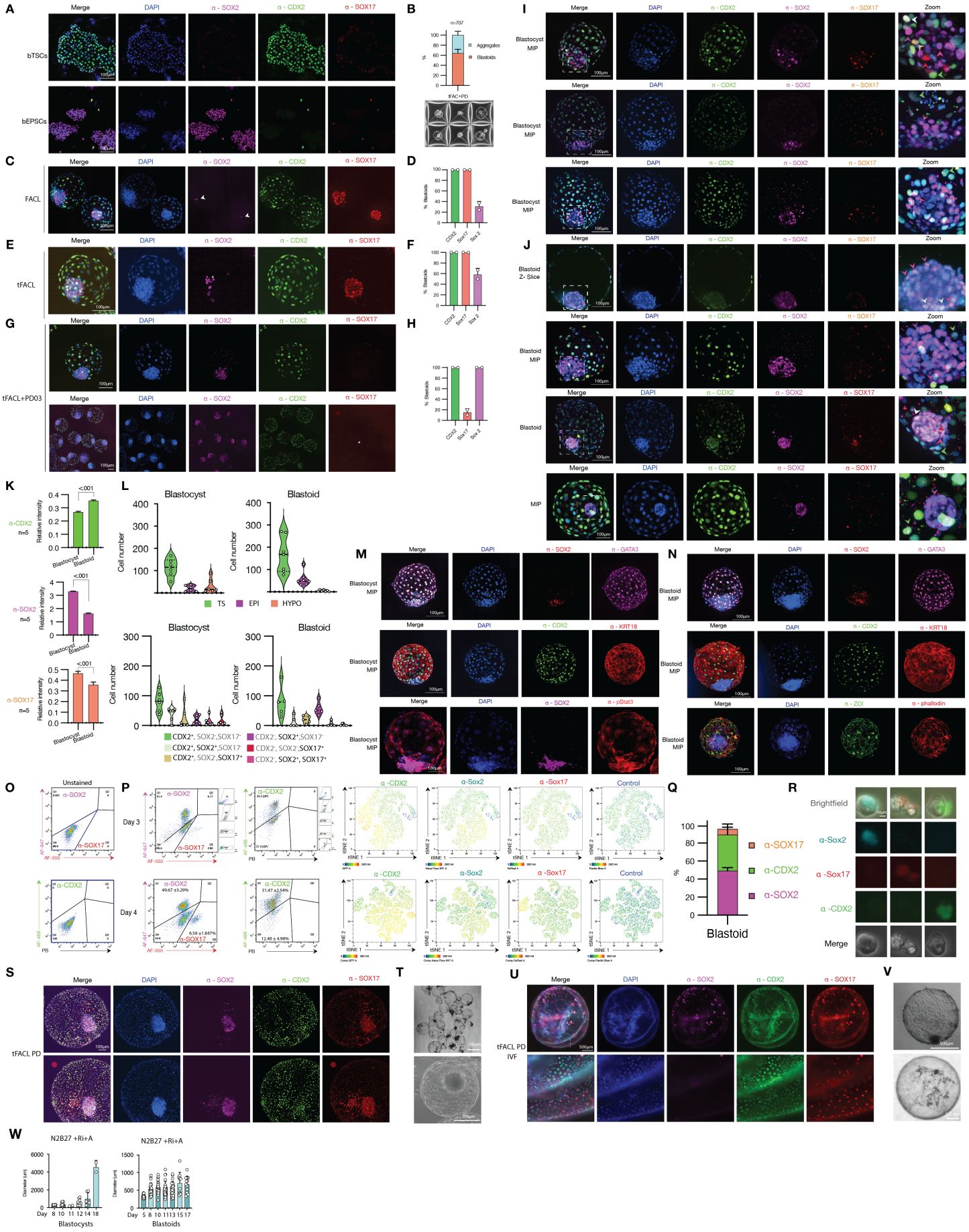
A
Merge
DAPI
α - SOX2
α - CDX2
α - SOX17
bTSCs
bEPSCs
B
n=707
Aggregates
Blastoids
% Blastoids
tFAC+PD
C
FACL
D
% Blastoids
CDX2
Sox17
Sox 2
E
tFACL
F
G
tFACL+PD03
H
I
Blastocyst MIP
Zoom
J
Blastoid Z- Slice
Blastoid MIP
Blastoid
MIP
K
α-CDX2
n=5
Relative intensity
<.001
Blastocyst
Blastoid
α-SOX2
α-SOX17
L
Blastocyst
Blastoid
Cell number
TS
EPI
HYPO
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
α-SOX2
α-SOX17
AF-555
AF-647
AF-488
PB
P
Day 3
Day 4
α-CDX2
49.67 ±3.29%
6.58 ±1.847%
31.47 ±2.54%
12.48 ±4.98%
α -CDX2
α -Sox2
α -Sox17
Control
tSNE 1
tSNE 2
Q
α-SOX17
α-CDX2
α-SOX2
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)
Day
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.

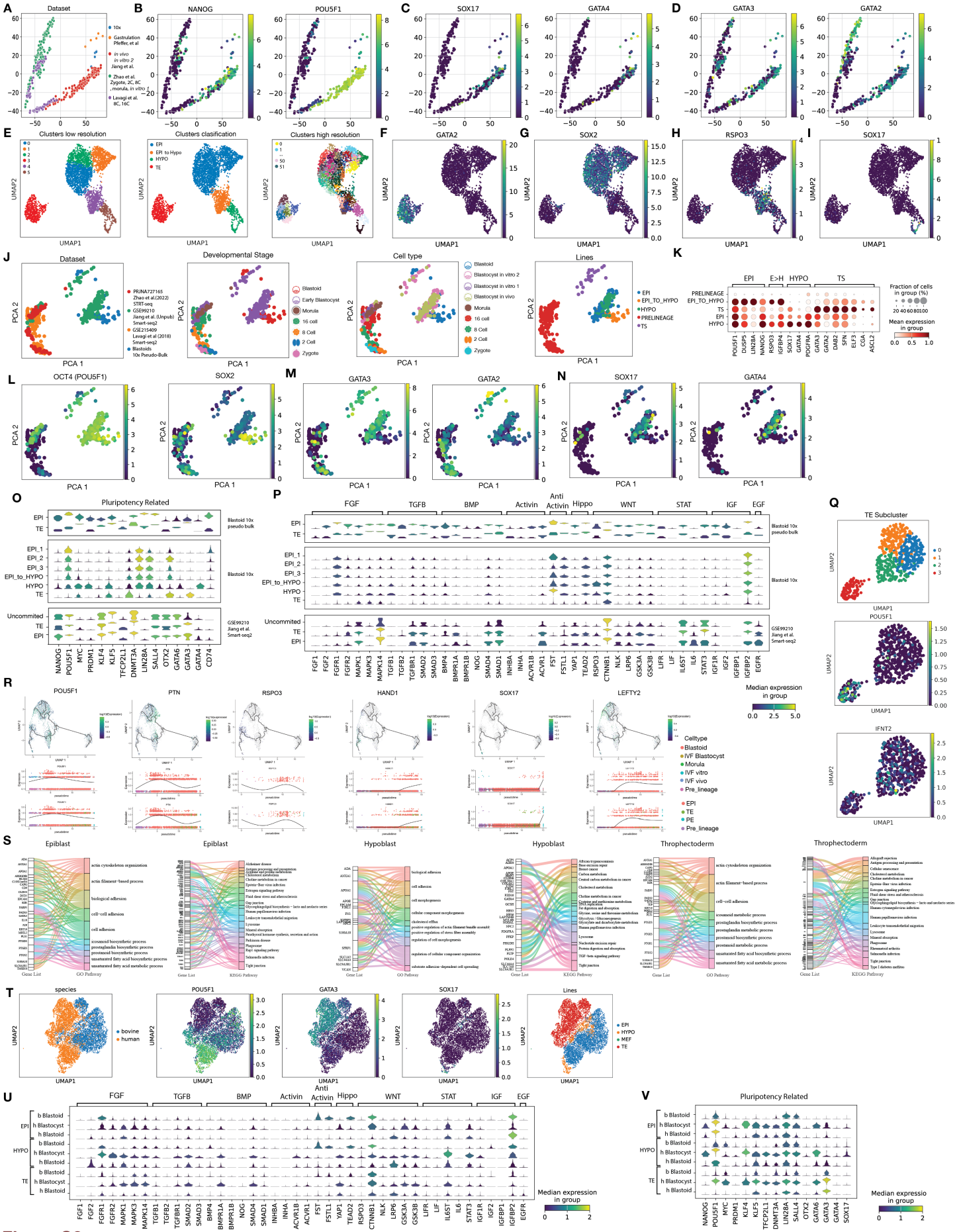

Figure S2.

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.