

1 **Supplementary Materials**

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16 **Branching development of early post-implantation human embryonic-like tissues in**
17 **3D stem cell culture**

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Table S1. Lists of primary antibodies.

Targeted protein	Species	Dilution	Catalog No.	Vendor
EZRIN	Mouse	1:2000	E8897	Sigma-Aldrich
E-CADHERIN	Mouse	1:500	610181	BD Biosciences
NANOG	Rabbit	1:500	4903S	Cell Signaling Technology
T (Brachyury)	Goat	1:100	PA5-46984	Thermo Fisher Scientific
GATA3	Mouse	1:100	MA1-028	Thermo Fisher Scientific
GATA3	Rabbit	1:500	D13C9	Cell Signaling Technology
SOX17	Goat	1:500	AF1924	R&D Systems
CDX2	Mouse	1:300	MU392AUC	Biogenex
SNAI2	Rabbit	1:100	SC-28199	Santa-Cruz Biotechnology

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Table S2. The morphological and intensity features of the pre-gastrulation tissues.

Feature name	Definition
Mean intensity \bar{t}	$\bar{t} = \frac{1}{N} \sum_{i=1}^N t\left(\frac{2\pi i}{N}\right)$, where $t(\theta)$ is the tissue thickness at θ .
Thickness standard deviation σ_t	$\sigma_t = \sqrt{\frac{1}{N-1} \sum_{i=1}^N \left(t\left(\frac{2\pi i}{N}\right) - \bar{t}\right)^2}$.
Thickness ratio r_t	$r_t = \frac{\max(t)}{\min(t)}$.
Thickness skewness	The skewness of the distribution of tissue thickness.
Area	The area of the tissue at the confocal imaging plane.
Lumen area	The area of the lumen.
Eccentricity	A measure of how non-circular the tissue boundary is. Eccentricity is calculated by python module Scikit-image.
Lumen eccentricity	A measure of how non-circular the inner tissue boundary (also the outer lumen boundary) is.
Diameter	The diameter of the smallest circle which can include the tissue inside.
Lumen diameter	The diameter of the smallest circle which can include the lumen inside.
Major axis length	The length of the major axis of the ellipse that has the same normalized second central moments as the tissue. The major axis length is calculated by python module Scikit-image.
Minor axis length	The length of the minor axis of the ellipse that has the same normalized second central moments as the tissue. The minor axis length is calculated by python module Scikit-image.
Perimeter	The perimeter of the tissue out boundary.
Cell number	The estimated number of cell nucleus counted based on nuclei segmentation.
GATA3 gyration	Intensity gyration of GATA3 marker, which is the distance between the mass center of GATA3+ cells and the mass center of the lumen.
GATA3 mean intensity	The mean intensity of the nucleus of GATA3+ cells.
GATA3 cell number	The number of GATA3+ cells.
GATA3 cell ratio	The percentage of GATA3+ cells.
NANOG gyration	Intensity gyration of Nanog marker, which is the distance between the mass center of NANOG+ cells and the mass center of the lumen.
NANOG mean intensity	The mean intensity of the nucleus of NANOG+ cells.
NANOG cell number	The number of NANOG+ cells.

NANOG cell ratio	The percentage of NANOG+ cells.
NANOG gyration	Intensity gyration of NANOG marker, which is the distance between the mass center of NANOG+ cells and the mass center of the lumen.
T mean intensity	The mean intensity of the nucleus of T+ cells.
T cell number	The number of T+ cells.
T cell ratio	The percentage of T+ cells.
GATA3-T gyration	The distance between the mass center of GATA3+ cells and the mass center of T+ cells.

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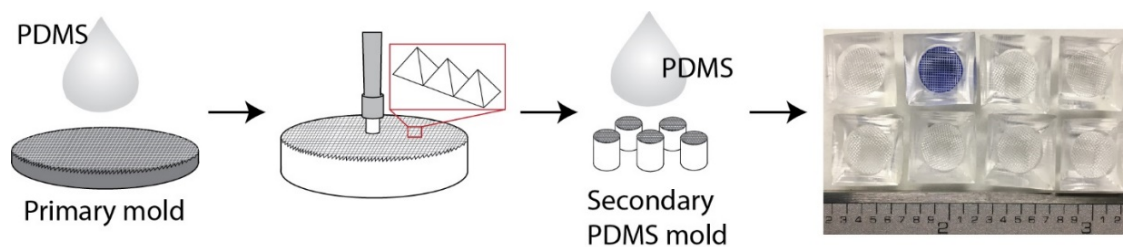


Fig. S1. Schematics of fabrication process of PDMS-based pyramidal well devices via replica-molding.

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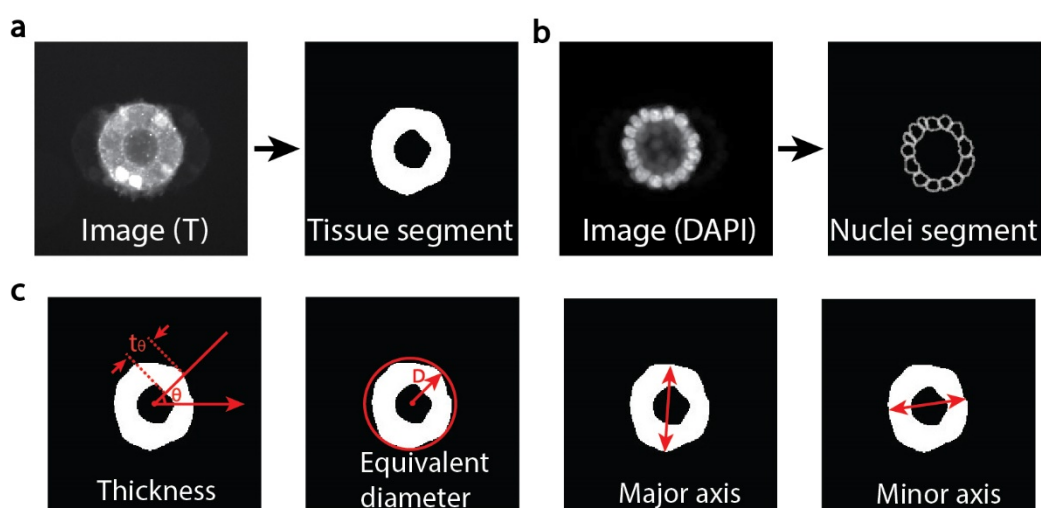


Fig. S2. Schematics of tissue segmentation, nucleus segmentation and morphological feature extraction. **a**, Tissue segmentation based on fluorescent image showing staining for T. Tissue area is labeled in white in the resulting image. **b**, Nucleus segmentation based on fluorescent image showing staining with DAPI. Boundaries of cell nuclei are labeled in white. **c**, Representative images of four morphological features, including tissue thickness, equivalent diameter, major axis and minor axis.

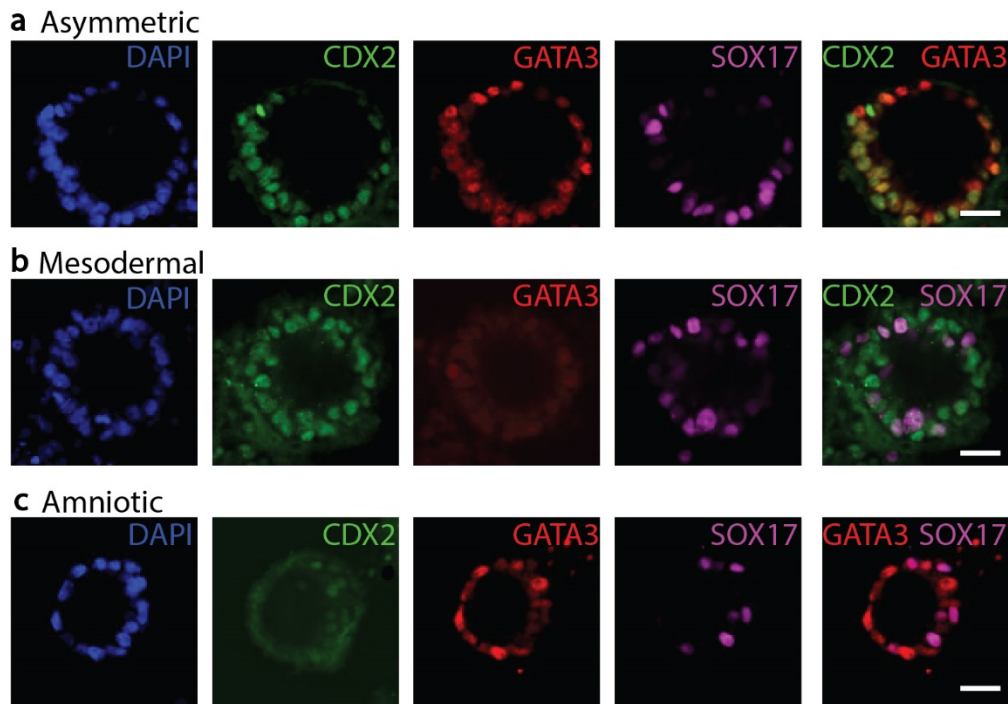


Fig. S3. Representative confocal images of asymmetric embryonic-like, mesodermal-like and amniotic-like tissues stained for CDX2, GATA3 and SOX17. Nuclei were stained with DAPI. Scale bars, 50 μ m.

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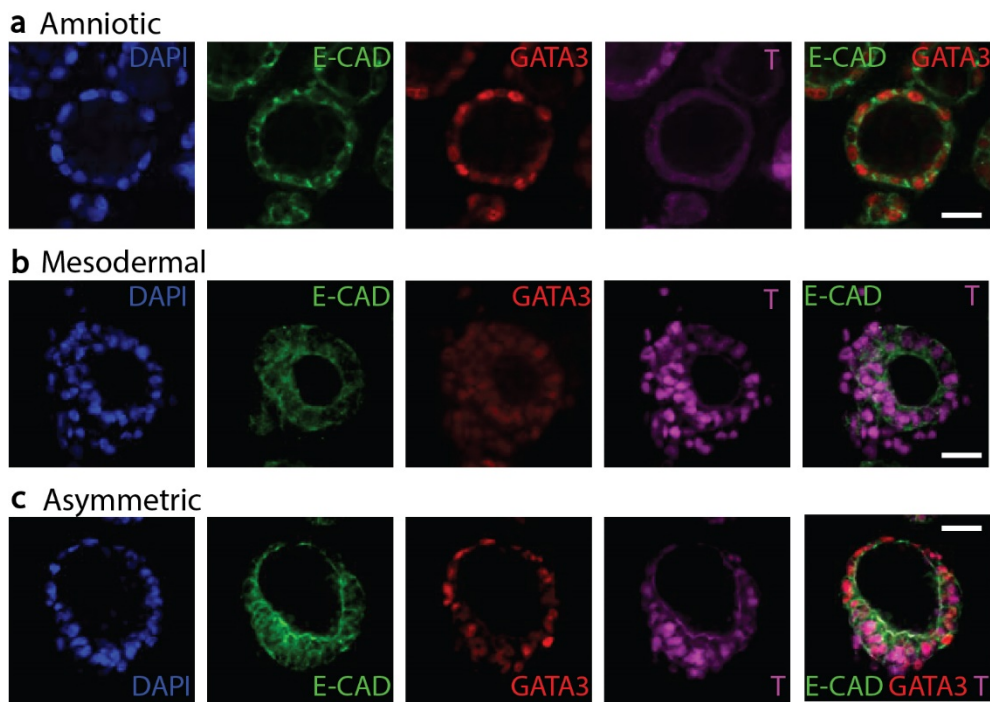


Fig. S4. Representative confocal images of asymmetric embryonic-like, mesodermal-like and amniotic-like tissues stained for E-CADHERIN, GATA3 and T. Nuclei were stained with DAPI. Scale bars, 50 μ m.

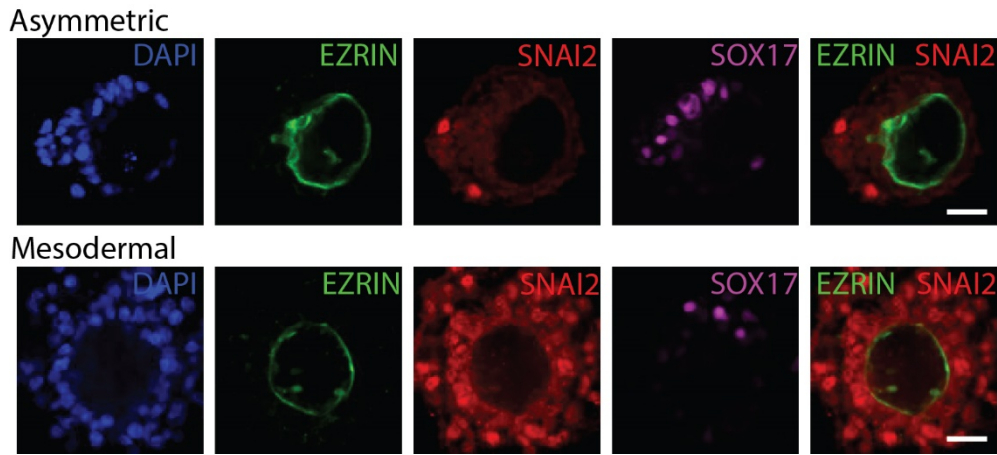


Fig. S5. Representative confocal images of asymmetric embryonic-like and mesodermal-like tissues stained for EZRIN, SNAI2 and SOX17. Nuclei were stained with DAPI. Scale bars, 50 μm .

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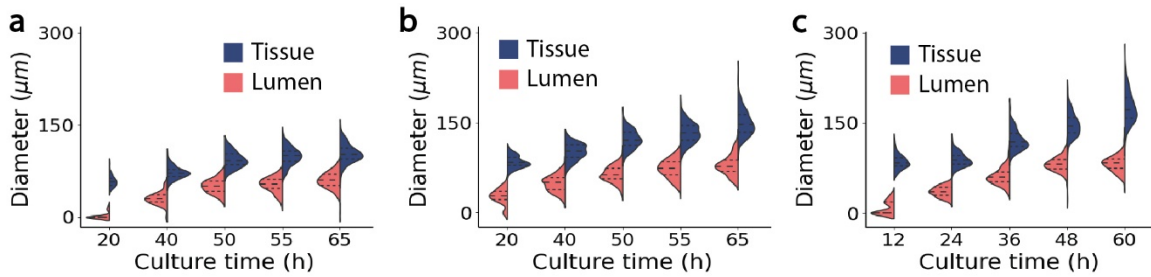


Fig. S6. The diameter of the tissue and the lumen over time when the initial seeding density is **a**, 1.8×10^4 cells/ml, **b**, 5.4×10^4 cells/ml and **c**, 9.0×10^4 cells/ml.

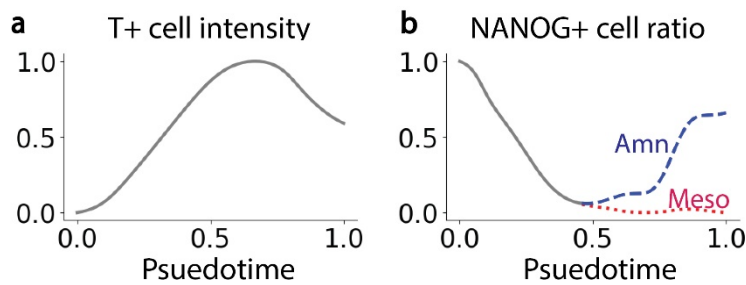


Fig. S7. **a**, T+ cell intensity along the asymmetric and mesodermal branches. **b**, NANOG+ cell ratio along the amniotic and mesodermal branches. The initial cell seeding density is 5.4×10^4 cells/ml.