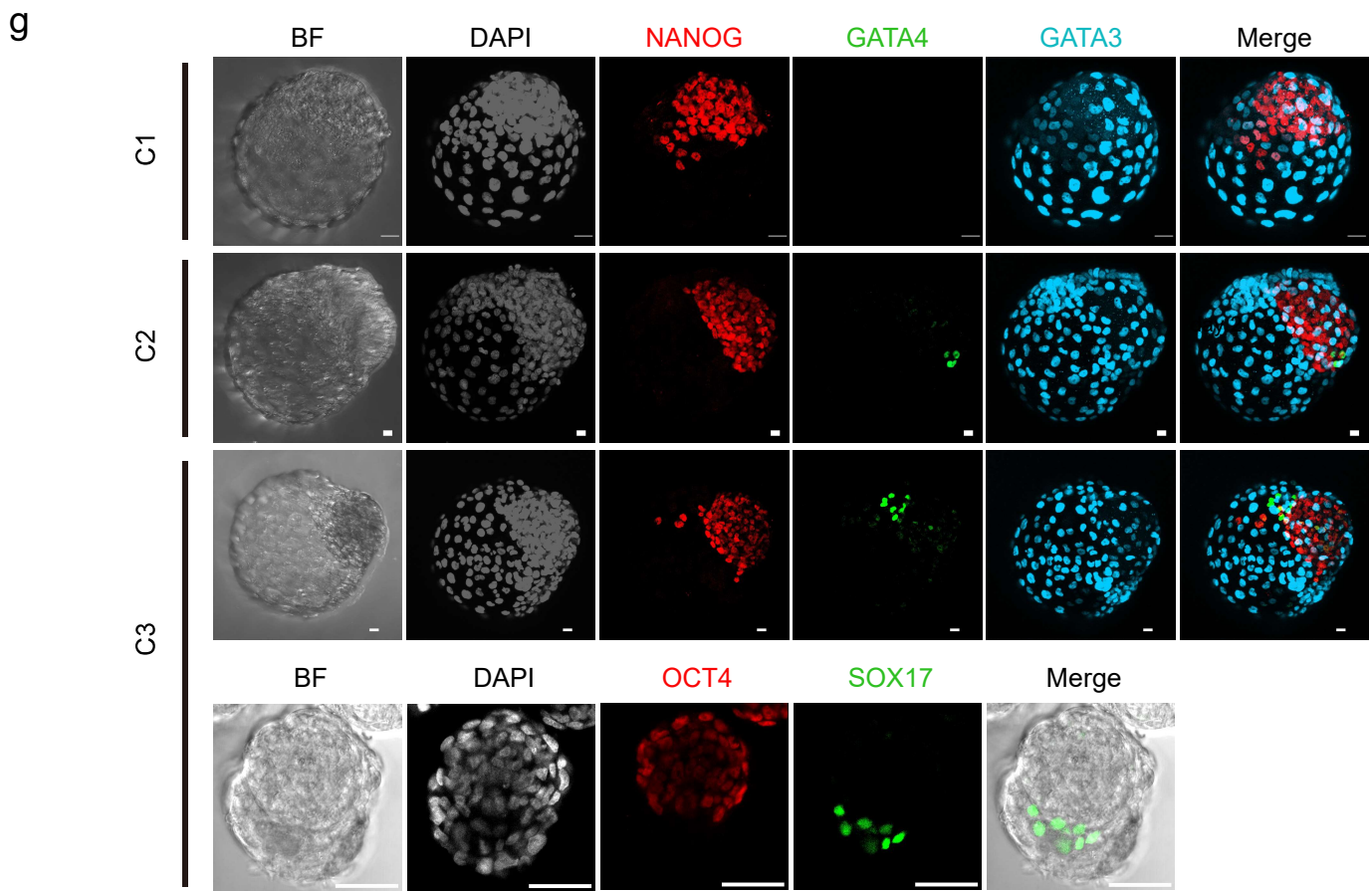
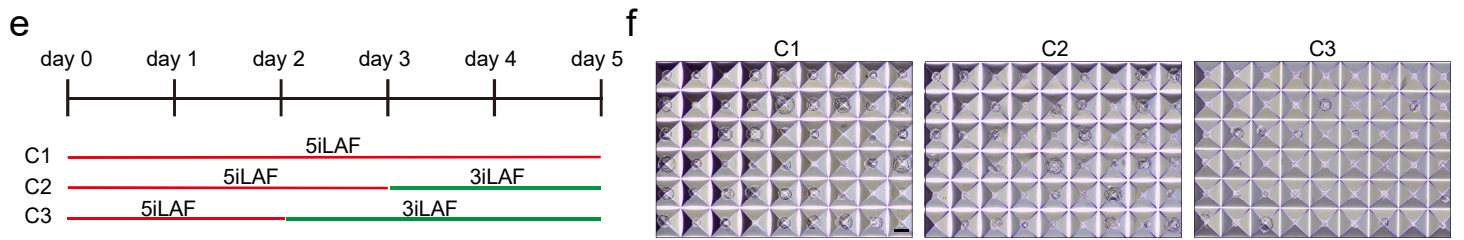
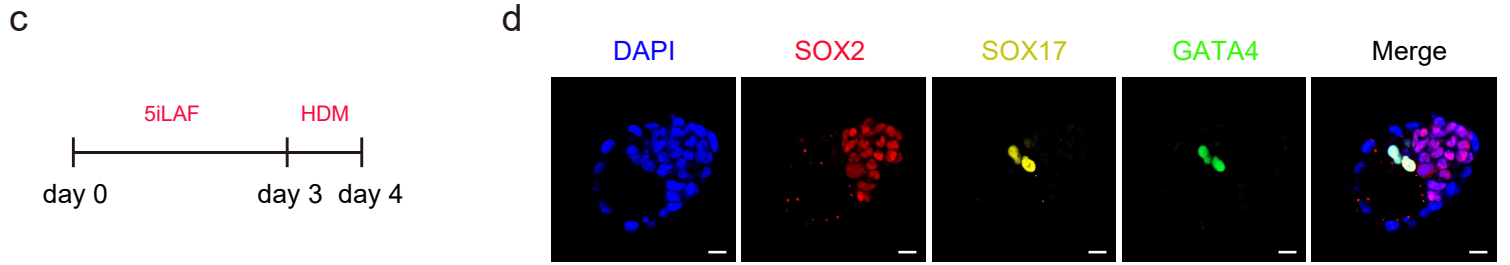
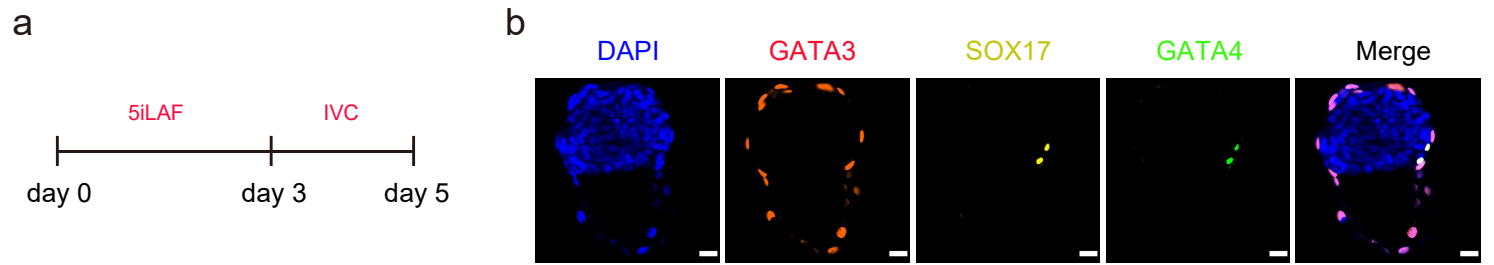
Supplementary Figure 3. **OXPHOS underlies the ability of hnESCs to generate blastoids spontaneously.** **a** Representative phase-contrast images of blastoids generated from CI-hnESCs (left) and C-hnESCs (right) with 5iLAF-I or 5iLAF-C medium in AggreWells. Scale bars, 200 μ m. **b** Representative phase-contrast images of blastoids generated from CI-hnESCs in 5iLAF-I with DMSO or OXPHOS inhibitor (Fig. 2h). **c** Blastoid inducing efficiency in the presence of DMSO or OXPHOS inhibitor (IACS-010759, 5 μ M) following reported eHT protocol.



d Human embryos (Petropoulos et al., 2016; Yanagida et al., 2021; Xiang et al., 2020; Tyser et al., 2021)

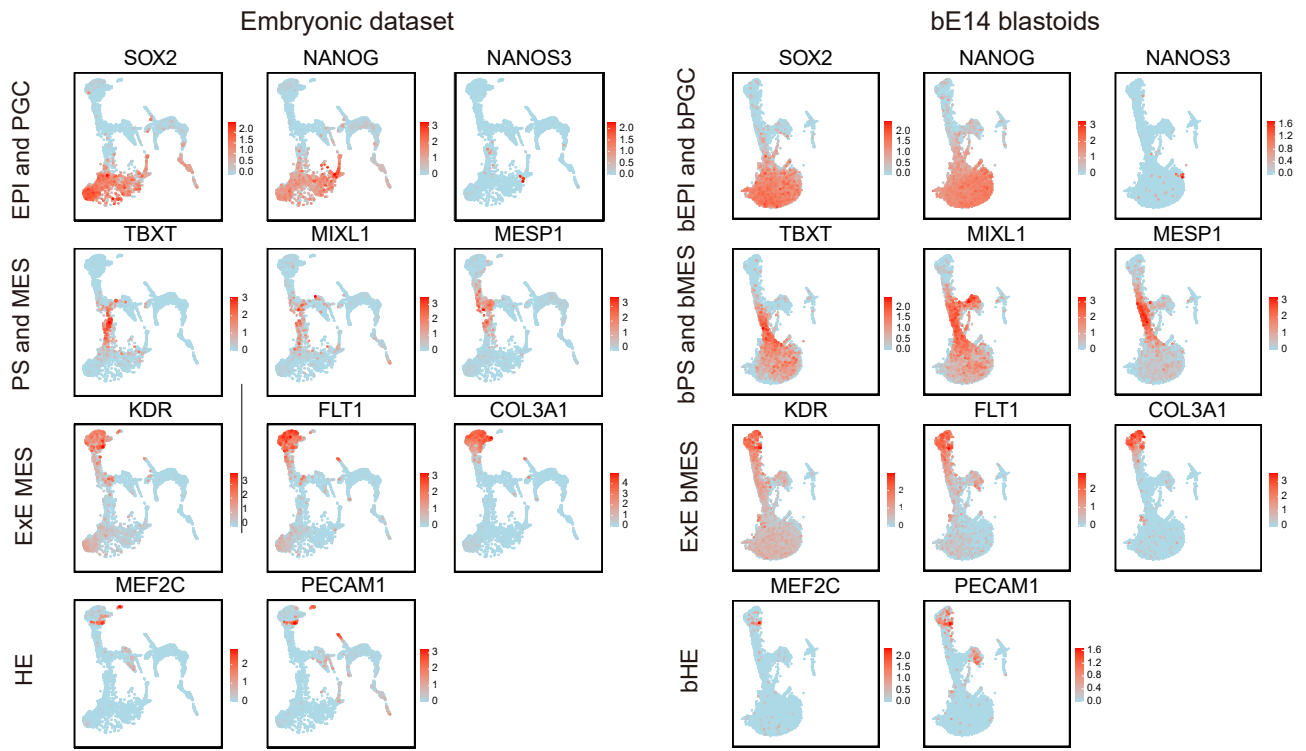


Supplementary Figure 4. **scRNA-seq analyses of human spontaneous blastoids.** **a** Expression levels of human blastocyst lineage markers. **b** Expression levels of representative genes enriched in clusters. **c** Joint UMAP of single-cell transcriptomes of cells from day6-blastoids (Fig.3f) integrated with published datasets from human early embryos (Fig.3e). **d-i** Joint UMAP of single-cell transcriptomes of cells from day6-blastoids (e) integrated with published datasets (d, f-i). PSA-EPI, primitive streak anlage stage epiblast. ExE, extraembryonic. AMLC, amniotic ectoderm-like cells. hPGCLC, human primordial germ cells -like cells. MeLC, Mesoderm-like cell.

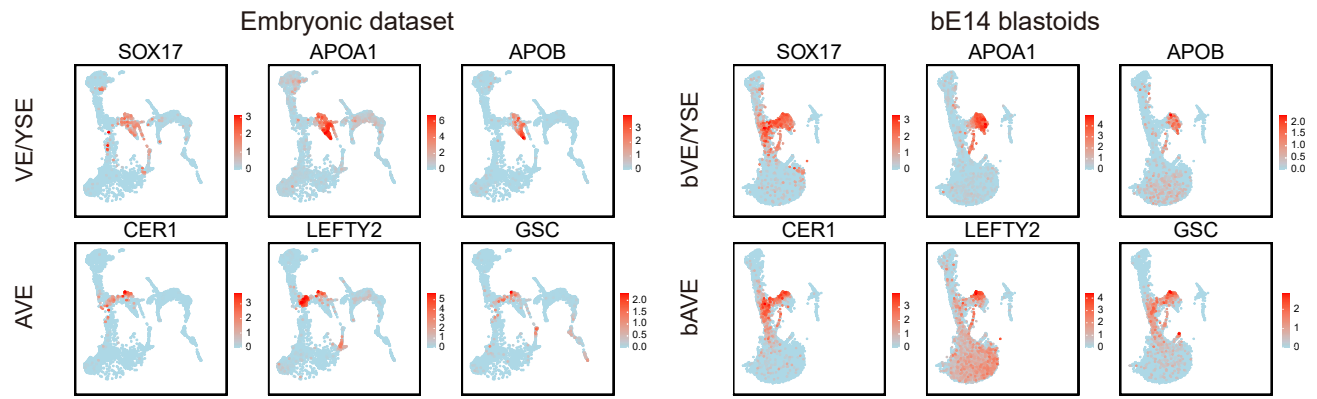


Supplementary Figure 5. **Spontaneous blastoids specify hypoblast-like cell fate.** **a-d** Schematic and representative immunofluorescence images of HYP-like cell containing blastoids generated using indicated culture conditions (a-d). Scale bars, 20 μ m. **e** Schematic of HYP-like cell induction by removing BRAF/MEK inhibitors. **f** Representative phase-contrast images showing blastoids in AggreWell. **g** Representative immunofluorescent images showing specification of GATA4+ or SOX17+ HYP-like cells in spontaneous blastoids. Scale bars, 20 μ m for GATA4 staining and 100 μ m for SOX17 staining. **h** Quantitative scoring of number of blastoids per AggreWell generated from the 3 different strategies. n = 3 (technical replicates). **i** Quantitative scoring of number of HYP cells per blastoid generated from the 3 different methods, n = 3 (technical replicates).

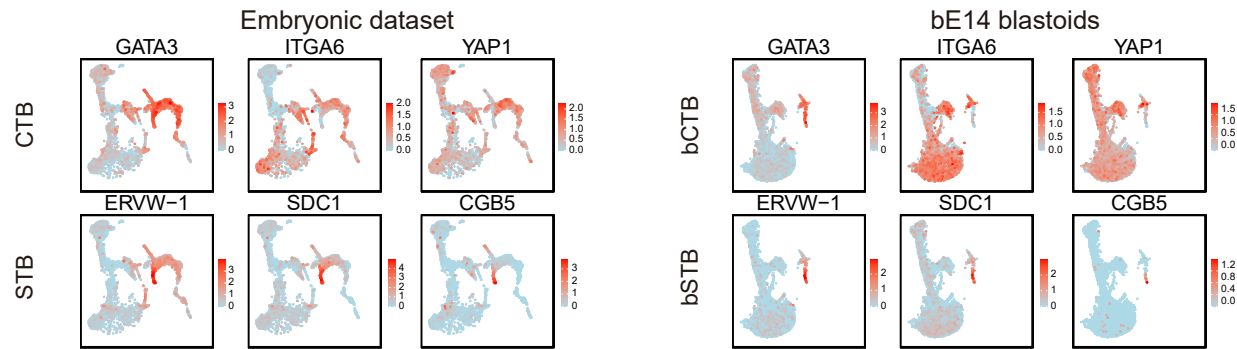
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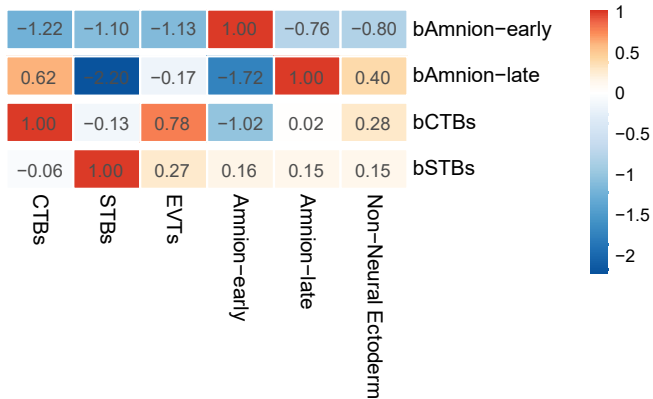
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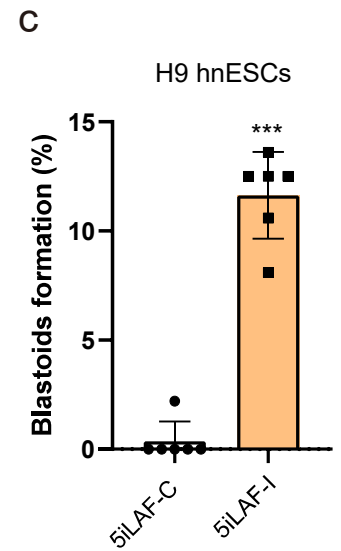
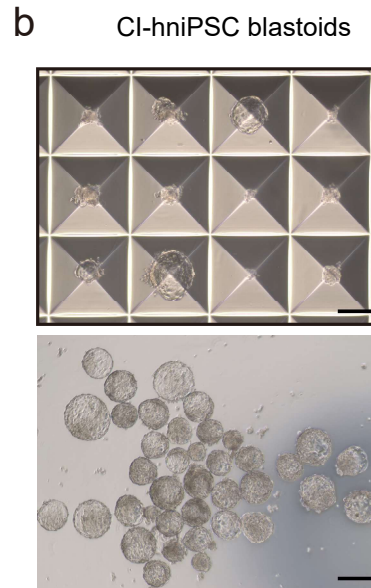
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Supplementary Figure 6. **Spontaneous blastoids have post-implantation development potential.** **a** Featureplot displaying key lineage markers between embryonic dataset (left) and bE14 blastoids. **b** Featureplot displaying key extraembryonic endodermal lineage markers between embryonic dataset (left) and bE14 blastoids. **c** Featureplot displaying key extraembryonic trophoblast lineage markers between embryonic dataset (left) and bE14 blastoids. **d** Gene set variance analysis (GSVA) comparing similarities of clusters bAmnion-early and -late to natural embryo trophoblast lineages.

a

	C-hniPSC		CI-hniPSC	
	number of colonies picked	number of blastoids formed	number of colonies picked	number of blastoids formed
5iLAF-C	53	0	44	1
	76	0	48	1
	68	0	45	0
5iLAF-I	48	3	38	11
	68	5	43	10
	40	3	41	7



Supplementary Figure 7. **Spontaneous blastoid generation in independent human PSCs.** **a** A table showing the exact statistics of picked colonies and formed blastoids number from C-hniPSC or CI hniPSC in 5iLAF-C or 5iLAF-I media. **b** Representative phase-contrast images of human spontaneous blastoids generated from dissociated CI-hniPSCs in AggreWell. Scale bars, 200 μ m. **c** Blastoids formation efficiency of picked H9 CI-hnESC colonies in 5iLAF-C (n = 6 technical replicates) and in 5iLAF-I (n = 6) media. mean \pm s.d.; unpaired two-tailed t-test; ***P < 0.001.