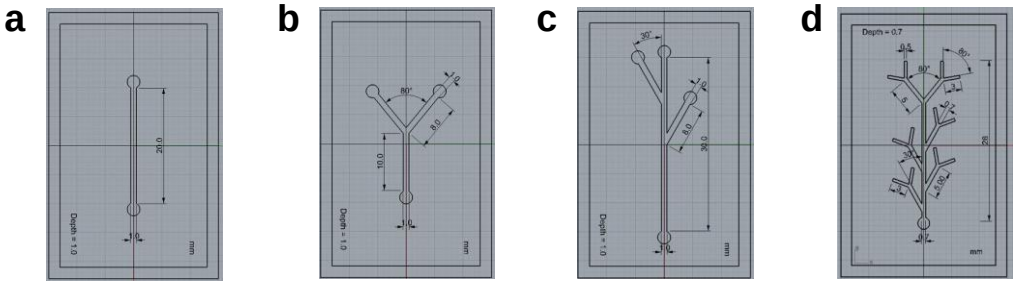
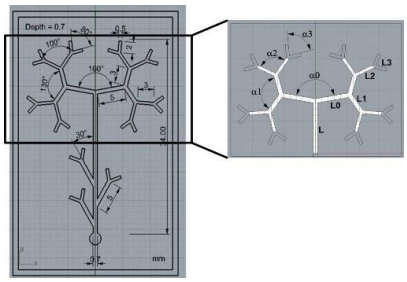
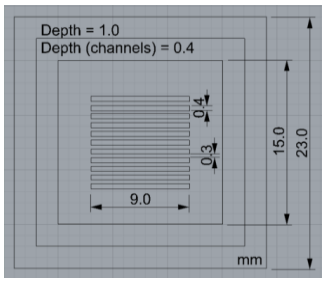
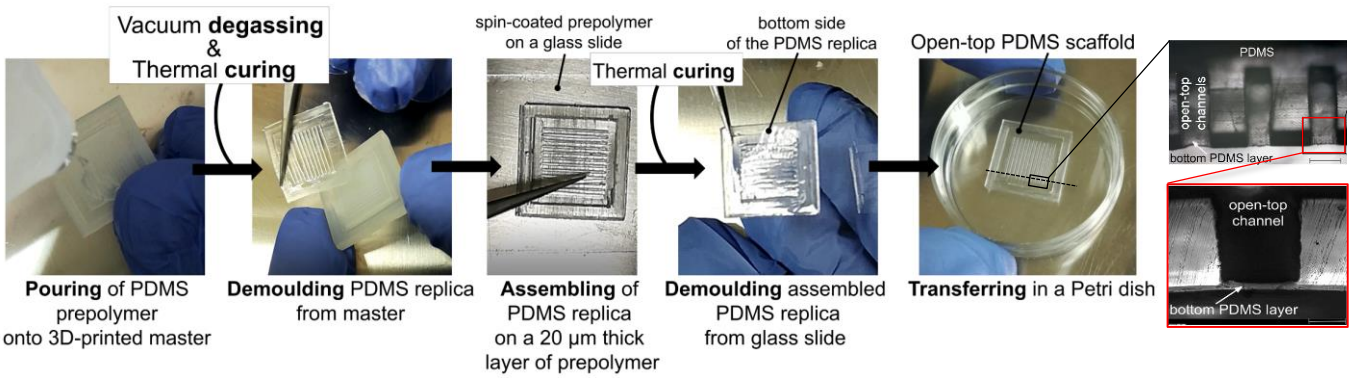


e

$$\begin{cases} \alpha_0 = 160° \\ \alpha_1 = 130° \\ \alpha_n = \dfrac{\alpha_{n-2}}{\varphi} \end{cases} \quad n = 2,3,\dots \qquad \begin{cases} L_0 = \dfrac{1}{\varphi} L \\ L_n = \dfrac{1}{\varphi} L_{n-1} \quad n = 1,3,5,\dots \\ L_n = L_{n-1} \quad n = 2,4,6,\dots \end{cases}$$







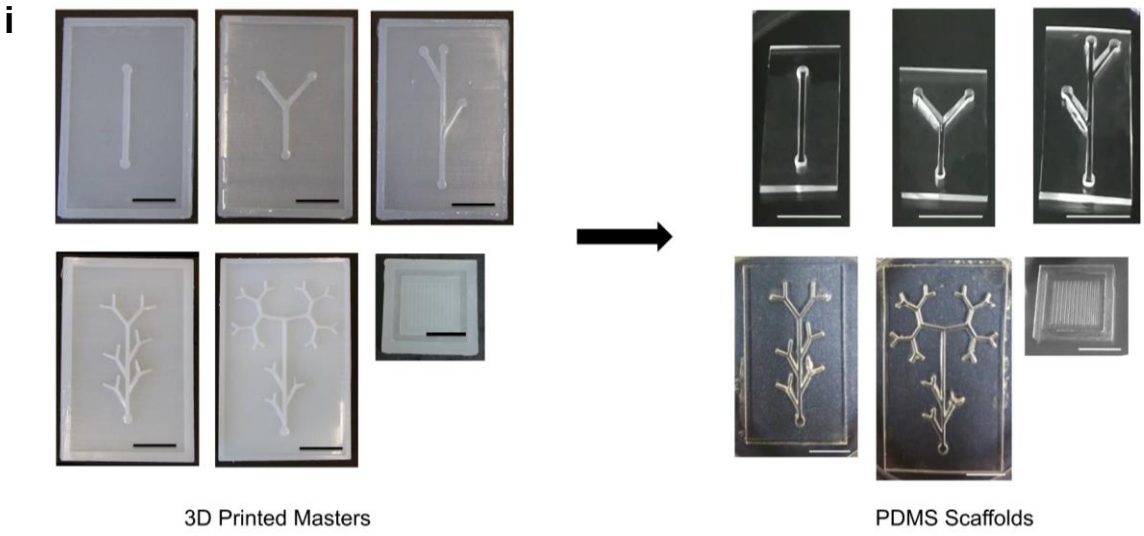


Figure S1

Supplementary Fig. 1. Design and fabrication of PDMS scaffolds. (A-D) Patterns designed for PDMS scaffold fabrication. Related lengths, depths, widths and angles are indicated for each pattern. Designed geometries: (A) linear, (B) bifurcated with an 80° terminal bifid branch, (C) asymmetrical with two 30° lateral branches arising from the central channel, and (D) ramified. (E) The 'golden fractal tree' formula. α_0 and α_1 represent the amplitude of first and second branching generation, respectively; *L* refers to central trunk length; *n* is the order of branching generation, and φ=1.618 is the 'golden ratio'. (F) The tree-like pattern consisting of fractal-like planar ramifications with different lengths and branching angles calculated by setting α_0=160° and α_1=130° and *n*=3. (G) Linear multichannel pattern for engineering multiple independent tubules at the same time. (H) Fabrication of PDMS micro-scaffold. The 3D-printed master is used to fabricate several copies of PDMS scaffolds through PDMS replica molding. PDMS prepolymer is poured onto the silanized master, the excess prepolymer is trimmed with a razor blade, and cured at 60°C for 1 h after removing bubbles in a vacuum chamber. The PDMS replica with void patterns is obtained by detaching the cured PDMS from the master. To control the thickness of the PDMS replica bottom side, first 1 ml of PDMS prepolymer is spin-coated on a glass slide at 2,500 rpm for 90 s to obtain a 20 μm-thick layer, and subsequently the PDMS replica is bounded against the coated glass at 60°C for 20 min. After curing and gentle cutting, the PDMS scaffold is separated from the glass slide and transferred into a standard Petri dish, with the open side facing up. To provide an insight into the scaffold geometry, the micro-scaffold was cut along the dashed line, showing tilted view of some PDMS microchannels. Scale bar: 400 μm. Cross section showing a rectangular microchannel with open-top geometry (red inset). Scale bar: 200 μm. All the scaffold types consist of the same rectangular cross-section grooves. The same fabrication process is applied to fabricate all the designed types of PDMS scaffold with linear, ramified and fractal-like patterns. (I) PDMS scaffolds types. Left: 3D-printed masters, right: PDMS patterned scaffolds. Scale bars: 10 mm.

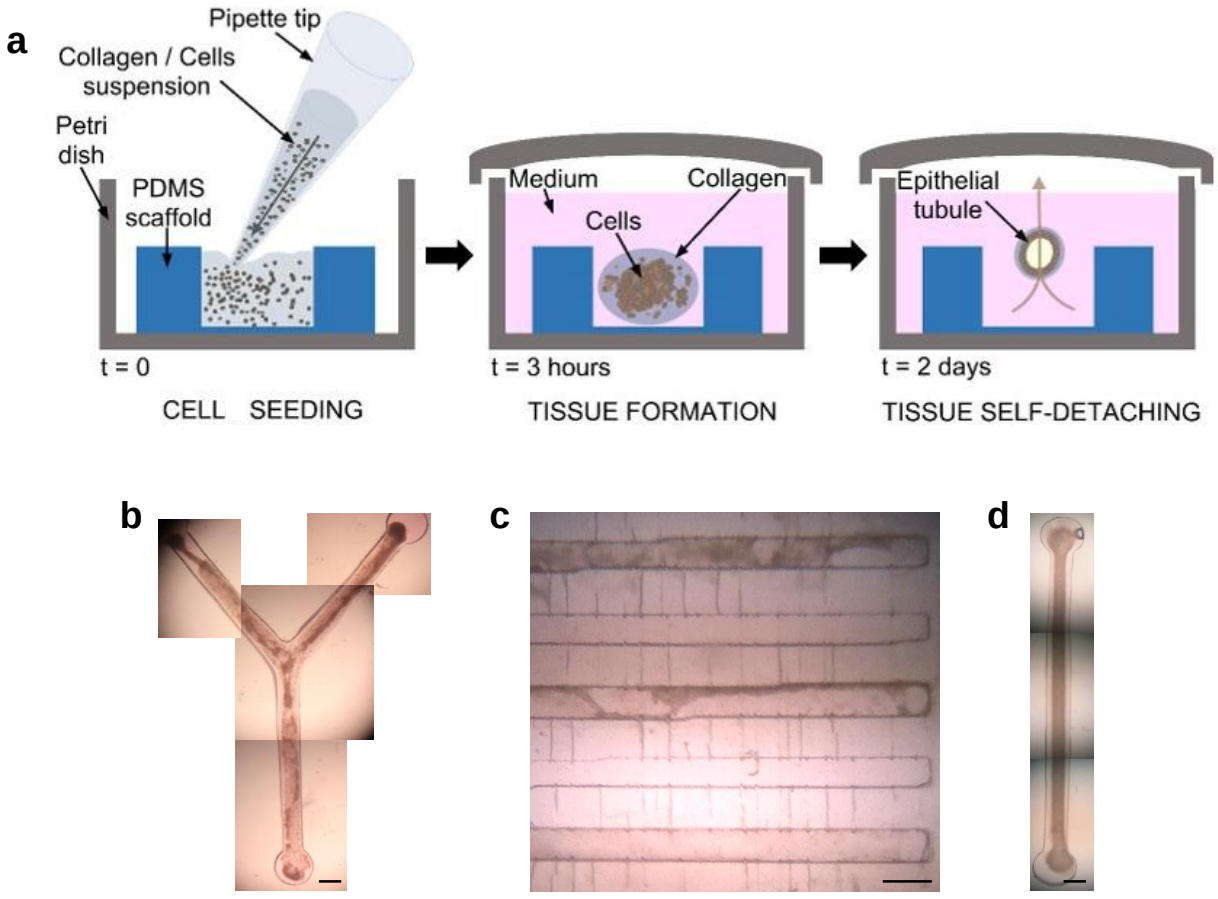

a
Pipette tip
Collagen / Cells suspension
Petri dish
PDMS scaffold
t = 0
CELL SEEDING
Medium
Collagen
Cells
t = 3 hours
TISSUE FORMATION
Epithelial tubule
t = 2 days
TISSUE SELF-DETACHING
b
c
d

Figure S2

Supplementary Fig. 2. Diagram of tissue engineering experiments and effects of cell densities on tubule formation. (A) The diagram shows the cell seeding and culture procedures from the section view. Cells suspended in collagen (with a density of $1.2x10^5$ and $2x10^5$ cells/µl collagen for macro- and micro-scaffolds, respectively) are seeded in the native PDMS channel, and the medium is added on top to form a submerged interface culture configuration. Collagen-embedded cells are cultured in a 5% CO_2, 20% O_2 incubator at 37°C under static conditions. Three hours after seeding, dispersed cells begin to self-organize to form tubular structures corresponding to the shape of the scaffold type, while the cell-containing collagen matrix forms a micrometer-sized envelope, separated from the channel walls. At 2 days, tubules self-detach from the scaffold. Open-top scaffolds allow the diffusion of oxygen and nutrients through the collagen matrix from the medium. The oxygen diffusion path length of the system is the thickness of the cell culture medium (~ 0.5 mm), and the depth of the collagen matrix in the channel. Frequent media changes compensate for the evaporation and depletion of nutrients and generation of waste products. Diagram not in scale. (B,C) Cell concentrations lower than those established as optimal completely prevent tubular structure formation both (B) in macro- and (C) in micro-scaffolds. (D) Three hours after seeding at the optimal cell concentration MDCK cells self-assemble into a tube-shaped aggregate. Scale bars: 1 mm (B,D), 500 µm (C).

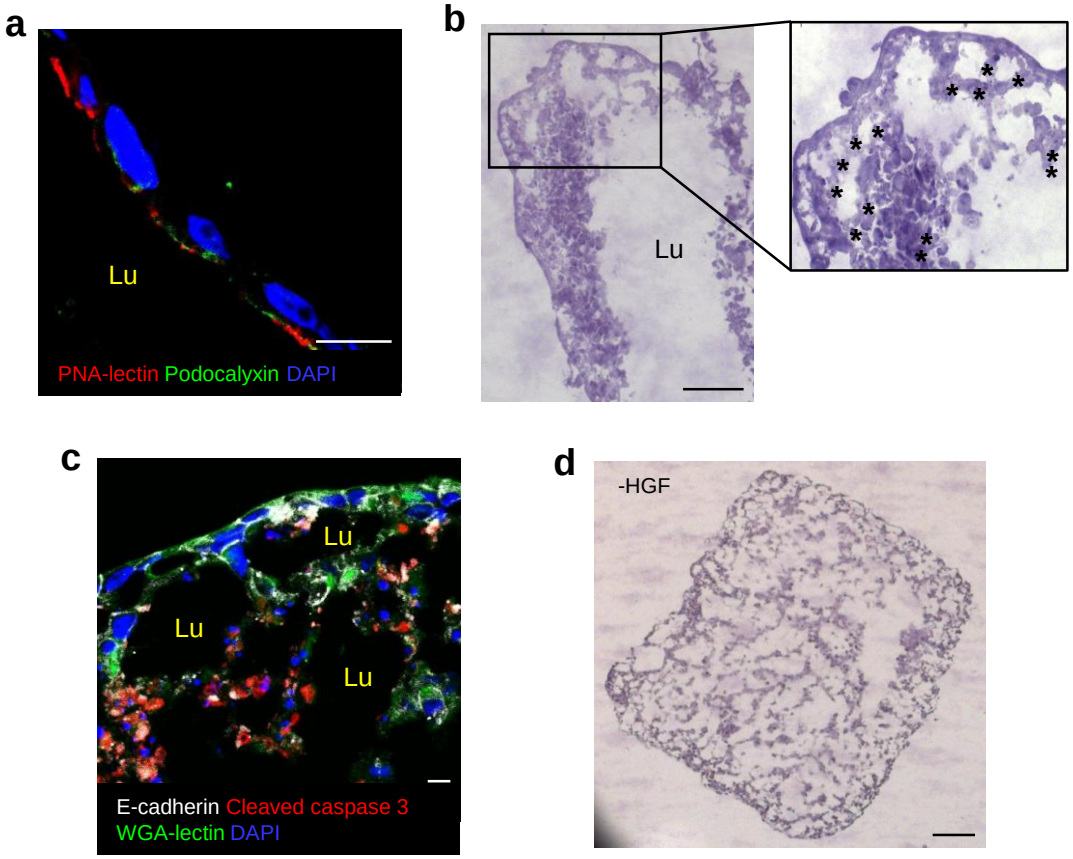

a
Lu
PNA-lectin Podocalyxin DAPI
b
Lu
c
Lu
Lu
Lu
E-cadherin Cleaved caspase 3
WGA-lectin DAPI
d
-HGF

Figure S3

Supplementary Fig. 3. MDCK cell-derived tubules display polarized epithelium establishment and apoptosis during lumen formation. (A) At 2 days, the monolayered epithelium in the tubular wall is double positive for PNA-lectin (red) and podocalyxin (green) in the apical domains. (B) At 1 day, macrotubules develop a major luminal space (Lu) bounded by aggregates of cells with condensed chromatin (inset, asterisks). (C) Cells forming aggregates in the tubular central areas express cleaved caspase 3 (red). (D) At 2 days, tubular structures do not develop a single lumen without HGF exposure. PNA-lectin, peanut agglutinin-lectin; WGA-lectin, wheat germ agglutinin-lectin; DAPI, blue-stained nuclei; HGF, hepatocyte growth factor. Scale bars: 10 μm (A,C), 100 μm (B,D).

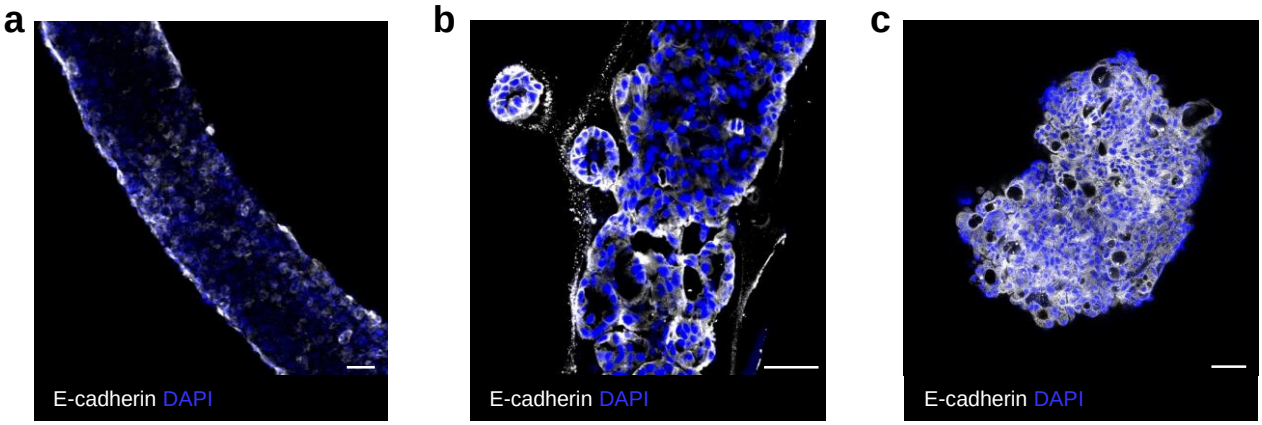


Figure S4

Supplementary Fig. 4. Engineering ADPKD patient-specific tubules and optimizing culture conditions for spontaneous cystogenesis. (A) A patient-derived microtubule, engineered by culturing huADPKD cells in collagen and in the presence of HGF for 2 days in PDMS micro-scaffolds (standard protocol), showing a continuous lumen lined by E-cadherin-positive epithelial cells. (B) A tubular structure generated by culturing huADPKD cells in Matrigel in PDMS micro-scaffold for 2 days, and cultured in a 3D Matrigel matrix for an additional 5 days. Under these conditions, tubular integrity was compromised, as several individual cysts emerged and detached from the tubuloid surface and invaded the surrounding matrix. (C) A patient-derived microtubule, engineered using the standard protocol, and cultured in a 3D Matrigel matrix for 8 days. Although several small cysts form spontaneously, these culture conditions cause excessive shrinkage of the structure, resulting in complete loss of the initial tubular configuration. DAPI, blue-stained nuclei. Scale bar: 50 μm.

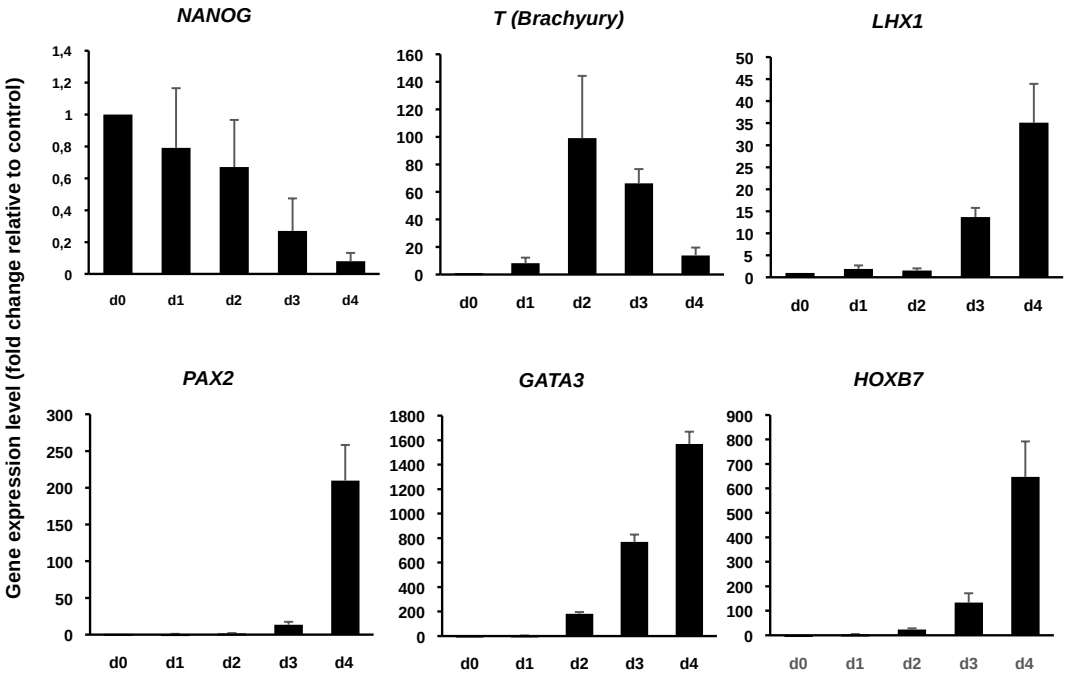

NANOG
T (Brachyury)
LHX1
PAX2
GATA3
HOXB7
Gene expression level (fold change relative to control)
d0
d1
d2
d3
d4

Figure S5

Supplementary Fig. 5. Gene expression profile of human iPSCs during differentiation into UB-like progenitor cells. *NANOG* (pluripotency marker) expression decreases progressively. *T* (*Brachyury*) (mesendoderm marker) expression peaks at d2, while *LHX1 (LIM1)* (intermediate mesoderm marker), *PAX2* (nephric lineage marker), *GATA3* (ureteric epithelium marker) and *HOXB7* (UB-related transcription factor) expression levels increase progressively up to d4. Values are relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Data shown are from three independent experiments and are expressed as mean±SD. d, day; *NANOG*, Nanog homeobox; *T*, brachyury transcription factor; *LHX1*, LIM homeobox 1; *GATA3*, GATA binding protein 3; *PAX2*, paired box 2; *HOXB7*, homeobox B7.

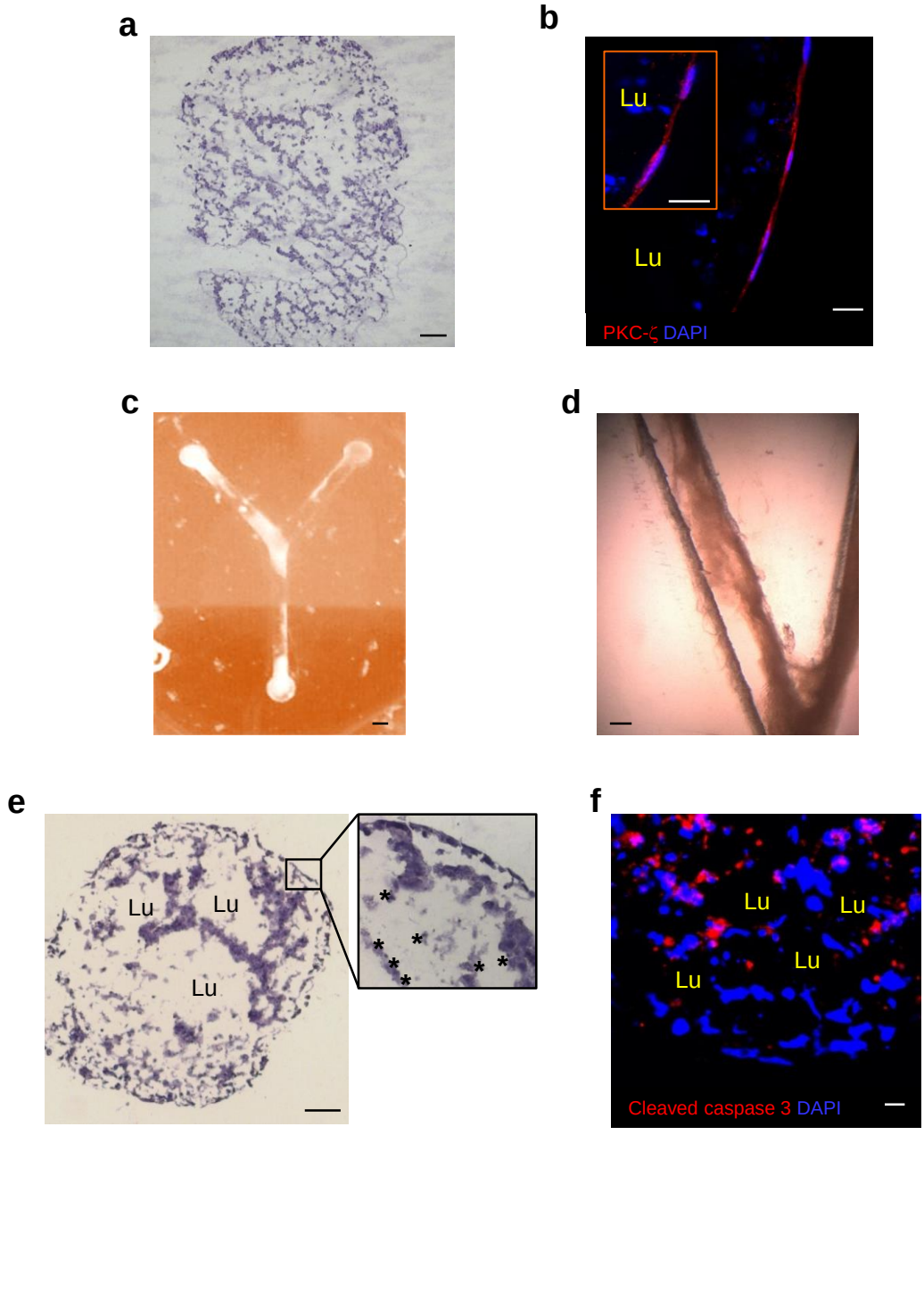

a
b
Lu
Lu
PKC-ζ DAPI
c
d
e
Lu
Lu
Lu
f
Lu
Lu
Lu
Lu
Cleaved caspase 3 DAPI

Figure S6

Supplementary Fig. 6. GDNF is indispensable for lumen formation in the human tubules derived from 3- and 4-day differentiated iPSCs. (A) Three-day cell-derived tubular aggregate cultured in the presence of HGF alone fails to develop a single lumen at 2 days. (B) An iPSC-derived tubule exposed to HGF and GDNF displays some cells in the tubular wall expressing PKC-ζ (red) in the apical domains. (C) Undifferentiated iPSCs proliferate massively and are scattered throughout the culture medium. (D) Two-day cells form aggregates that detach from the mold and fail to form tubules. (E) At 1 day, a macrotubule as that in (B) displays multiple lumens (Lu) surrounded by apoptotic cells with chromatin condensation (inset, asterisks). (F) These cells express cleaved caspase 3 (red). PKC-ζ, protein kinase C isoform-zeta; DAPI, blue-stained nuclei. Scale bars: 100 μm (A,E), 10 μm (B,F), 1 mm (C), 500 μm (D).

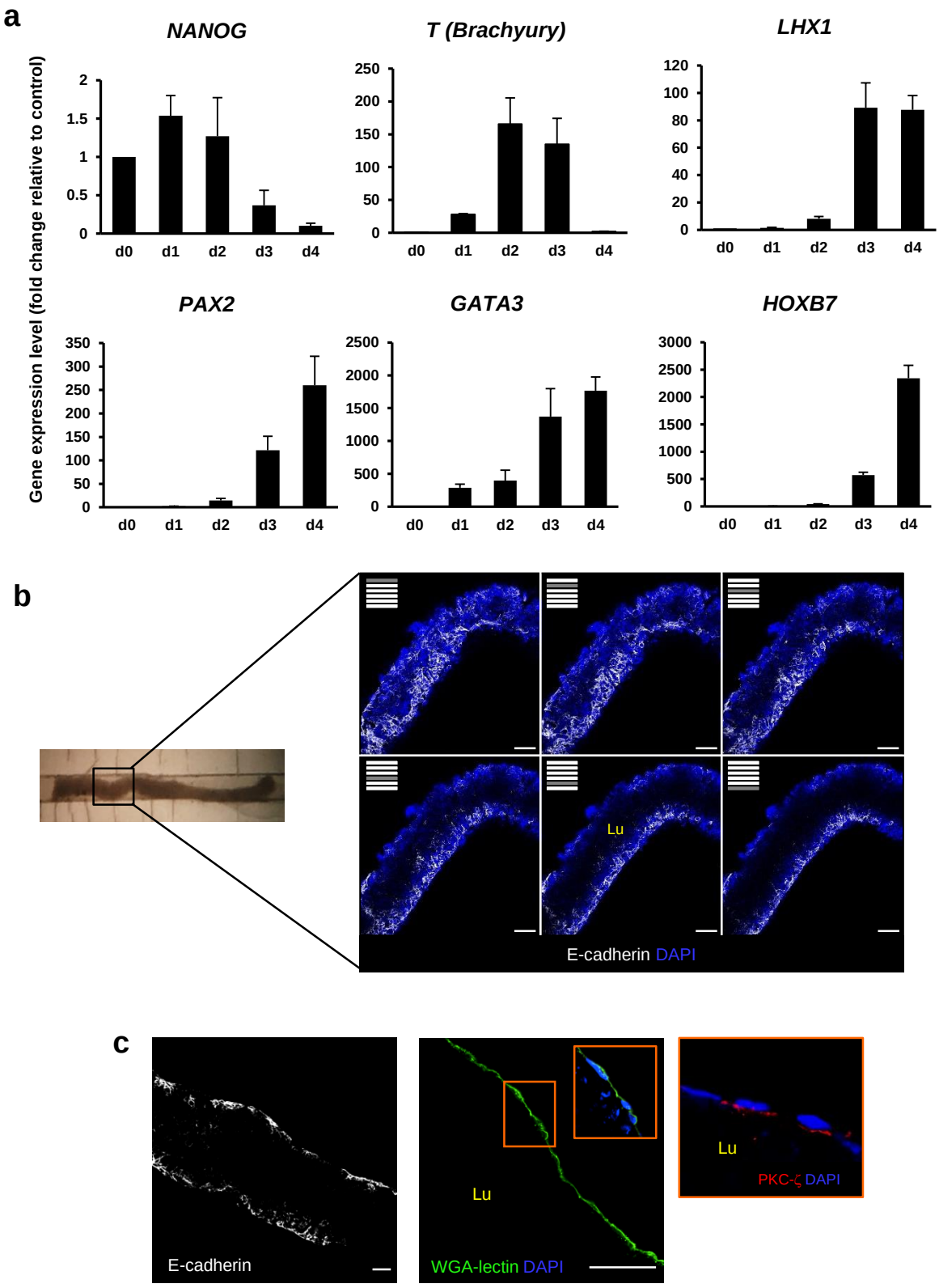

a
NANOG
T (Brachyury)
LHX1
PAX2
GATA3
HOXB7
Gene expression level (fold change relative to control)
d0
d1
d2
d3
d4
b
Lu
E-cadherin DAPI
c
E-cadherin
Lu
WGA-lectin DAPI
Lu
PKC-ζ DAPI

Figure S7

Supplementary Fig. 7. Differentiation of healthy donor-derived iPSCs toward UB-like progenitor cells and tubule engineering. (A) Gene expression profile of healthy donor-derived iPSCs during differentiation. *NANOG* expression decreases up to d4, while *T* (*Brachyury*) peaks at d2. *LHX1* (*LIM1*) is markedly upregulated from d3, while *PAX2*, *GATA3* and *HOXB7* expression levels increase up to d4. Values are relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Data shown are from three independent experiments and are expressed as mean ± SD. (B) Z-stack images of healthy donor-derived microtubule display formation of a single lumen (Lu) in the central region. (C) Healthy donor-derived microtubules showing a continuous lumen (Lu) lined by a monolayered epithelium positive for E-cadherin, WGA-lectin and PKC-ζ (red). d, day; *NANOG*, Nanog homeobox; *T*, brachyury transcription factor; *LHX1*, LIM homeobox 1; *PAX2*, paired box 2; *GATA3*, GATA binding protein 3; *HOXB7*, homeobox B7; WGA-lectin, wheat-germ agglutinin lectin; PKC-ζ, protein kinase C isoform-zeta; DAPI, blue-stained nuclei. Scale bars: 100 μm (B), 50 μm (C).

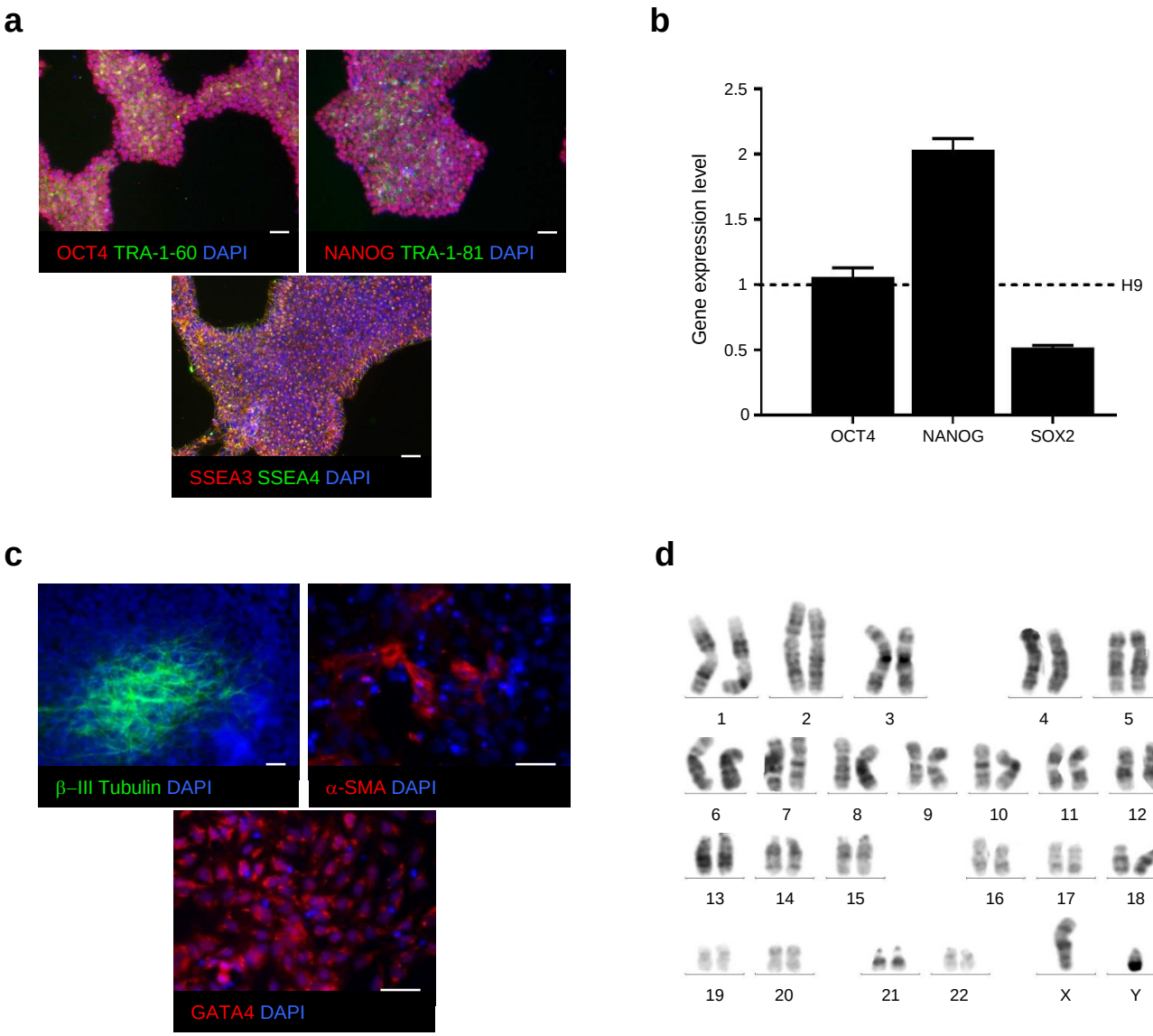

a
OCT4 TRA-1-60 DAPI
NANOG TRA-1-81 DAPI
SSEA3 SSEA4 DAPI
b
Gene expression level
2.5
2
1.5
1
0.5
0
H9
OCT4
NANOG
SOX2
c
β–III Tubulin DAPI
α-SMA DAPI
GATA4 DAPI
d
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Figure S8

Supplementary Fig. 8. Characterization of patient-derived iPSCs. (A) Patient-derived iPSCs are positive for the pluripotency markers OCT4, TRA-1-60, NANOG, TRA-1-81, SSEA3 and SSEA4. (B) Pluripotency genes' expression level in patient-derived iPSCs is similar to that in human embryonic stem cell line H9, used as control. Data are expressed as means ± SEM from three independent experiments. (C) iPSC *in vitro* differentiation into all three germ layers through EBs formation: β-Tubulin III (ectoderm marker), α-SMA (mesoderm marker) and GATA4 (endoderm marker). (D) Karyotype analysis revealing normal 46,XY chromosomal number and structure. TRA-1-60, Tumor-related antigen-1-60; NANOG, Nanog homeobox; TRA-1-81, Tumor-related antigen-1-81; SSEA3, Stage-specific embryonic antigen 3; SSEA4, Stage-specific embryonic antigen 4; SOX2, SRY-box2: α-SMA, alpha-smooth muscle actin; GATA4, GATA binding protein 4; EBs, Embryoid bodies; DAPI, blue-stained nuclei. Scale bars: 50 μm (A,C).

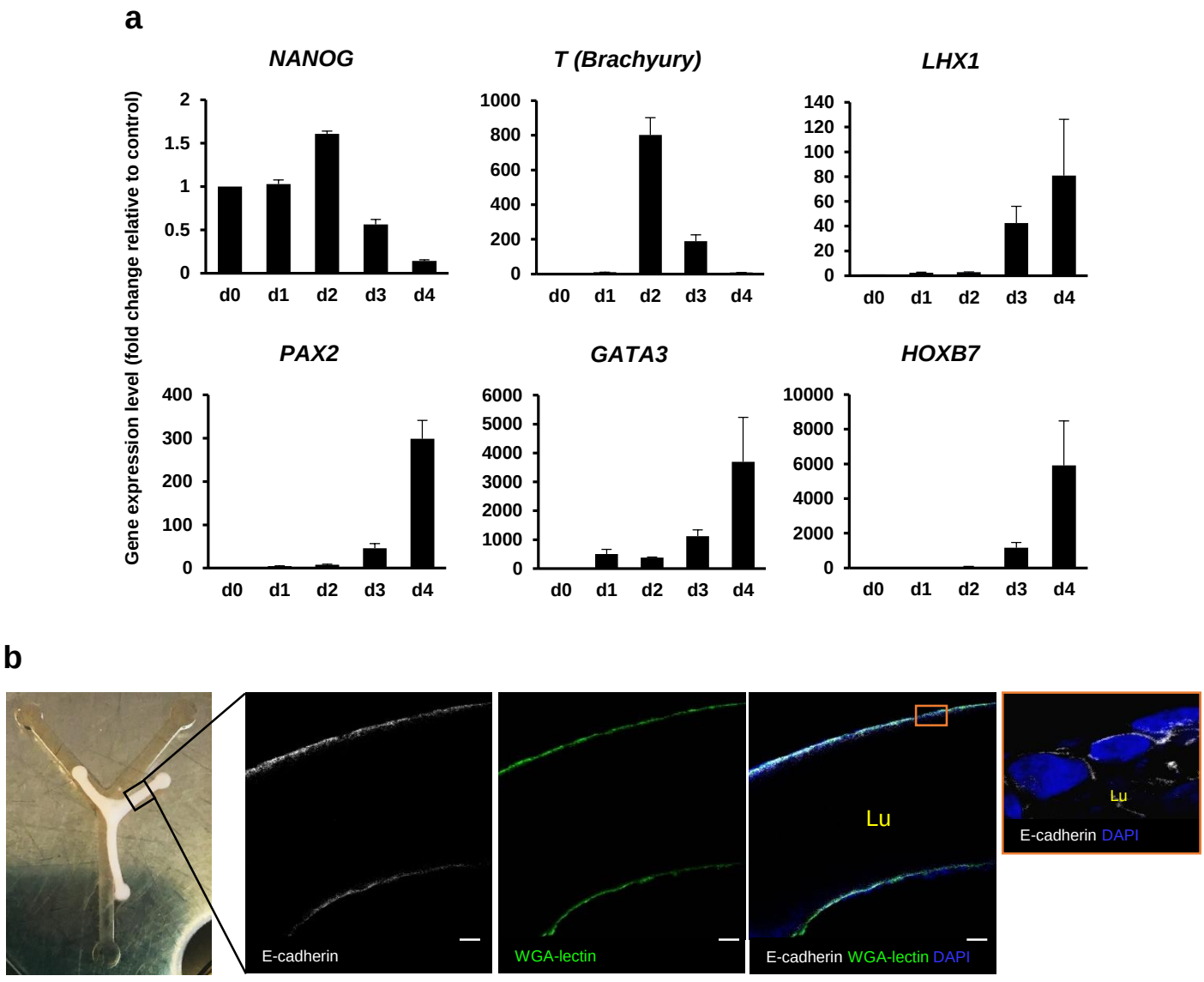

a
NANOG
T (Brachyury)
LHX1
PAX2
GATA3
HOXB7
Gene expression level (fold change relative to control)
d0
d1
d2
d3
d4
b
E-cadherin
WGA-lectin
E-cadherin WGA-lectin DAPI
Lu
E-cadherin DAPI

Figure S9

Supplementary Fig. 9. Differentiation of patient-derived iPSCs toward UB-like progenitor cells and tubule engineering. (A) Gene expression profile of patient-derived iPSCs during differentiation. *NANOG* expression decreases up to d4, while *T* (*Brachyury*) peaks at d2. *LHX1 (LIM1)* is markedly upregulated from d3, while *PAX2*, *GATA3* and *HOXB7* expression levels increase up to d4. Values are relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Data shown are from three independent experiments and are expressed as mean ± SD. (B) Patient-derived macrotubule showing a continuous lumen (Lu) lined by a monolayered epithelium positive for E-cadherin in the basolateral membranes (inset). d, day; *NANOG*, Nanog homeobox; *T*, brachyury transcription factor; *LHX1*, LIM homeobox 1; *PAX2*, paired box 2; *GATA3*, GATA binding protein 3; *HOXB7*, homeobox B7; WGA-lectin, wheat-germ agglutinin lectin; DAPI, blue-stained nuclei. Scale bars: 100 μm (B).