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Supplemental Information

3D ECM-rich environment sustains the identity of naive human iPSCs

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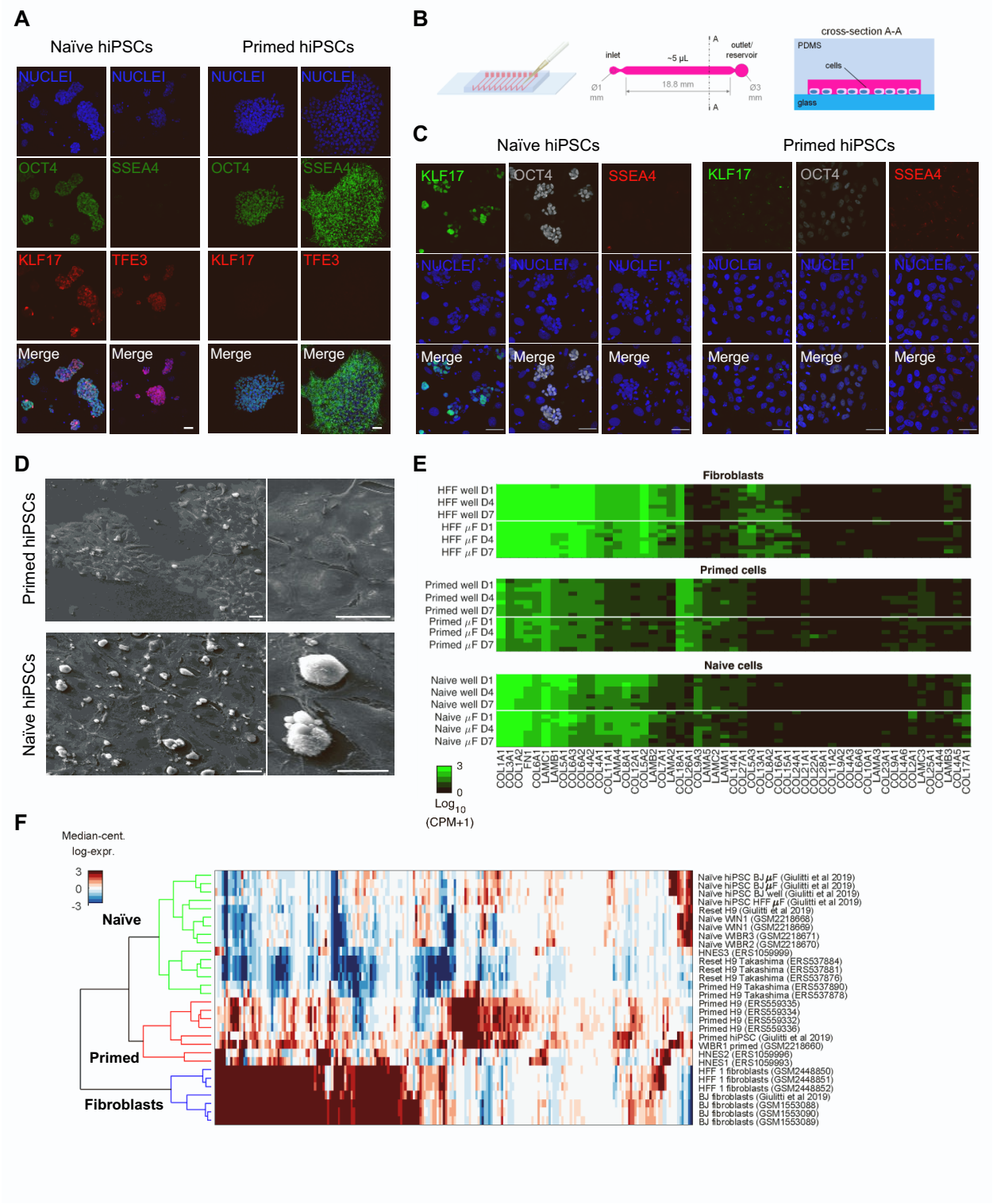
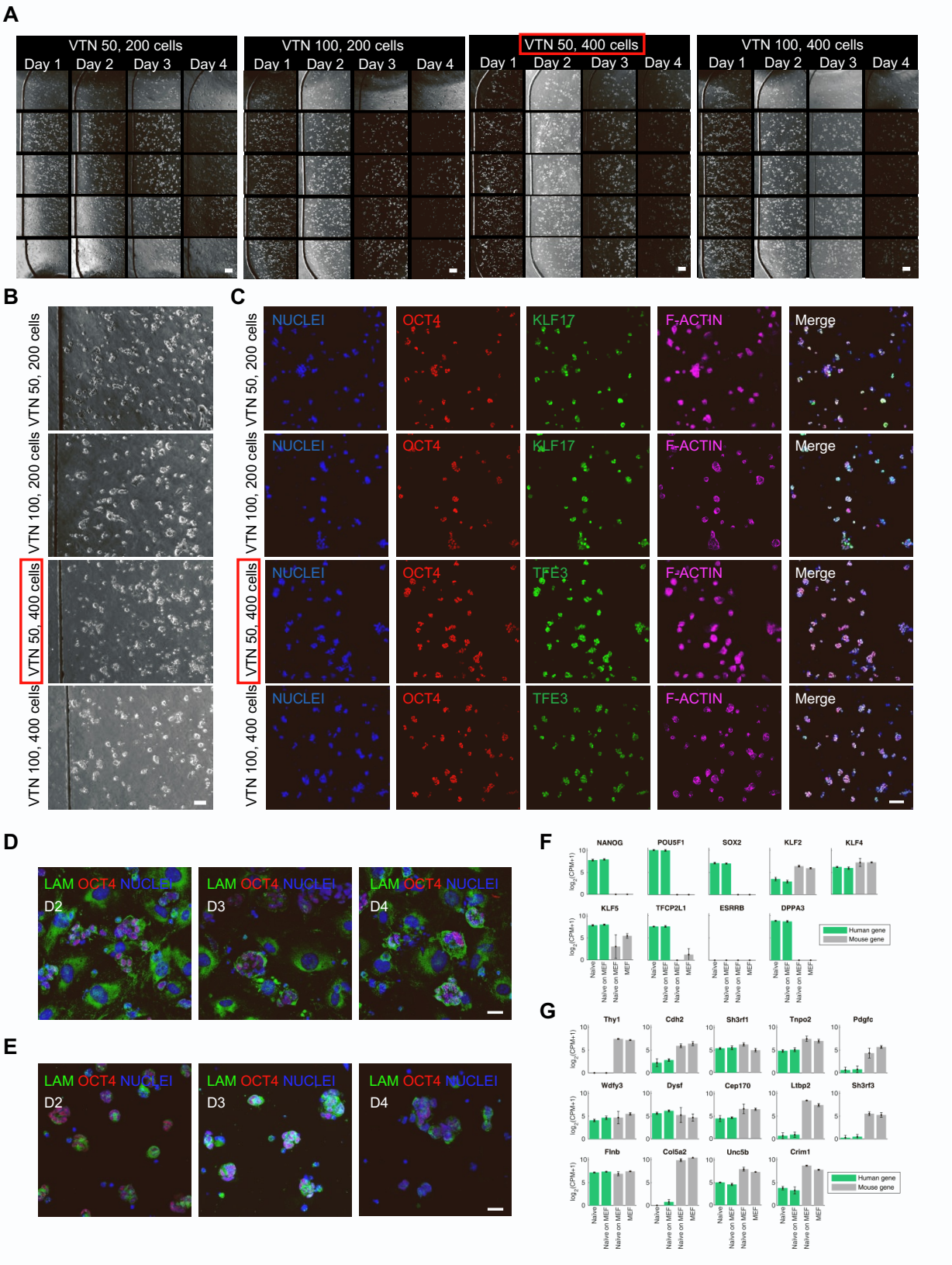

A
Naïve hiPSCs
Primed hiPSCs
NUCLEI
OCT4
SSEA4
KLF17
TFE3
Merge
B
inlet
~5 µL
outlet/
reservoir
18.8 mm
Ø1
mm
Ø3
mm
cross-section A-A
PDMS
cells
glass
C
Naïve hiPSCs
Primed hiPSCs
KLF17
OCT4
SSEA4
NUCLEI
Merge
D
Primed hiPSCs
Naïve hiPSCs
E
Fibroblasts
HFF well D1
HFF well D4
HFF well D7
HFF µF D1
HFF µF D4
HFF µF D7
Primed cells
Primed well D1
Primed well D4
Primed well D7
Primed µF D1
Primed µF D4
Primed µF D7
Naive cells
Naive well D1
Naive well D4
Naive well D7
Naive µF D1
Naive µF D4
Naive µF D7
3
0
Log10
(CPM+1)
F
Median-cent.
log-expr.
3
0
-3
Naïve
Primed
Fibroblasts
Naïve hiPSC BJ µF (Giulitti et al 2019)
Naïve hiPSC BJ µF (Giulitti et al 2019)
Naïve hiPSC BJ well (Giulitti et al 2019)
Naïve hiPSC HFF µF (Giulitti et al 2019)
Reset H9 (Giulitti et al 2019)
Naïve WIN1 (GSM2218668)
Naïve WIN1 (GSM2218669)
Naïve WIBR3 (GSM2218671)
Naïve WIBR2 (GSM2218670)
HNES3 (ERS1059999)
Reset H9 Takashima (ERS537884)
Reset H9 Takashima (ERS537881)
Reset H9 Takashima (ERS537876)
Primed H9 Takashima (ERS537890)
Primed H9 Takashima (ERS537878)
Primed H9 (ERS559335)
Primed H9 (ERS559334)
Primed H9 (ERS559332)
Primed H9 (ERS559336)
Primed hiPSC (Giulitti et al 2019)
WIBR1 primed (GSM2218660)
HNES2 (ERS1059996)
HNES1 (ERS1059993)
HFF 1 fibroblasts (GSM2448850)
HFF 1 fibroblasts (GSM2448851)
HFF 1 fibroblasts (GSM2448852)
BJ fibroblasts (Giulitti et al 2019)
BJ fibroblasts (GSM1553088)
BJ fibroblasts (GSM1553090)
BJ fibroblasts (GSM1553089)

Supplementary Figure 1.

Derivation and characterization of naïve and isogenic primed hiPSCs in standard culture system and in microfluidic devices. Related to Figure 1.

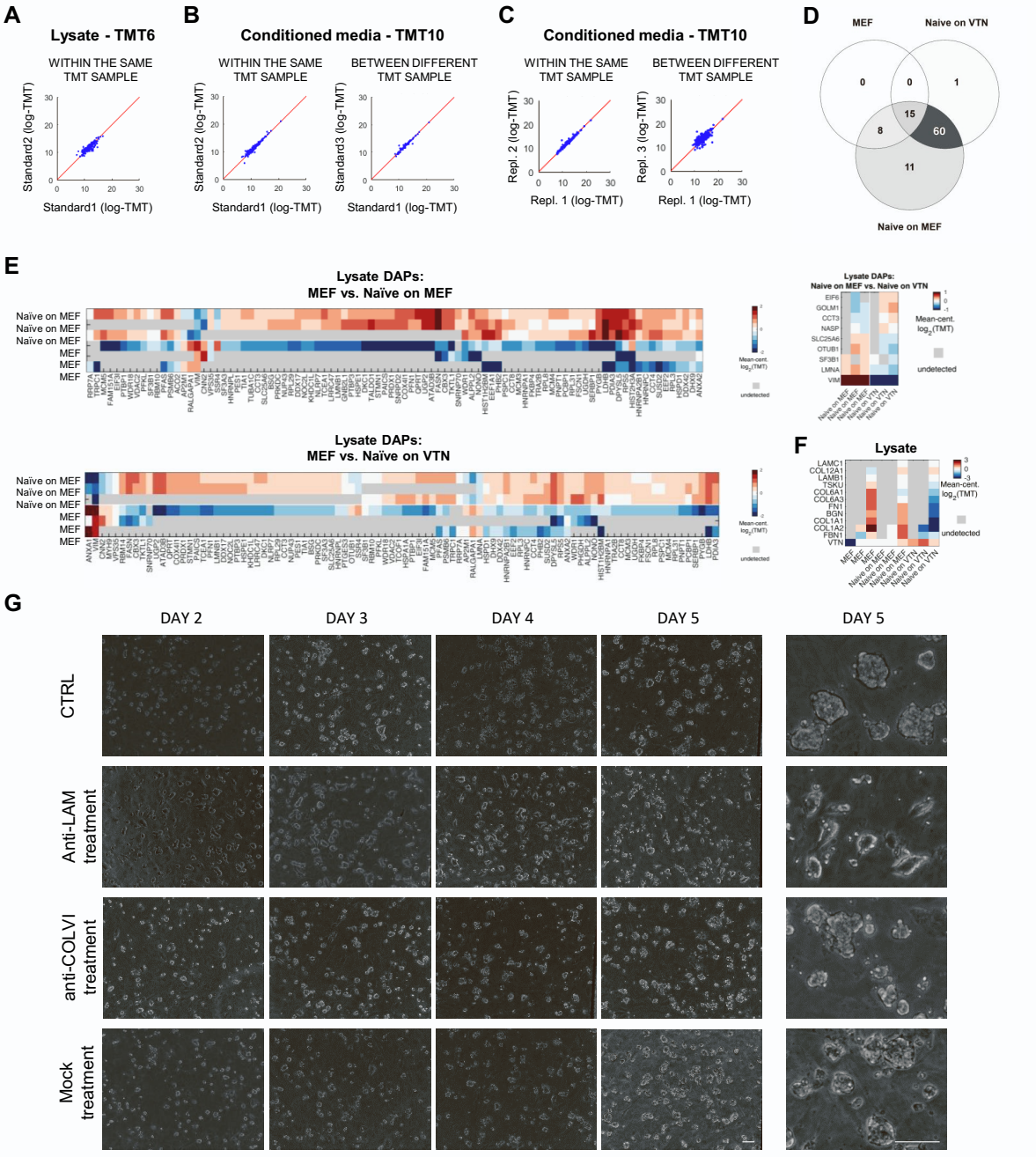
A. Representative Z-stack confocal images of naïve (left panel) and isogenic primed hiPSCs (Primed hiPSCs P1, middle panel) immunostained for OCT4 (green) and KLF17 (red) or SEEA4 (green) and TFE3 (red). Nuclei were stained with Hoechst (blue). Scale bars, 100 µm. **B.** Representative drawing of a microfluidic device during (left panel), and dimensions of a single channel-shaped microfluidic culture chamber. Cell size is not to scale. Adapted from Gagliano et al., 2019[1]**. C.** Representative Z-stack confocal images of primed (left panel) or naïve (right panel) hiPSCs immunostained for KLF17 (green), OCT4 (grey) and SEEA4 (red). Nuclei were stained with Hoechst (blue). Scale bars, 50 µm. **D.** Representative scanning electron microscopy (SEM) images showing primed (upper panel) or naïve (lower panel) hiPSCs. Scale bars, 50 µm. **E**. Heat map of transcriptional profiles of collagens, laminins, and FN of naïve cells, isogenic primed and parental fibroblasts after 1, 4, or 7 days of normoxic culture in conventional well or microfluidics. Each experimental condition shown is the mean of three replicates. **F**. Hierarchical clustering of published RNA-seq data sets of fibroblasts, primed and naïve cells, including only core ECM gene expression.


A
VTN 50, 200 cells
VTN 100, 200 cells
VTN 50, 400 cells
VTN 100, 400 cells
Day 1 Day 2 Day 3 Day 4
B
C
VTN 50, 200 cells
VTN 100, 200 cells
VTN 50, 400 cells
VTN 100, 400 cells
NUCLEI
OCT4
KLF17
TFE3
F-ACTIN
Merge
D
E
LAM OCT4 NUCLEI
D2
D3
D4
F
NANOG
POU5F1
SOX2
KLF2
KLF4
KLF5
TFCP2L1
ESRRB
DPPA3
Human gene
Mouse gene
Naive
Naive on MEF
MEF
G
Thy1
Cdh2
Sh3rf1
Tnpo2
Pdgfc
Wdfy3
Dysf
Cep170
Ltbp2
Sh3rf3
Flnb
Col5a2
Unc5b
Crim1

Supplementary Figure 2.

Optimization of Naïve hiPSC culture in microfluidic devices and feeder free condition and evaluation of ECM production. Related to Figure 2.

A. Representative bright field images of naïve hiPSC colonies seeded at 200 cell/mm^2 or 400 cell/mm^2 into microfluidic devices coated with 50 µg/mL (left panels) or 100 µg/mL (right panels) of vitronectin (VTN). Cultures are shown 1, 2, 3 and 4 days after cell seeding. Scale bars, 200 µm. **B.** Representative bright field images of naïve hiPSC colonies seeded at 200 cell/mm^2 or 400 cell/mm^2 into microfluidic devices coated with 50 µg/mL or 100 µg/mL of vitronectin (VTN) 4 days after seeding. Scale bar, 100 µm. **C.** Representative Z-stack confocal images of naïve hiPSCs seeded at 200 cell/mm^2 or 400 cell/mm^2 into microfluidic devices coated with 50 µg/mL or 100 µg/mL of vitronectin (VTN) and immunostained 4 days after seeding for OCT4 (red), KLF17 (green) and F-actin (magenta) or OCT4 (red), TFE3 (green) and F-actin (magenta). Nuclei were stained with Hoechst (blue). Scale bar, 100 µm. Optimal naïve hiPSC culture was defined as 400 cell/mm^2 into microfluidic devices coated with 50 µg/mL vitronectin (red squares). **D.** Representative Z-stack confocal images of naïve hiPSCs cultured on MEF at 2 (D2), 3 (D3) and 4 (D4) days after seeding and immunostained for laminins (LAM, green) and OCT4 (red). Nuclei were stained with Hoechst (blue). Scale bar, 25 µm. **E.** Representative Z-stack confocal images of naïve hiPSCs cultured in feeder free conditions 2 (D2), 3 (D3) and 4 (D4) days after seeding and immunostained for laminins (LAM, green) and OCT4 (red). Nuclei were stained with Hoechst (blue). Scale bar, 25 µm. **F-G.** Expression of selected genes from the transcriptomic data results of the samples naïve hiPSCs cultured feeder-free on VTN (Naïve), naïve hiPSCs cultured on MEFs (Naïve on MEF) and MEF-only cultures (MEF), all cultured in microfluidics. As indicated in the legend both human and mouse orthologs are shown, thus naïve on MEF is shown twice displaying the two different transcriptome alignments. **F**. Expression of human or murine ortholog naïve genes. **G**. Expression of human or murine ortholog fibroblast genes.

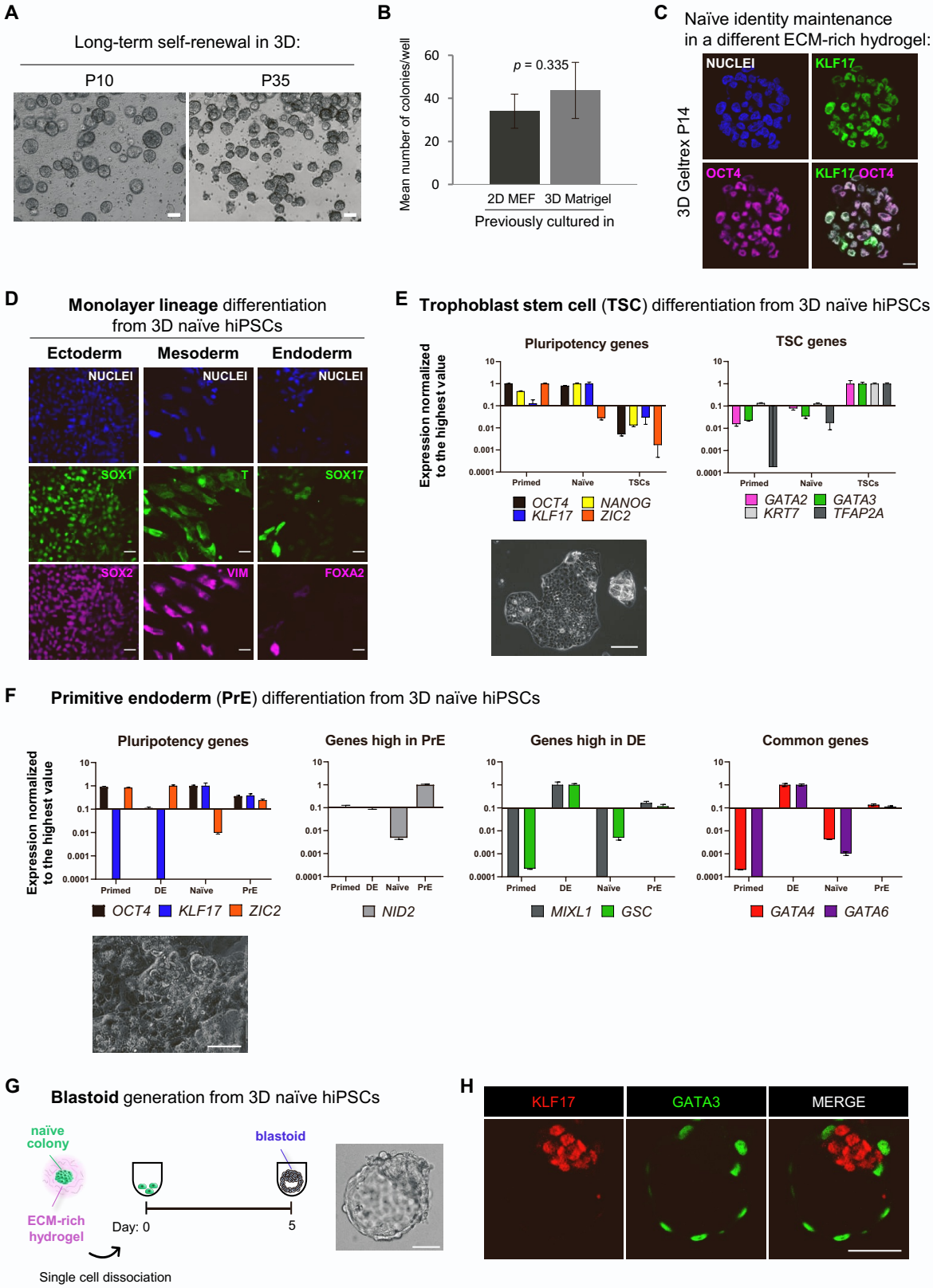

A
Lysate - TMT6
WITHIN THE SAME TMT SAMPLE
Standard2 (log-TMT)
Standard1 (log-TMT)
B
Conditioned media - TMT10
WITHIN THE SAME TMT SAMPLE
BETWEEN DIFFERENT TMT SAMPLE
Standard2 (log-TMT)
Standard3 (log-TMT)
Standard1 (log-TMT)
C
Conditioned media - TMT10
WITHIN THE SAME TMT SAMPLE
BETWEEN DIFFERENT TMT SAMPLE
Repl. 2 (log-TMT)
Repl. 3 (log-TMT)
Repl. 1 (log-TMT)
D
MEF
Naive on VTN
Naive on MEF
0
0
1
15
8
60
11
E
Lysate DAPs:
MEF vs. Naïve on MEF
Naïve on MEF
Naïve on MEF
Naïve on MEF
MEF
MEF
MEF
Mean-cent. log2(TMT)
undetected
Lysate DAPs:
MEF vs. Naïve on VTN
Naïve on MEF
Naïve on MEF
Naïve on MEF
MEF
MEF
MEF
Lysate DAPs:
Naive on MEF vs. Naive on VTN
EIF5
GOLM1
CCT3
NASP
SLC25A6
OTUB1
SF3B1
LMNA
VIM
Mean-cent. log2(TMT)
undetected
F
Lysate
LAMC1
COL12A1
LAMB1
TSKU
COL6A1
COL6A3
FN1
BGN
COL1A1
COL1A2
FBN1
VTN
Mean-cent. log2(TMT)
undetected
G
DAY 2
DAY 3
DAY 4
DAY 5
DAY 5
CTRL
Anti-LAM treatment
anti-COLVI treatment
Mock treatment

Supplementary Figure 3.

Proteomic data analysis and treatments to interfere with the interaction between Naïve hiPSCs and ECM proteins. Related to Figures 3 and 4.

A. Technical variability in lysate TMT6. Standard samples 1 and 2 indicate the same biological sample labelled with different TMT tags and analyzed within the same TMT pool of samples. Each dot represents a protein, whose coordinates are given by log-scale TMT intensity. **B**. Technical variability in conditioned media TMT10. (Left) Standard samples 1 and 2 indicate the same biological sample labelled with different TMT tags and analyzed within the same TMT pool of samples. (Right) Standard samples 1 and 3 indicate the same biological sample labelled with different TMT tags and analyzed in two different TMT pool of samples. **C**. Biological variability in conditioned media TMT10. (Left) Replicate samples 1 and 2 indicate two biological replicates obtained under the same experimental conditions, labelled with different TMT tags and analyzed within the same TMT pool of samples. (Right) Replicate samples 1 and 3 indicate two biological replicates obtained in the same experimental condition, labelled with different TMT tags and analyzed in two different TMT pool of samples. **D**. Venn diagram of proteins significantly accumulated in the samples indicated at day 2 or 3, as compared to RSeT™ samples.

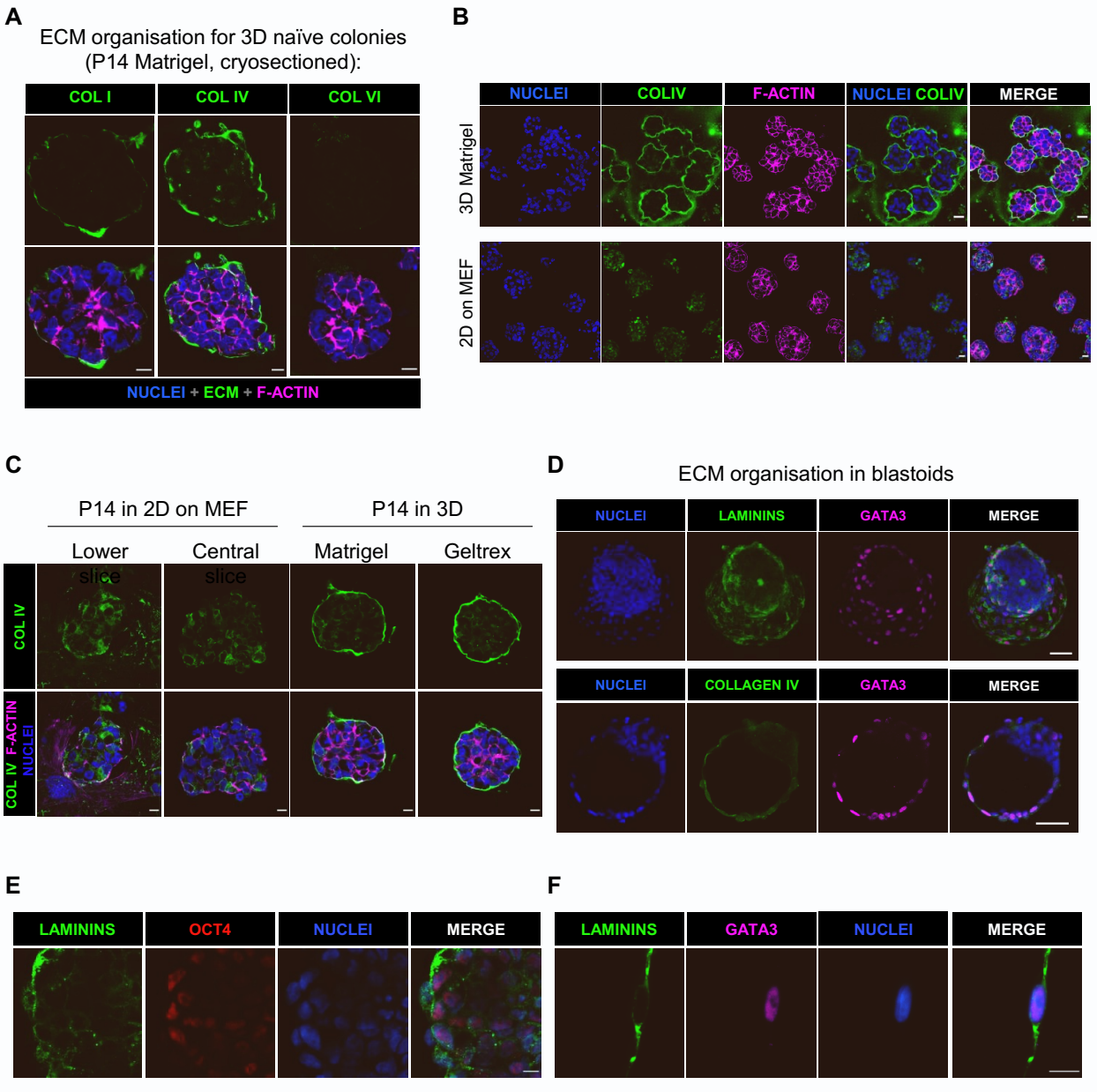
E. Heat map showing the relative expression of differentially abundant proteins in MEF vs. naïve on MEF (upper panel), MEF vs. naïve on VTN (middle panel) and naïve on MEF vs. naïve on VTN (lower panel). Undetected proteins are shown in grey. These datasets were used to obtain plots in Fig. 3A and B. **F**. Heat map showing the relative expression of core ECM proteins in MEF, naïve on MEF, and naïve on VTN samples for each of the replicates. Mean values of the three replicates are shown in the heat map in fig. 3C. Undetected proteins are shown in grey. **G**. Representative bright field images of naïve hiPSC colonies seeded on feeders at 400 cell/mm^2 into microfluidic devices, untreated (CTRL, upper panel) or treated with anti-laminins (second panel), anti-collagen VI (third panel) or mock (bottom panel) antibodies. Cultures are shown 2, 3, 4 and 5 days after cell seeding (left panel). For each condition, a magnified view of colonies at day 5 is represented in the panel on the right. Scale bars, 100 µm.


A
Long-term self-renewal in 3D:
P10
P35
B
Mean number of colonies/well
60
40
20
0
p = 0.335
2D MEF
3D Matrigel
Previously cultured in
C
Naïve identity maintenance
in a different ECM-rich hydrogel:
3D Geltrex P14
NUCLEI
KLF17
OCT4
KLF17 OCT4
D
Monolayer lineage differentiation
from 3D naïve hiPSCs
Ectoderm
Mesoderm
Endoderm
NUCLEI
SOX1
T
SOX17
SOX2
VIM
FOXA2
E
Trophoblast stem cell (TSC) differentiation from 3D naïve hiPSCs
Pluripotency genes
TSC genes
Expression normalized to the highest value
Primed
Naïve
TSCs
OCT4
NANOG
KLF17
ZIC2
GATA2
GATA3
KRT7
TFAP2A
F
Primitive endoderm (PrE) differentiation from 3D naïve hiPSCs
Pluripotency genes
Genes high in PrE
Genes high in DE
Common genes
Primed
DE
Naïve
PrE
OCT4
KLF17
ZIC2
NID2
MIXL1
GSC
GATA4
GATA6
G
Blastoid generation from 3D naïve hiPSCs
naïve colony
ECM-rich hydrogel
Day: 0
5
blastoid
Single cell dissociation
H
KLF17
GATA3
MERGE

Supplementary Figure 4.

3D ECM-rich environment supports long-term self-renewal and totipotency of naïve hiPSCs. Related to Figure 5.

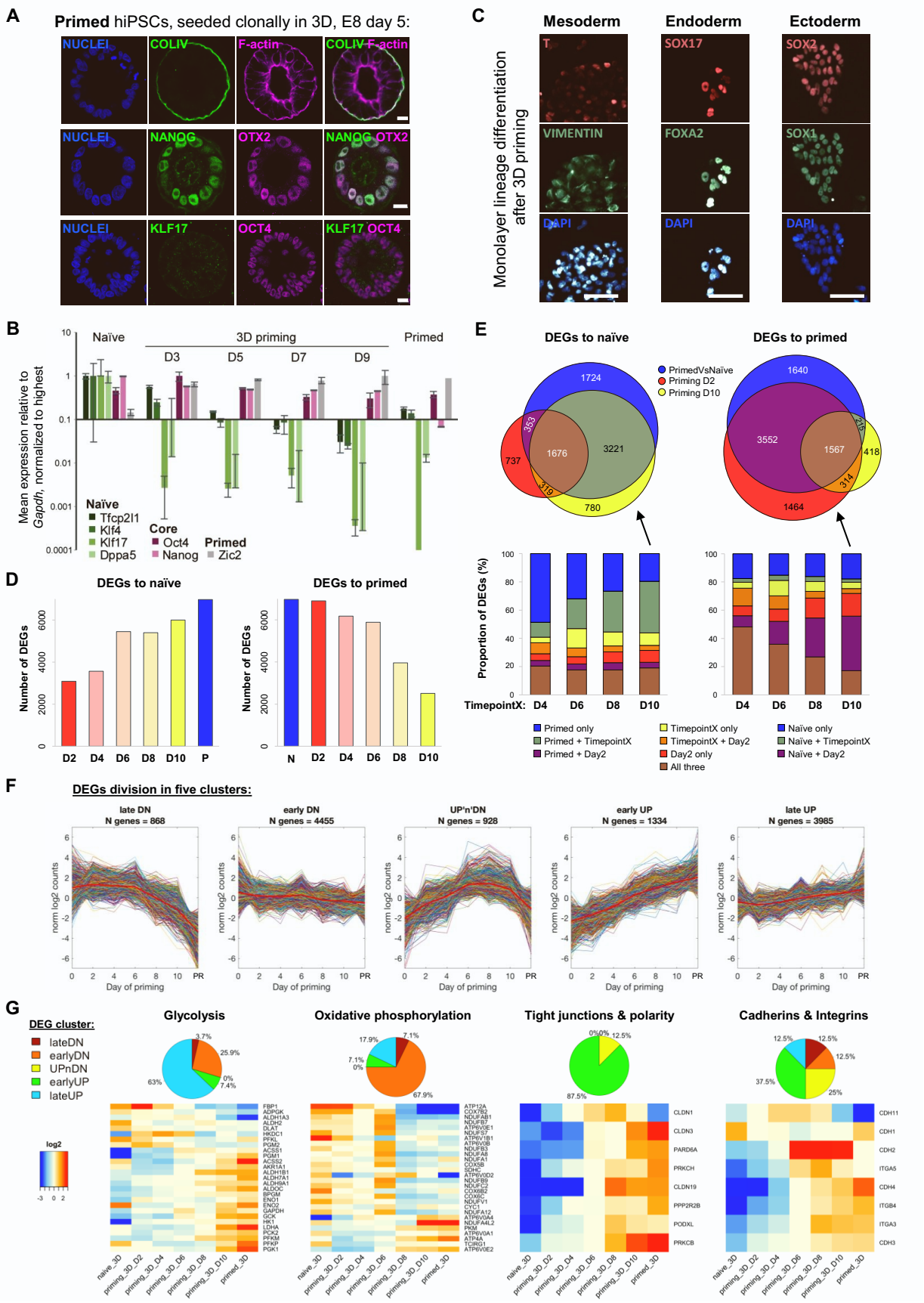
A. Representative bright field images showing naïve hiPSCs colonies at 2 different passages in 3D Matrigel. Scale bars, 100 µm. **B**. Clonal assay performed on conventional 2D MEF feeder layer, for naïve hiPSCs previously cultured in 2D standard system or 3D Matrigel in parallel. Quantification was performed by scoring KLF17-OCT4 double-positive colonies. Data are presented as mean ± standard deviation, n = 3. p = 0.335. **C.** Representative single confocal slices of 3D naïve hiPSCs after 14 passages in Geltrex, immunostained for KLF17 (green) and OCT4 (magenta). Nuclei were stained with Hoechst (blue). Scale bar, 10 µm**. D**. Representative images of monolayer lineage differentiation of 3D naïve hiPSCs after 14 passages in 3D Matrigel, immunostained for ectodermal markers (left panel) SOX1 (green) and SOX2 (magenta), mesodermal markers (middle panel) Brachyury (T, green) and Vimentin (VIM, magenta) and endodermal markers (right panel) SOX17 (green) and FOXA2 (magenta). Nuclei were stained with Hoechst (blue). Scale bars, 20 µm. **E**. Differentiation of 3D naïve hiPSCs into Trophoblast stem cells (TSCs). In the upper panel, qRT-PCR for core pluripotency and TSCs markers, normalised to *GAPDH* then to highest, displayed as mean ± standard deviation, n = 3. In the lower panel, representative bright field image of a TSC colony. Scale bar, 100 µm. **F**. Differentiation of 3D naïve hiPSCs into primitive endoderm cells. In the upper panel, qRT-PCR for core pluripotency, DE-associated, PrE-associated and common endodermal markers, normalised to *GAPDH* then to highest, displayed as mean ± standard deviation, n = 3. In the lower panel, representative bright field image of a primitive endoderm colony. Scale bar, 75 µm. **G**. Experimental design: 3D-cultured naïve hiPSCs were passaged as single cells after 5 days of culture in 3D Matrigel. Cells were seeded at the density of 500-1000 cells per well in U-bottom suspension culture plates. Blastoids were fixed at day 5 for immunofluorescence analysis. On the right panel, a representative bright field image of a blastoid. Scale bar, 50 µm. **H**. Representative single confocal slices of a blastoid derived from 3D naïve hiPSCs, immunostained for KLF17 (red) and GATA3 (green). Scale bar, 50 µm.


A
ECM organisation for 3D naïve colonies
(P14 Matrigel, cryosectioned):
COL I
COL IV
COL VI
NUCLEI + ECM + F-ACTIN
B
NUCLEI
COLIV
F-ACTIN
NUCLEI COLIV
MERGE
3D Matrigel
2D on MEF
C
P14 in 2D on MEF
P14 in 3D
Lower slice
Central slice
Matrigel
Geltrex
COL IV
COL IV F-ACTIN NUCLEI
D
ECM organisation in blastoids
NUCLEI
LAMININS
GATA3
MERGE
NUCLEI
COLLAGEN IV
GATA3
MERGE
E
LAMININS
OCT4
NUCLEI
MERGE
F
LAMININS
GATA3
NUCLEI
MERGE

Supplementary Figure 5.

Characterization of the ECM in 3D naïve hiPSCs and in naïve-derived Blastoids. Related to Figure 5.

A. Representative single confocal slices of cryosectioned 3D naïve hiPSCs colonies, after 14 passages in Matrigel, immunostained for ECM proteins collagen type I, IV, and VI (green) and F-actin (magenta) showing the differential organization of the ECM around the 3D colonies. Nuclei were stained with Hoechst (blue). Scale bars, 10 μm **B**. Representative single confocal slices of 3D (upper panel) and parallel 2D (lower panel) naïve hiPSCs colonies immunostained for collagen type IV (COLIV, green) and F-actin (magenta) showing marked organization of a layer of collagen type IV around the 3D colony edges. Both slices were taken from the middle of the z-stack. Nuclei were stained with Hoechst (blue). Scale bars, 20 μm. **C**. Representative single confocal slices of 3D naïve hiPSCs colonies, after 14 passages in Geltrex or Matrigel (upper panels), and control 2D naïve cultures (bottom panels, slices taken from the middle and the bottom of the stack, showing also MEF feeders) immunostained for collagen type IV (green) and F-actin (magenta) showing the differential organization of collagen type IV in 3D and 2D naïve colonies. Nuclei were stained with Hoechst (blue). Scale bars, 10 μm. **D**. Representative confocal images of blastoids immunostained for laminins (upper panel, z-stack) and collagen type IV (lower panel, single slices) (green) and GATA3 (magenta) showing the organization of laminins on both the ICM-like and Trophoblast-like components of the blastoids, and a less organized deposition of collagen type IV on the trophoblast component of the blastoid. Nuclei were stained with Hoechst (blue). Scale bars, 50 μm. **E**. Representative single confocal slices of blastoids immunostained for laminins (green), OCT4 (red) and GATA3 (magenta) showing the organization of laminins on the surface and between cells of the ICM-like (OCT4 positive cells) part of the blastoid. Scale bar, 12 μm **F**. Representative single confocal slices of blastoids immunostained for laminins (green), OCT4 (red) and GATA3 (magenta) showing the presence of laminins for the GATA3 positive trophoblast-like cells of the blastoid. Nuclei were stained with Hoechst (blue). Scale bar, 6 μm.


A
Primed hiPSCs, seeded clonally in 3D, E8 day 5:
NUCLEI
COLIV
F-actin
COLIV F-actin
NANOG
OTX2
NANOG OTX2
KLF17
OCT4
KLF17 OCT4
B
Naïve
3D priming
Primed
D3
D5
D7
D9
Mean expression relative to Gapdh, normalized to highest
Naïve
Tfcp2l1
Klf4
Klf17
Dppa5
Core
Oct4
Nanog
Primed
Zic2
C
Mesoderm
Endoderm
Ectoderm
Monolayer lineage differentiation after 3D priming
T
VIMENTIN
SOX17
FOXA2
SOX2
SOX1
DAPI
D
DEGs to naïve
DEGs to primed
Number of DEGs
D2
D4
D6
D8
D10
P
N
E
DEGs to naïve
DEGs to primed
PrimedVsNaïve
Priming D2
Priming D10
1724
353
1676
3221
737
319
780
1640
3552
1567
418
314
1464
Proportion of DEGs (%)
TimepointX:
Primed only
Primed + TimepointX
Primed + Day2
TimepointX only
TimepointX + Day2
Day2 only
All three
Naïve only
Naïve + TimepointX
Naïve + Day2
F
DEGs division in five clusters:
late DN
N genes = 868
early DN
N genes = 4455
UP'n'DN
N genes = 928
early UP
N genes = 1334
late UP
N genes = 3985
norm log2 counts
Day of priming
PR
G
DEG cluster:
lateDN
earlyDN
UPnDN
earlyUP
lateUP
log2
Glycolysis
Oxidative phosphorylation
Tight junctions & polarity
Cadherins & Integrins
CLDN1
CLDN3
PARD6A
PRKCH
CLDN19
PPP2R2B
PODXL
PRKCB
CDH11
CDH1
CDH2
ITGA5
CDH4
ITGB4
ITGA3
CDH3

Supplementary Figure 6.

Identity transition and gene expression dynamics of 3D naïve hiPSCs during 3D priming. Related to Figure 6.

A. Representative single confocal slices at day 5 after single isogenic primed hiPSCs were seeded in 3D Matrigel and cultured in E8, immunostained for KLF17 (green) and OCT4 (magenta) (left panel), OTX2 (magenta) and NANOG (green) (middle panel), Collagen type IV (COLIV, green) and F-actin (magenta) (right panel) showing primed identity and primed morphology in 3D. Nuclei were stained with Hoechst (blue). Scale bars, 10 μm. **B.** Identity characterization of 3D naïve hiPSCs during the priming process at different timepoints (day 3, 5, 7 and 9). qRT-PCR for naïve-, primed-associated, and core pluripotency markers, normalized to *GAPDH*, displayed as mean ± standard deviation, n = 3 (n = 2 for day 7). **C.** Representative images of monolayer lineage differentiation of 3D naïve hiPSCs after priming in 3D Matrigel, immunostained for ectodermal markers (upper panel) SOX2 (red) and SOX1 (green), endodermal markers (middle panel) SOX17 (red) and FOXA2 (green) and mesodermal markers (lower panel) Brachyury (T, red) and Vimentin (VIM, green). Nuclei were stained with Hoechst (blue). Scale bars, 75 μm. **D.** Bar charts showing the number of differentially expressed genes (DEGs) obtained from pairwise comparison between each timepoint versus naïve (upper panel) or versus primed (lower panel) samples as reference. FDR lower than 0.01 and absolute log2 (fold change) higher than 2 were used as thresholds for DEG analysis. **E.** Upper panel: Venn diagrams showing the intersection between DEG lists for the indicated samples compared to naïve (left) or compared to primed (right), with the number of unique and shared DEGs annotated. Lower panel: Bar charts showing the proportion of unique and shared DEGs for the indicated samples compared to naïve (left) or compared to primed (right), coloured as per the parent Venn diagrams. **F.** Temporal expression profiles of DEGs compiled from all pairwise comparisons between any two samples (11570 DEGs in total), divided by clustering analysis into five dynamic clusters. **G.** Heatmaps showing the median-centred expression of selected genes, ordered by their belonging to the clusters shown in panel F. The related pie charts represent the percentage of genes that belong to each of cluster 1–5. Only DEGs are presented in heatmaps. Gene lists were compiled from KEGG pathways and published literature[44, 49, 53].

Supplementary Table 4. List of all primary and secondary antibodies used in this work, related to STAR Methods.

Antibodies	Diluition	Code	Company
POU5F1	1:200	Sc-5279	Santa Cruz Biotechnology
SSEA-4	1:250	Sc-21704	Santa Cruz Biotechnology
KLF17	1:250	HPA024629	Atlas Antibodies
TFE3	1:250	HPA023881	Atlas Antibodies
SUSD2	1:100	327401	BioLegend
SUSD2-PE	5 µl/million cells	327406	BioLegend
GATA3	1:100	AF2605	R&D Systems
SOX1	1:50	AF3369	R&D Systems
SOX17	1:50	AF1924	R&D Systems
T	1:50	AF2085	R&D Systems
OTX2	1:100	AF1979	R&D Systems
NANOG	1:100	4903S	Cell Signaling Technology
FOXA2	1:300	D56D6	Cell Signaling Technology
SOX2	1:500	NB110-37235	Novus Biologicals
FOXG1	1:500	Ab18259	Abcam
COLI	1:1000	Ab34710	Abcam
COLIV	1:1000	Ab6586	Abcam
COLVI	1:250-500	70R-CR009x	Fitzgerald
INTEGRIN β1	1:10	MABT821	Sigma-Aldrich
LAMININS	1:250	L9393	Sigma-Aldrich
VIMENTIN	1:50	SAB1305445	Sigma-Aldrich

Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 488	1:200	A-21206	ThermoFisher Scientific
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 647	1:200	A-31573	ThermoFisher Scientific
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 594	1:200	A-21203	ThermoFisher Scientific
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488	1:200	A-11055	ThermoFisher Scientific
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor 647	1:200	A-21447	ThermoFisher Scientific

Supplementary Table 5. List of all TaqMan assay probes and real-time qPCR primers used in this work, related to Figure 5 and S4.

For 3D naïve hiPSCs analysis (Related to Figure 5)

Gene name	Product code	Species	Dye	Company
POU5F1	Hs00999632_g1	Hs	FAM	ThermoFisher Scientific
NANOGP1	Hs04399610_g1	Hs	FAM	ThermoFisher Scientific
KLF17	Hs00702999_m1	Hs	FAM	ThermoFisher Scientific
KLF4	Hs00358836_m1	Hs	FAM	ThermoFisher Scientific
DPPA5	Hs00988349_g1	Hs	FAM	ThermoFisher Scientific
TFCP2L1	Hs00232708_m1	Hs	FAM	ThermoFisher Scientific
ZIC2	Hs00600845_m1	Hs	FAM	ThermoFisher Scientific
GATA 4	Hs01034629_m1	Hs	FAM	ThermoFisher Scientific
GATA 6	Hs00232018_m1	Hs	FAM	ThermoFisher Scientific
GSC	Hs00418279_m1	Hs	FAM	ThermoFisher Scientific
NID2	Hs00201233_m1	Hs	FAM	ThermoFisher Scientific
MIXL1	Hs04400364_m1	Hs	FAM	ThermoFisher Scientific

GAPDH	Hs02786624_g1	Hs	VIC	ThermoFisher Scientific

For Trophoblast Stem Cell analysis (Related to Supplementary Figure 4)

Gene name	**Forward primer (5'-3')**	**Reverse primer (3'-5')**
GAPDH	CGAGATCCCTCCAAAATCAA	GGCAGAGATGATGACCCTTT
OCT4	GTGGAGGAAGCTGACAACAA	ATTCTCCAGGTTGCCTCTCA
NANOG	TTTGTGGGCCTGAAGAAAACT	AGGGCTGTCCTGAATAAGCAG
KLF17	CACACAGGTGAGAGGCCATA	TATGCGGGTACACACCAGAT
ZIC2	CATGCACGGTCCACACCTC	CTCATGGACCTTCATGTGCTT
GATA2	GACTACAGCAGCGGACTCTT	GCCTTCTGAACAGGAACGAG
GATA3	ACAGAAGGCAGGGAGTGTGT	TCCGTTCATTTTGTGATAGAGC
TFAP2A	TCCCAATGAGCAAGTGACAA	CAGCAGGTCGGTGAACTCTT
KRT7	AGGAGAGCGAGCAGATCAAG	CTTGGTCTCCAGCAGCTTGT