



Supplementary information, Fig. S1 Single-cell transcriptomic profiling of 3D-cultured human post-implantation embryos, related to Fig. 1. **a** Diameters of 3D-cultured human embryos used for 10x Genomics sequencing. From embryonic day (E10-14), the number of 3D-cultured human embryos (corresponding thawed blastocysts) collected at each time point was 6 (10), 5 (10), 5 (12), 4 (14) and 4 (17) at each time point, respectively. **b** and **c** Numbers and proportions of different subtypes of cells in extended cultured human embryos at different developmental time points according to the results of scRNA-seq data. **d** Violin plots of indicated genes expressed in EPI by scRNA-seq data. The smart-seq2 results are from published data.¹ **e** Dot plots of candidate genes specific for different cell subtypes. **f** and **g** Immunostaining of EPI, XEN and ExM markers in three E13 extended cultured human embryos. White ordinal numbers indicate section numbers, arrowheads indicate ExM

cells, and red arrowheads indicate few OCT4⁻GATA6⁻KDR⁺ ExM cells. **f** a twin embryo; **g** two singleton embryos. We cultured ten E6 blastocysts and obtained three normally developed E13 embryos for immunostaining. **h** The inferred WNT signaling pathway network. Circle sizes are proportional to the number of cells in each subpopulation and line weight represents the communication probability. E, embryonic day; EPI, epiblast; PostE–EPI and PostL–EPI, post-implantation early and late EPI; UC, undefined cell-type; AME, amniotic epithelium; PS, primitive streak; ExM, extraembryonic mesoderm; VE/YE, visceral/yolk sac endoderm; AVE, anterior visceral endoderm; XEN, extraembryonic endoderm. Scale bars, 100 μ m.

Reference

- 1 Xiang, L. *et al.* A developmental landscape of 3D-cultured human pre-gastrulation embryos. *Nature* **577**, 537-542 (2020).