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Supplemental Information

Bovine blastocyst-like structures derived from stem cell cultures

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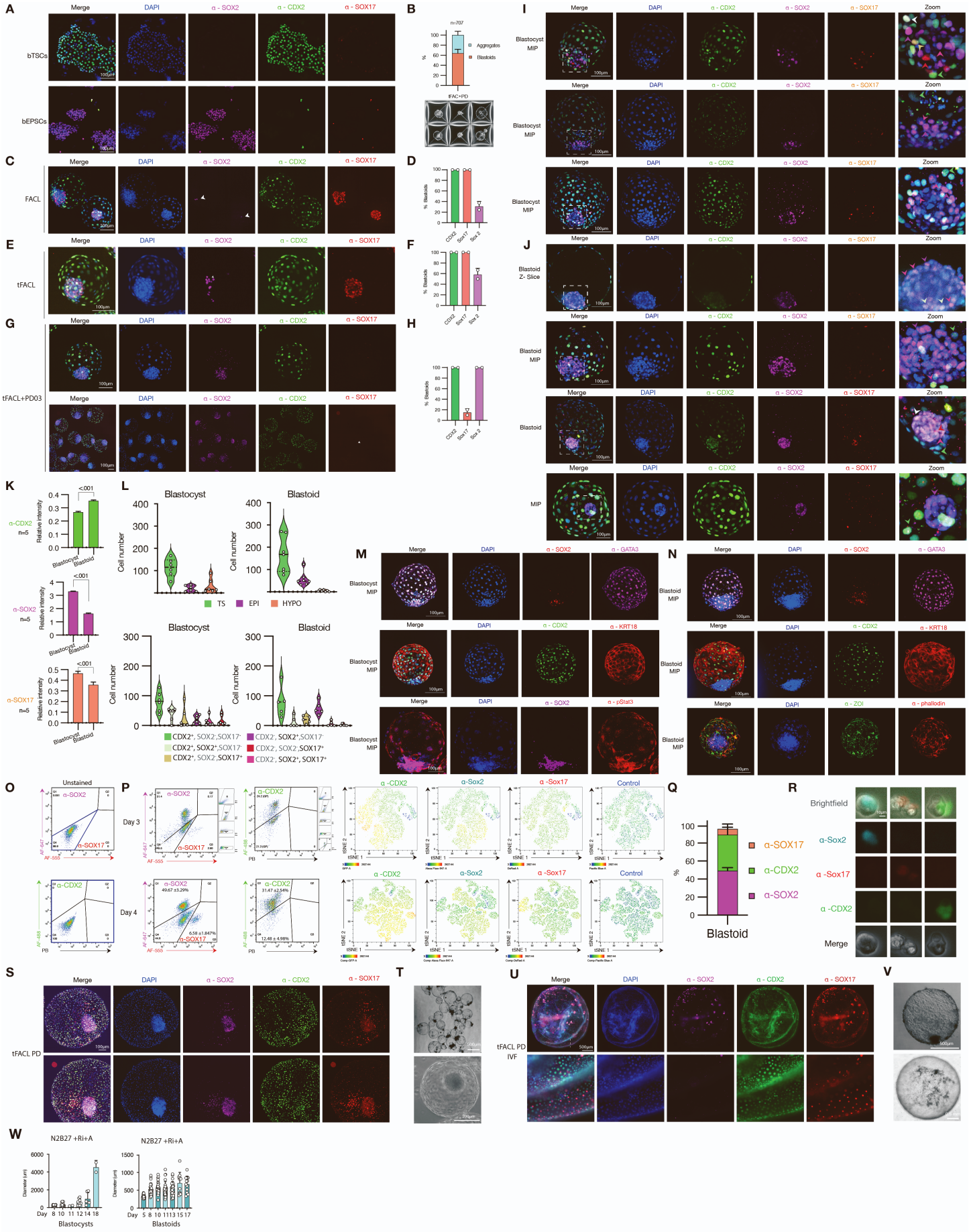

A
Merge
DAPI
α - SOX2
α - CDX2
α - SOX17
bTSCs
bEPSCs
B
n=707
Aggregates
Blastoids
IFAC+PD
C
FACL
D
% Blastoids
CDX2
Sox17
Sox 2
E
tFACL
F
G
tFACL+PD03
H
I
Blastocyst MIP
Zoom
J
Blastoid Z- Slice
Blastoid MIP
Blastoid
MIP
K
α-CDX2
n=5
α-SOX2
α-SOX17
Relative intensity
<.001
Blastocyst
Blastoid
L
Blastocyst
Blastoid
Cell number
TS
EPI
HYPO
CDX2+, SOX2-,SOX17-
CDX2-, SOX2+,SOX17-
CDX2+, SOX2+,SOX17-
CDX2-, SOX2-,SOX17+
CDX2+, SOX2-,SOX17+
CDX2-, SOX2+, SOX17+
M
α - GATA3
α - KRT18
α - pStat3
N
α - ZO1
α - phallodin
O
Unstained
P
Day 3
Day 4
α-CDX2
α-SOX2
α-SOX17
AF-555
AF-647
AF-488
PB
α -CDX2
α -Sox2
α -Sox17
Control
tSNE 1
tSNE 2
Q
%
Blastoid
R
Brightfield
α-Sox2
α -Sox17
α -CDX2
Merge
S
tFACL PD
T
U
tFACL PD
IVF
V
W
N2B27 +Ri+A
Diameter (um)
Blastocysts
Blastoids

Figure S1.

Figure S1. Media optimization and blastoid characterization. Related to Figure 1.

(A) Immunostaining of bovine EPSC and TSCs for epiblast marker SOX2 (cyan), hypoblast marker SOX17(red) and trophectoderm marker CDX2 (green).

(B) Quantification of blastoid formation efficiency and representative image. Immunostaining and quantification of epiblast marker SOX2 (magenta), hypoblast marker SOX17(red) and trophectoderm marker CDX2(green) in (C) FACL, (E) tFACL, and (G) FACL+ PD.

Quantifications in (D-H) n=2, mean ± s.d. Immunostaining for SOX2 (magenta), SOX17(red) and CDX2(green) of (I) IVF Blastocysts and (J) Blastoids, arrows depict marker color scheme in L.

(K) DAPI normalized relative intensity quantification of side-by-side staining and imaging of blastocysts and blastoids n=5, mean ± s.d.

(L) Blastocyst and Blastoid lineage composition quantified via confocal microscopy 3D reconstruction and spots colocalization quantification using IMARIS.

(M) Blastocyst and (N) Blastoid immunostaining for epiblast marker SOX2 and trophectoderm markers gata3, Keratin 18; phospho-STAT3 (Blastocyst), and tight junction marker ZO1(TJP1) and apical marker F-actin (Phallodin) (Blastoid).

Blastoids lineage quantification for epiblast marker SOX2 (AF-647), hypoblast marker SOX17(AF-555, DsRed channel) and trophectoderm marker CDX2(AF-488, GFP channel).
(O) Unstained control (P) days 3 and 4 of protocol together with tSNE plots of each of the quantified markers.

(Q) lineage quantification via flow cytometry averages n=3, mean ± s.d. (R) Imaging examples of stained cells used in FACS quantification.

(S) Immunostaining of bovine blastoids grown in the 3D ClinoStar incubator at day 16 for epiblast marker SOX2 (magenta), hypoblast marker SOX17(red) and trophectoderm marker CDX2(green) in tFACL+PD media.

(T) Phase-contrast image of bovine blastoids grown in the ClinoStar incubator.

(U) Bovine IVF blastocyst grown in in the ClinoStar incubator at day 16 for stained as in S.

(V) Phase-contrast image of in vitro grown bovine blastocyst.

(V, W) Quantification of invitro grown blastoids and blastocysts on N2NB27 with rock inhibitor (Y27632) and activin A as reported in 39.

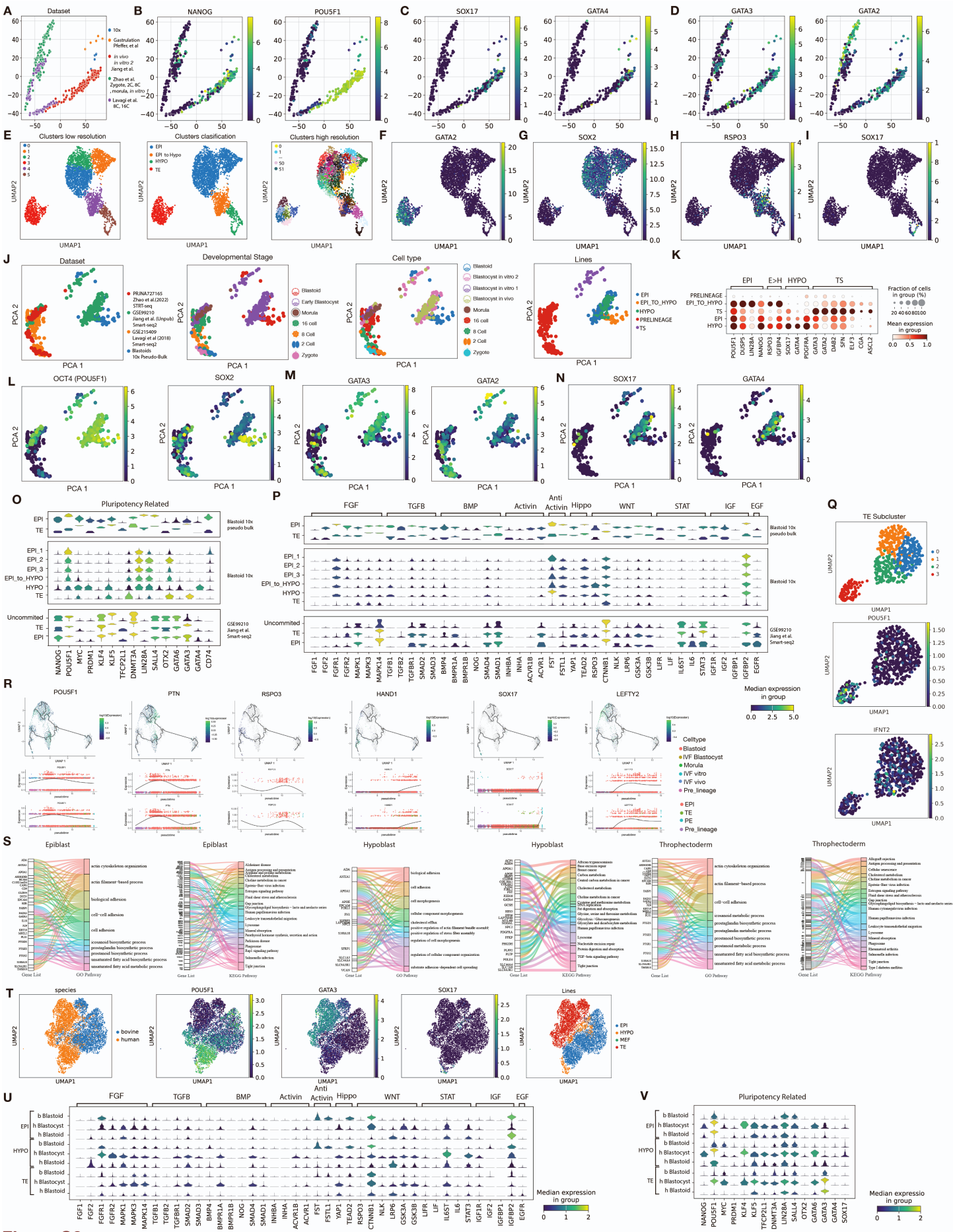

A
Dataset
10x
Gastrulation
Pfeffer, et al
in vivo
in vitro 2
Jiang et al.
Zhao et al.
Zygote, 2C, 8C
, morula, in vitro 1
Lavagi et al.
8C, 16C
B
NANOG
POU5F1
C
SOX17
GATA4
D
GATA3
GATA2
E
Clusters low resolution
Clusters clasification
Clusters high resolution
EPI
EPI to Hypo
HYPO
TE
UMAP1
UMAP2
F
GATA2
G
SOX2
H
RSPO3
I
SOX17
J
Dataset
PRJNA727165
Zhao et al.(2022)
STRT-seq
GSE99210
Jiang et al. (Unpub)
Smart-seq2
GSE215409
Lavagi et al (2018)
Smart-seq2
Blastoids
10x Pseudo-Bulk
Developmental Stage
Blastoid
Early Blastocyst
Morula
16 cell
8 Cell
2 Cell
Zygote
Cell type
Blastoid
Blastocyst in vitro 2
Blastocyst in vitro 1
Blastocyst in vivo
Morula
16 cell
8 Cell
2 Cell
Zygote
Lines
EPI
EPI_TO_HYPO
HYPO
PRELINEAGE
TS
PCA 1
PCA 2
K
EPI
E>H
HYPO
TS
PRELINEAGE
EPI_TO_HYPO
TS
EPI
HYPO
POU5F1
DUSP5
LIN28A
NANOG
RSPO3
IGFBP4
SOX17
GATA4
PDGFRA
GATA3
GATA2
DAB2
SFN
ELF3
CGA
ASCL2
Fraction of cells
in group (%)
20 40 60 80 100
Mean expression
in group
0.0 0.5 1.0
L
OCT4 (POU5F1)
SOX2
M
GATA3
GATA2
N
SOX17
GATA4
O
Pluripotency Related
EPI
TE
EPI_1
EPI_2
EPI_3
EPI_to_HYPO
HYPO
Uncommited
Blastoid 10x
pseudo bulk
Blastoid 10x
GSE99210
Jiang et al.
Smart-seq2
NANOG
POU5F1
MYC
PRDM1
KLF4
KLF5
TFCP2L1
DNMT3A
LIN28A
SALL4
OTX2
GATA6
GATA3
GATA4
CD74
P
FGF
TGFB
BMP
Activin
Anti Activin
Hippo
WNT
STAT
IGF
EGF
FGF1
FGF2
FGFR1
FGFR2
MAPK1
MAPK3
MAPK14
TGFB1
TGFB2
TGFBR1
SMAD2
SMAD3
BMP4
BMPR1A
BMPR1B
NOG
SMAD4
SMAD1
INHBA
INHA
ACVR1B
ACVR1
FST
FSTL1
YAP1
TEAD2
RSPO3
CTNNB1
NLK
LRP6
GSK3A
GSK3B
LIFR
LIF
IL6ST
IL6
STAT3
IGF1R
IGF2
IGFBP1
IGFBP2
EGFR
Median expression
in group
0.0 2.5 5.0
Q
TE Subcluster
POU5F1
IFNT2
R
POU5F1
PTN
RSPO3
HAND1
SOX17
LEFTY2
Celltype
Blastoid
IVF Blastocyst
Morula
IVF vitro
IVF vivo
Pre_lineage
EPI
TE
PE
Pre_lineage
S
Epiblast
Epiblast
Hypoblast
Hypoblast
Throphectoderm
Throphectoderm
Gene List
GO Pathway
KEGG Pathway
T
species
bovine
human
POU5F1
GATA3
SOX17
Lines
EPI
HYPO
MEF
TE
U
FGF
TGFB
BMP
Activin
Anti Activin
Hippo
WNT
STAT
IGF
EGF
b Blastoid
h Blastocyst
h Blastoid
EPI
HYPO
TE
Median expression
in group
0 1 2
V
Pluripotency Related
b Blastoid
h Blastocyst
h Blastoid
EPI
HYPO
TE
NANOG
POU5F1
MYC
PRDM1
KLF4
KLF5
TFCP2L1
DNMT3A
LIN28A
SALL4
OTX2
GATA6
GATA3
GATA4
SOX17
Median expressio
in group
0 1 2

Figure S2.

Figure S2. Single-cell RNA-seq characterization of blastoids. Related to Figure 2.

(A)Principal component analysis (PCA) heatmaps of first pseudo bulk conversion of blastoid data colored by dataset and heatmaps of (B) epiblast markers: NANOG, POU5F1(OCT4); (C) hypoblast markers: SOX17, GATA4; and (D)trophectoderm markers: GATA3, GATA2.

(E) UMAP of blastoid 10X data and clustering analysis. Cluster allocation and heatmaps of (F) trophectoderm marker (GATA2), (G) epiblast marker POU5F1(OCT4), (H) epiblast to hypoblast transition marker RSPO3, and (I) hypoblast marker SOX17.

(J) Principal component analysis (PCA) of second pseudo bulk conversion of blastoid data based on the 52 clusters of (E), showing annotations for datasets, developmental stages, cell types and assigned lines.

(K) Dot plot indicating the expression of markers of pre lineage cells, epiblast (EPI), epiblast to hypoblast transitioning cells (E>H), trophectoderm (TE) and hypoblast (HYPO).

Heatmaps of (L), epiblast markers: POU5F1(OCT4) and SOX2; (M) trophectoderm markers: GATA3, GATA2; and (N) hypoblast markers: SOX17, GATA4. (O) Violin plot heatmap of pluripotency related genes in three datasets.

(P) Violin plot heatmap of signaling pathways key genes.

(Q) UMAP of trophectoderm subclusters and heatmaps of epiblast marker POU5F1(OCT4) within the TSC subcluster indicating an early blastocyst like subpopulation, and INFt transcript INFT2 expression within TSC subcluster.

(R) Expression heatmap and pseudotime analysis of different markers.

(S) Alluvial diagram of Go pathways and KEGG pathway of differentially expressed genes (DEG) in each cluster.

(T) UMAP of Human blastoid, blastocyst and bovine blastoid scRNA-seq comparison.

(U)Violin plot heatmap of pluripotency related genes in three datasets. (V) Violin plot heatmap of signaling pathways key genes.