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Supplementary Materials for

Early developmental plasticity enables the induction of an intermediate extraembryonic cell state

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary figure legends

Figure S1. iXTE and PE cells incorporation into pre-implantation embryos, related to Figure 2.

(A) TE excision from E4.5 blastocysts (n embryos = 30) and culture of the dissected TE (n TE = 30) in iXTE medium and MEFs.

(B) E2.5 embryos were cultured for 48h in the presence of DMSO (control) or PD325901 and stained for Nanog, Cdx2 and Sox17. The embryos were then placed in individual wells of 96-well plate and cultured in the presence of MEFs and iXTE medium for derivation of iXTE cells.

(C) Number of TE-like (iXTE) cells derived from control (DMSO, n = 5) or PD325901 pre-treated embryos (n = 9). Error bars represent SEM, unpaired Student's t test.

(D) Chimeric embryos stained for mTom, Troma1, Sox2 and DAPI. Arrows indicate the mTom-expressing iXTE cells.

(E) Quantification of iXTE cells incorporation into host blastocysts (n embryos = 15).

(F) Schematic representation of the conversion of the ICM into PE, the subsequent isolation of the PE cells and their incorporation into pre-implantation embryos.

(G) Incorporation of PE cells in chimeric blastocyst (n = 8) stained for Gata6, Cdx2 and DAPI.

(H) Quantification of incorporation of PE cells into PE, mural TE or Polar TE in chimeric blastocysts (n embryos = 8).

Scale bar = 20 um

Figure S2. XEN cells reprograming into iXTE cells, related to Figure 3.

(A) FACS analysis of XEN cells grown in iXTE medium for the surface markers PDGFRα and CD40 across consecutive passages. XEN cells maintained in basal N2B27 medium was used as control. Error bars represent SEM, n=3.

(B) qPCR analysis of Cdx2, Gata6, Sox17 and Nanog expression in XEN cells and iXTE cells derived from XEN cells. β-actin was used as an internal control. Error bars represent SEM, n=3.

(C) PDGFRα and CD40 surface marker expression FACS analysis of XEN cells cultured in the presence of 50 ng/ml Bmp4, compared to control, untreated cells.

(D) Quantification of PDGFRα and CD40 FACS analysis of XEN cells cultured in the presence of Bmp4, compared to control, untreated cells. Three independent experiments.

(E) Control untreated XEN cells and XEN cells treated with Bmp4 stained for Cdx2, Gata6 and DAPI.

(F) Quantification of the incorporation into host embryos of iXTE cells established from XEN cells (n embryos = 21).

(G) Incorporation of Venus+ XEN cells in chimeric blastocyst (n = 23).

(H) Quantification XEN cells incorporation into host embryos (n embryos = 23).
(I) Incorporation of GFP+ TSC cells in chimeric blastocyst (n = 22).
(J) Quantification TSC incorporation into host embryos (n embryos = 22).
Scale bar = 20 μm.

Figure S3. Establishment of XEN state is required for the activation of the TE program, related to Figure 3.
(A) qPCR analysis of Cdx2, Gata6, Sox17 and Nanog expression in iXTE cells derived from ESC in comparison to the expression levels in ESC and XEN-like cells. β-actin was used as an internal control. Error bars represent SEM and significance was calculated using unpaired student's t-test, n=3.
(B) FACS analysis of PDGFRα and CD40 expression in ESC grown in iXTE medium.
(C) ESC grown in 2i/Lif medium or iXTE medium and stained for Cdx2, Oct4, Sox17 and DAPI.
(D) FACS analysis of PDGFRα and CD40 expression in TSC grown in iXTE medium.
(E) ESC grown in TSC medium or iXTE medium and stained for Cdx2, Gata6, Sox17 and DAPI.
(F) FACS analysis for expression of PDGFRa and CD40 in iXTE cells derived from ES cells. After conversion of ESC into XEN-like cells, they were cultured in iXTE medium either for 4 days (Passage 1, Day 4), 5 days (passage 1, Day 5) or passaged on day 4 and maintained for 24 h more (Passage 2, Day 1) and then analysed by FACS.
(G) Quantification of PDGFRα+/Gata6-H2B:Venus+ (XEN-like) cells generated after Dox induction for 24, 48 or 72h, followed by incubation in iXTE medium for 5 days and reprograming to PDGFRα+/CD40+ cells; one-way ANOVA, error bars represent SEM, n=3.
(H) Incorporation of Venus+ ESC-derived iXTE cells in chimeric blastocyst (n = 18) stained for Gata4 and DAPI.
(I) Quantification of the incorporation into host embryos of iXTE cells established from XEN-like cells (n embryos = 20), unpaired student's t-test, error bars represent SEM.
(J) Quantification of the incorporation of CD40 low (n embryos = 9) and CD40 high cells (n embryos = 9) in chimeric blastocysts.
Scale bar C, E, H = 20 μm

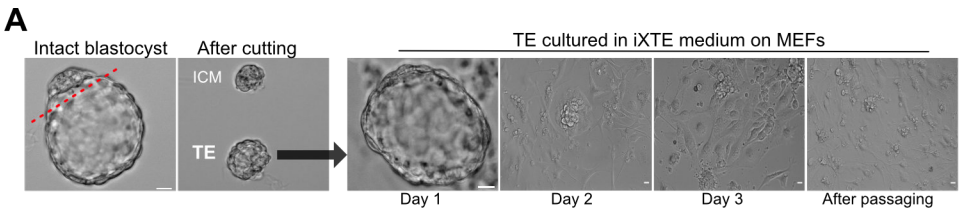
Figure S4. Differentiation potential of iXTE cells, related to Figure 4.
(A) E5.5 chimeric embryo with intact (left panel) and peeled RM (right panel), containing mTom-positive cells (n chimeric E5.5 embryos = 14).
(B) E5.5 chimeric embryo with intact (left panel) and peeled RM (right panel), containing Gata6-H2B:Venus positive cells (n chimeric E5.5 embryos = 5).
Scale bar A, B = 40 μm

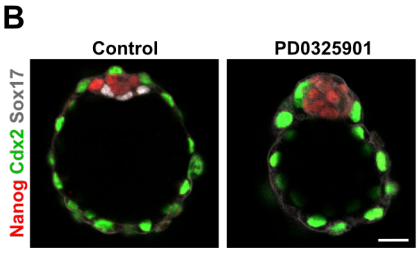
Figure S5. Transcriptional state of the iXTE cells, related to Figure 5.
(A) "Elbow plot" method assessing the appropriate number of clusters for the gene expression clustering shown in Figure 5B. The variability within clusters was measured using the total within-cluster sum of squares.
(B) GO "biological process" enrichment analysis for each cluster in Figure 5B.
(C) GO "molecular function" enrichment analysis for each cluster in Figure 5B.

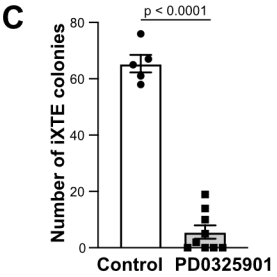
Figure S6. Mechanism of XEN-state reprogramming to iXTE cells, related to Figure 6.
(A) Embryo-derived iXTE cells stained for E-cad and DAPI.
(B) XEN cells and iXTE cells derived from XEN cells stained for β-catenin and DAPI.
(C) XEN cells and iXTE cells derived from ESC cells stained for β-catenin and DAPI.
(D) Genotyping PCR for recombination of the conditional E-cad allele. GAPDH was used as loading control and 1Kb ladder was used for determining the size of the PCR products.
(E) Control and E-cad deficient cells stained for Ezrin, Par6 and DAPI, yellow lines indicate orthogonal sections.
(F) XEN cells and iXTE cells derived from XEN cells stained for Yap and DAPI.
(G) Genotyping PCR for recombination of the conditional Yap allele. GAPDH was used as loading control and 1Kb ladder was used for determining the size of the PCR products.
(H) Genotyping PCR for recombination of the conditional Taz allele. GAPDH was used as loading control and 1Kb ladder was used for determining the size of the PCR products.
(I) Control and Peptide 17 treated iXTE cells stained for Gata6 H2B:Venus, Yap, Cdx2 and DAPI.
(J) CBF:H2B-Venus embryos stained for Venus, Cdx2 and DAPI.
(K) Live imaged iXTE cells derived from CBF:H2B-Venus blastocyst.
Scale bars = 20 μm.

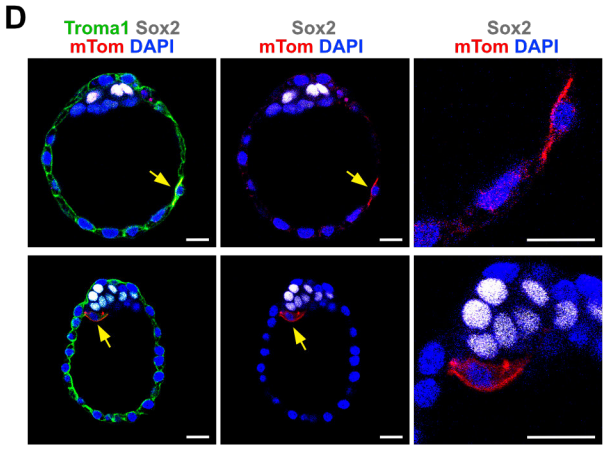
Figure S7. Tissue-scale organization of iXTE cells, related to Figure 7 and Figure 8.
(A) iXTE vesicles stained for E-cad (magenta) and DAPI (cyan).
(B) Snapshot images of time lapse microscopy of blastocyst and iXTE vesicles cultured in the presence of serum containing medium
(C) FACS analysis for DNA content indicating the ploidy of TSC and iXTE cells cultured in serum containing medium for 5 days, 4 independent experiments.
(D) Quantification of the 4N+ polyploid population in TSC and iXTE cells grown in serum containing medium; error bars represent SEM, unpaired Student's t test. 4 independent experiments.
(E) Embryoids stained for Nanog, Sox2, Par6, Gata6, Oct4 and Cdx2. Nuclei counterstained with DAPI.
Scale bars A, E = 20 μm, B = 50 μm.

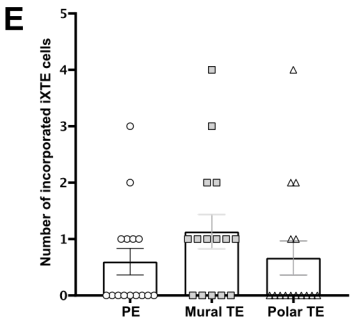
Figure S8. Singe cell RNA-seq analysis, related to Figure 8.
(A) Hierarchical clustering of cell populations identified in the scRNA-seq of the current study and the scRNA-seq datasets of Pijuan-Sala et al. *(27)*.
(B) Violin plot of Gata3, Enpep and Tfap2c expression in the different cell clusters.

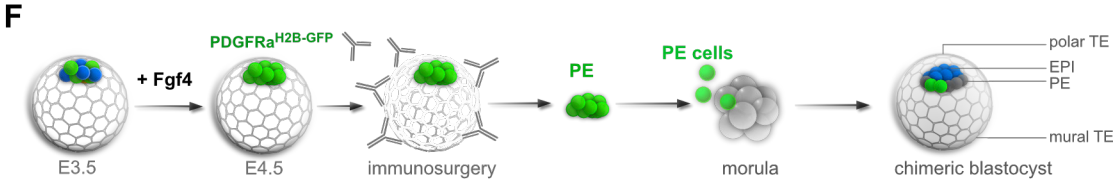


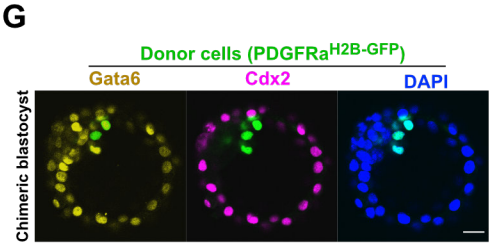












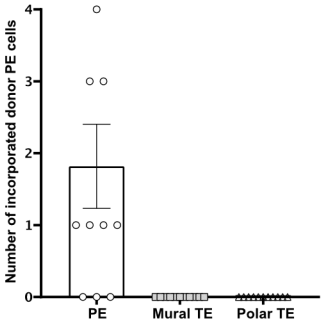


FIGURE S1 Sathyanarayanan et al

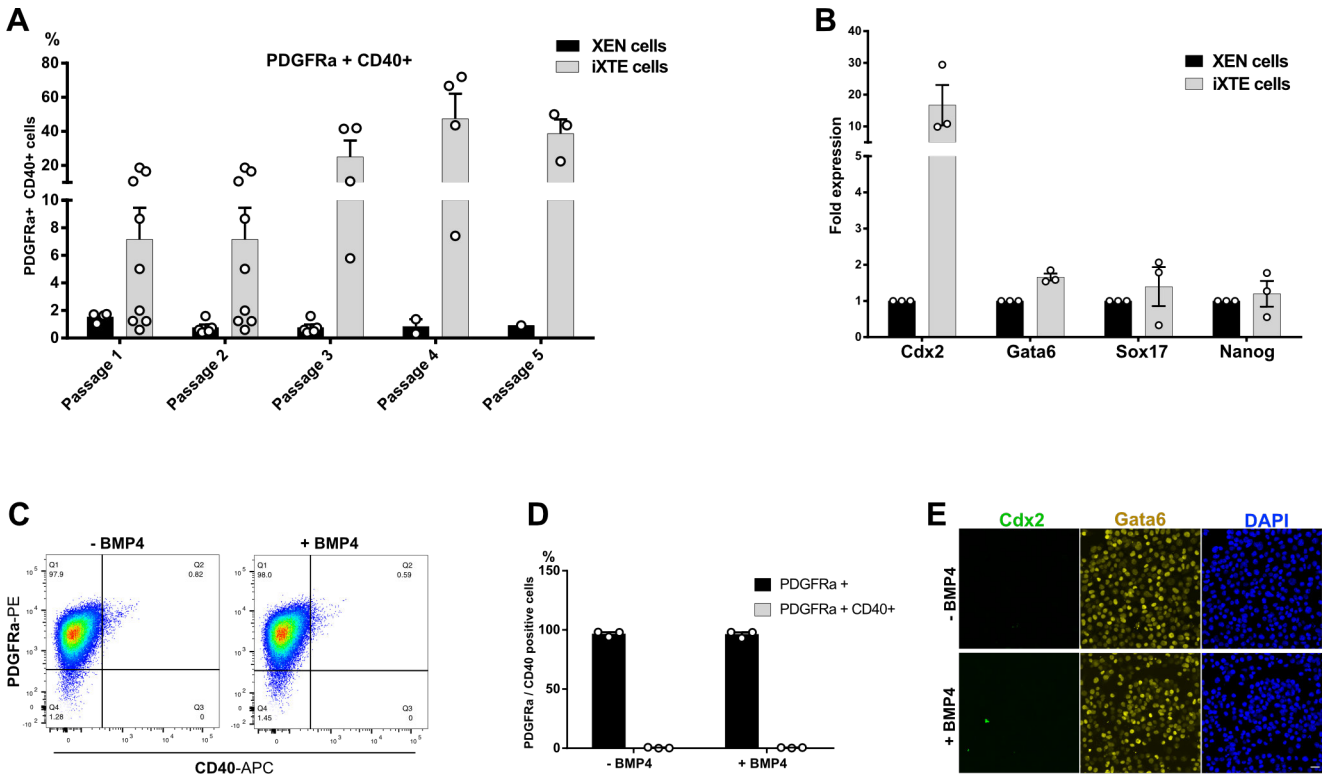


FIGURE S2 Sathyanarayanan et al

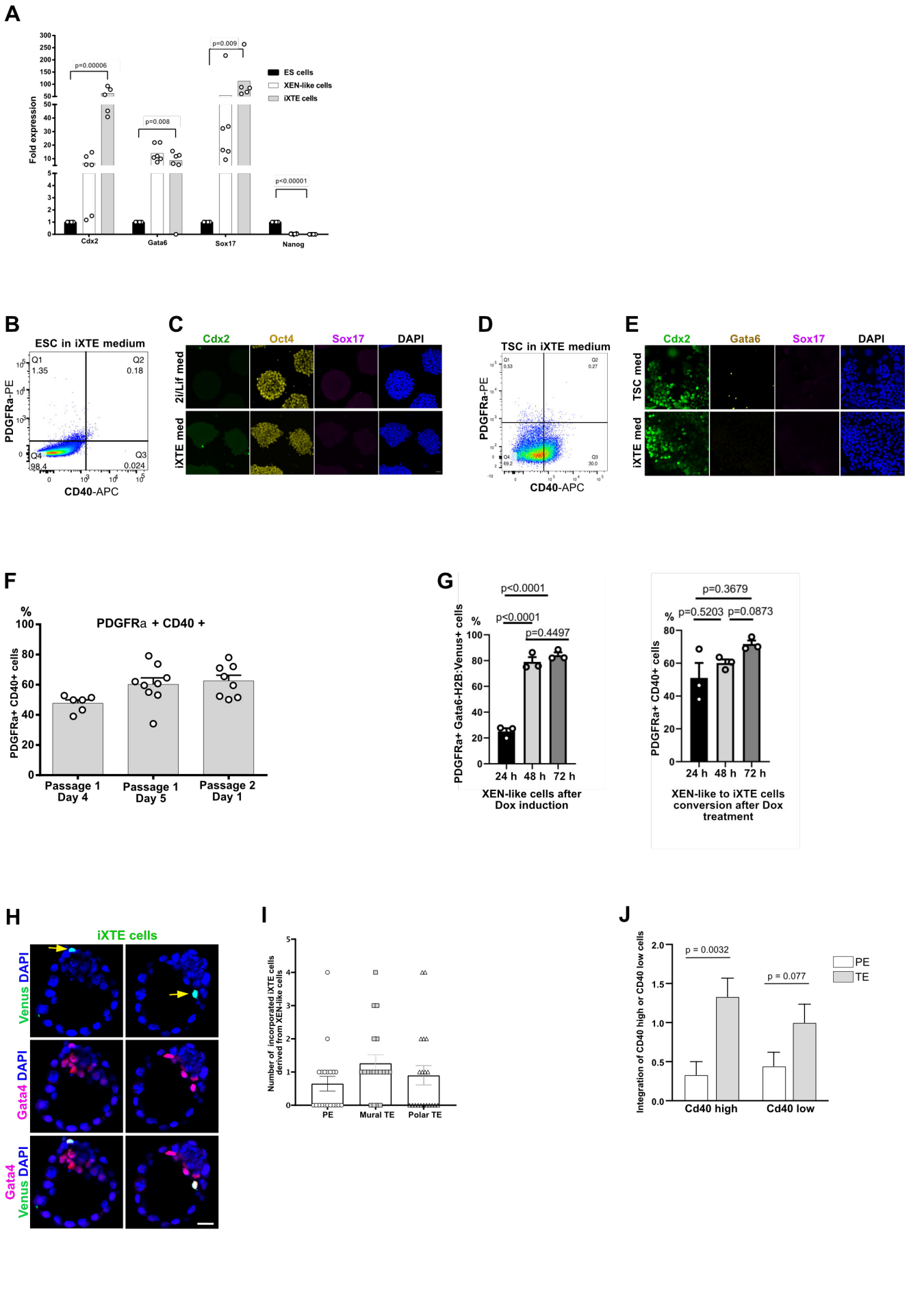


FIGURE S3 Sathyanarayanan et al

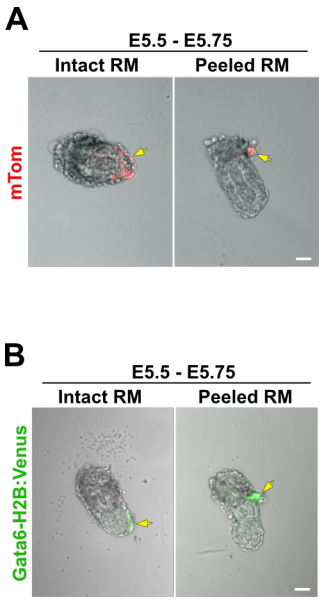
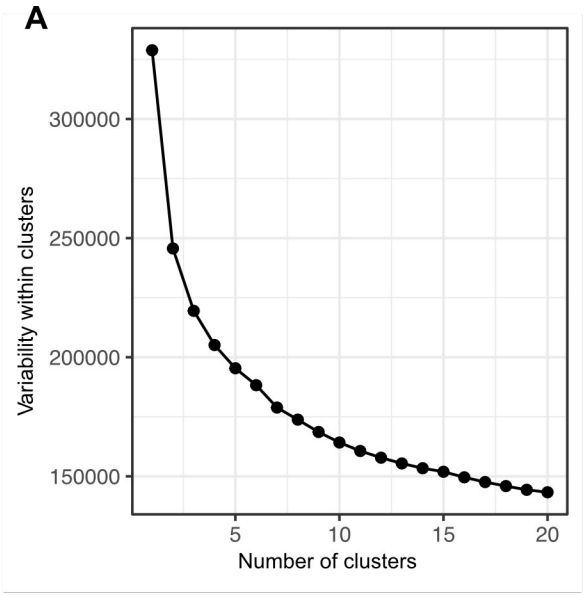
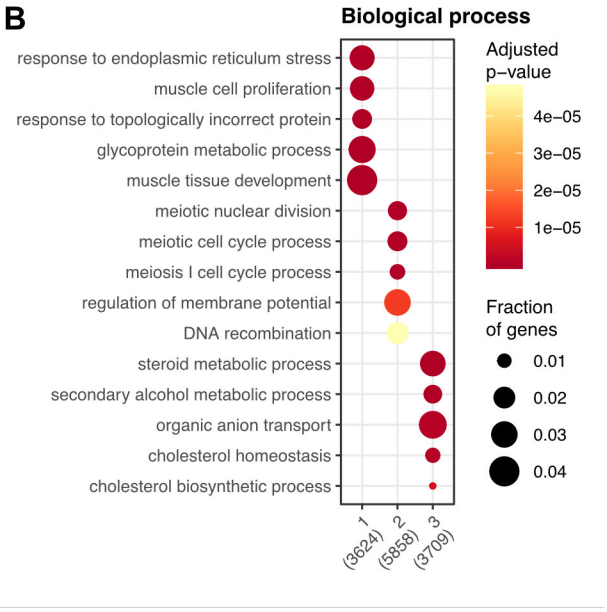
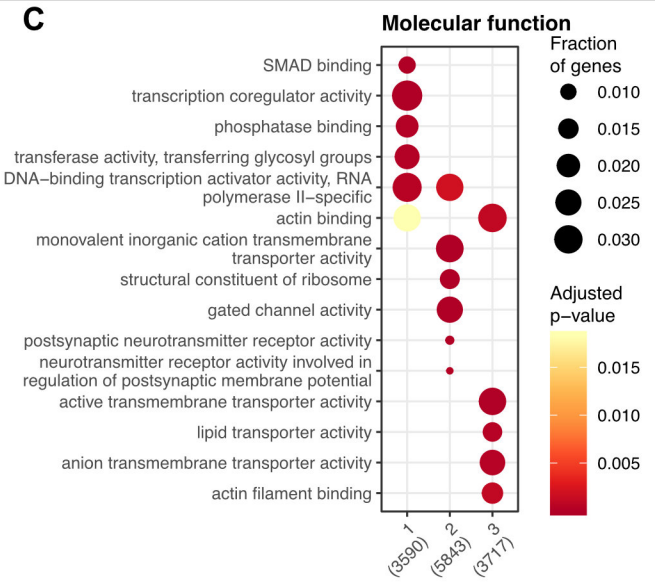


FIGURE S4 Sathyanarayanan et al







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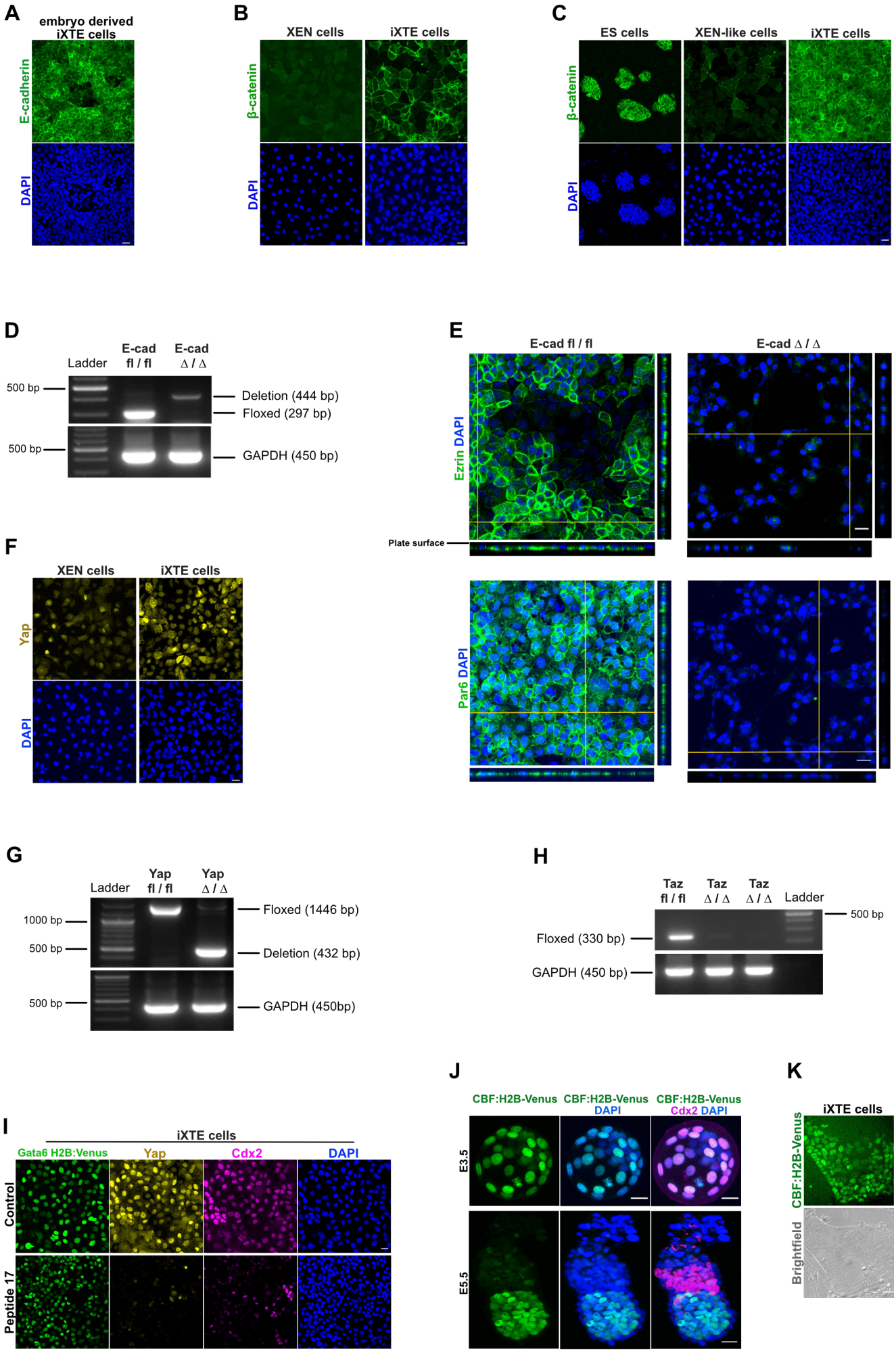

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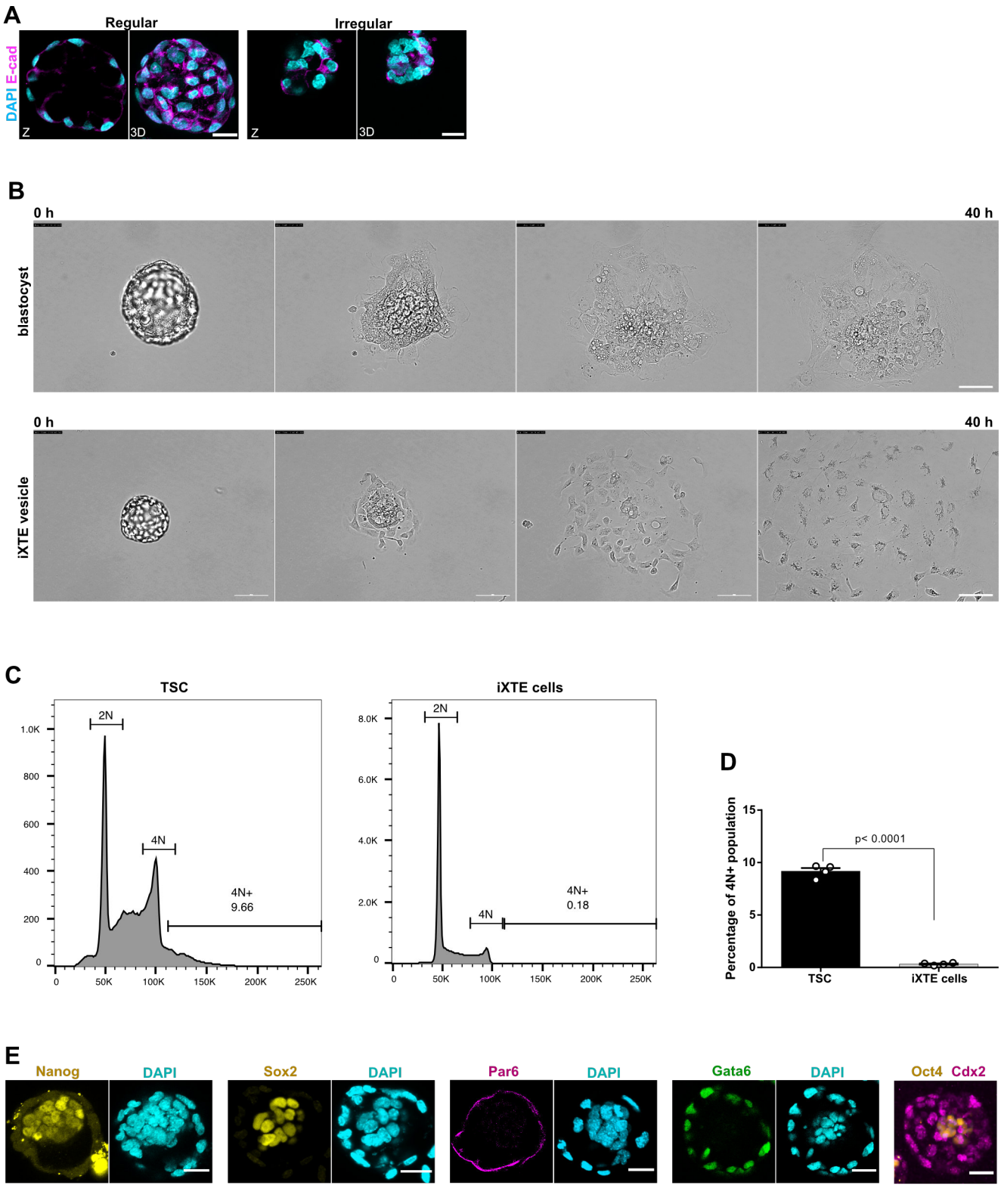
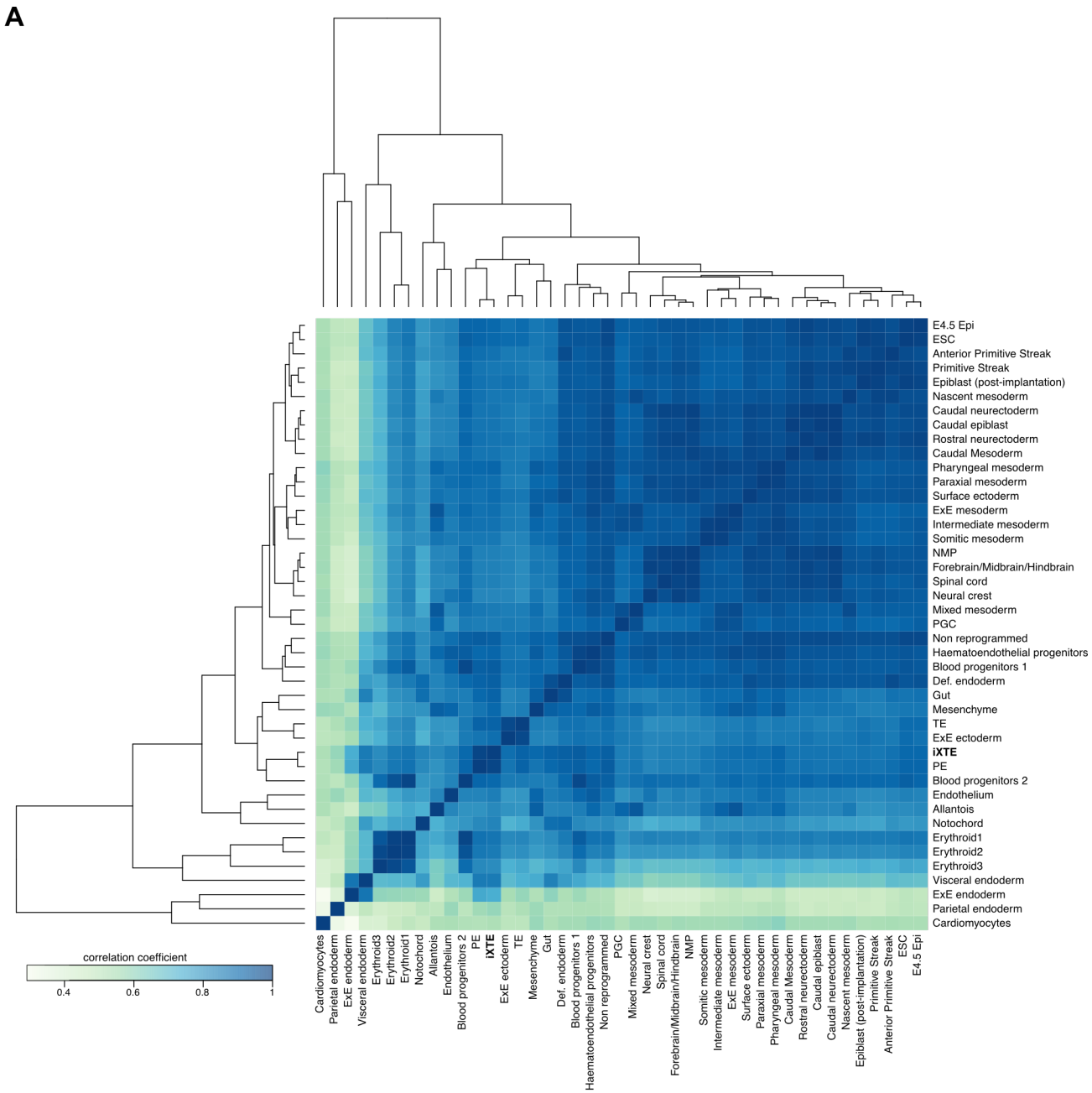


FIGURE S7 Sathyanarayanan et al



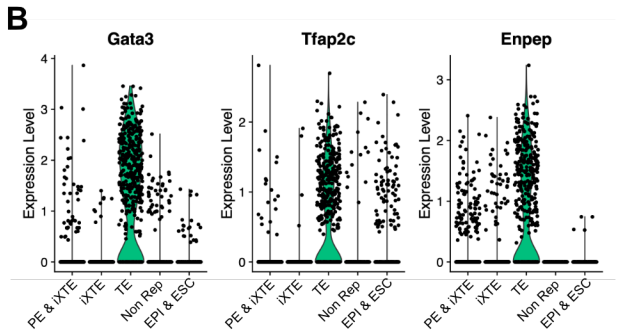


FIGURE S8 Sathyanarayanan et al

Supplementary tables

Table S1. qPCR primers used in this study.

Gene	Forward	Reverse
Cdx2	ACCGGAATTGTTTGCTGCTGT	TCCCGACTTCCCTTCACCAT
Gata6	AGACGGCACCGGTCATTACC	TCACCCTCAGCATTTCTACGCC
Sox17	GCTAGGCAAGTCTTGGAAGG	CTTGTAGTTGGGGTGGTCCT
Nanog	AGGGTCTGCTACTGAGATGCTCTG	CAACCACTGGTTTTTCTGCCACCG
β-actin	GGTCATCACTATTGGCAACG	TCCATACCCAAGAAGGAAGG
Krt18	ACAAGTACTGGTCTCAGCAG	GCATGGAGTTGCTGGAGTC
E-cad	CACCTGGAGAGAGGCCATGT	TGGGAAACATGAGCAGCTCT
Gata4	GGGCCCTCTTTGTCATTCTTC	TCCTTGCTTTCTGCCTGCTAC
Lamb1	CAGAATGCAGACGATGTTAAGAA	GGCATCTGCTGACTCTTCAGT
Sparc	AGGGCCTGGATCTTCTTTCTC	CAAATTCTCCCATTTCCACCT
tPa	CTGACTGGACAGAGTGTGAGCTT	ACAGAT GCT GTGAGGTGCAG

Table S2. Primers used for genotyping.

Gene	Forward	Reverse	Product size
Yap	CTTTTGTCCCTCACCCAGC TA	GCTGAAAGAATGCACAAGG TCC	Floxed - 1446bp Del - 432bp
Taz	AAGCAGTTTCCACTTCATG AAAC	AGTCAAGAGGGGCAAAGTT GTGA	Floxed- 330bp
E-cad	CTTATACCGCTCGAGAGC CGGA; TGTTCCAAGCCTGCTTTCT T	TGACACATGCCTTTACTTTA GT	Floxed - 297bp Del - 444bp
GAPDH	ACCACAGTCCATGCCATCA CT	GTCCACCACCCTGTTGCTG TA	450bp

Table S3. List of antibodies.

Antibody	Vendor	Catalogue number	Dilution
Cdx2 (mouse)	Biogenex	MU392A-5UC	1:300
β-catenin (mouse)	BD Biosciences	610154	1:300
E-cadherin (mouse)	BD Biosciences	610182	1:300
Nanog (mouse)	Cell signaling technology	8822	1:300

Oct4 (mouse)	Santa Cruz Biotechnology	sc-5279	1:300
Oct4 (rabbit)	Cell signaling technology	83932S	1:300
Yap (rabbit)	Cell signaling technology	14074S	1:300
RFP (rabbit)	Biomol	600-401-379	1:300
Eomes (rabbit)	Abcam	ab23345	1:300
Pard6B1 (rabbit)	Santa Cruz Biotechnology	sc-67393	1:300
Sox2 (rabbit)	Cell signaling technology	23064S	1:300
Gata6 (rabbit)	Thermo Fisher Scientific	PA1-104	1:300
Gata4 (rabbit)	Santa Cruz Biotechnology	SC-9053	1:300
Sox17 (goat)	R&D	AF1924	1:300
Ezrin	Cell signaling technology	3726S	1:300
GFP (goat)	R&D	AF4240	1:200
Troma1 (rat)	Home made		1:200
Podocalyxin (rat)	R&D systems	MAB1556	1:300
Laminin (rabbit)	Sigma	L9393	1:400
F-actin (Alexa Fluor® 647 Phalloidin)	Cell signaling technology	8940S	1:200
Alexa 594 (donkey Anti-Rabbit)	Thermo Fisher Scientific	A-21207	1:200
Alexa 488 (donkey Anti-Rabbit)	Thermo Fisher Scientific	A-21208	1:200
Alexa 488 (donkey Anti-Mouse)	Thermo Fisher Scientific	A-21202	1:200
Alexa 647 (donkey Anti-Rat)	Thermo Fisher Scientific	A-21247	1:200
Alexa 647 (donkey Anti-mouse)	Thermo Fisher Scientific	A-31571	1:200
Alexa 488 (donkey Anti-Goat)	Thermo Fisher Scientific	A-11055	1:200
Alexa 647 (donkey Anti-Goat)	Thermo Fisher Scientific	A-21447	1:200
Alexa 488 (donkey Anti-Rabbit)	Thermo Fisher Scientific	A-21206	1:200
Pdgfrα (mouse, PE-tagged)	E-biosciences	12-1401-81	1:200
CD40 (rabbit, APC-tagged)	Thermo Fisher Scientific	17-0401-82	1:100
α-tubulin (mouse)	Sigma	T6199	1:500
Secondary HRP Anti-Mouse IgG	Jackson	115-035-044	1:10000

Captions for Movies S1 and S2

Movie S1. Time lapse microscopy of mouse blastocyst cultured in serum containing medium, related to Figure 6.

Movie S2. Time lapse microscopy of iXTE vesicle cultured in serum containing medium, related to Figure 6.