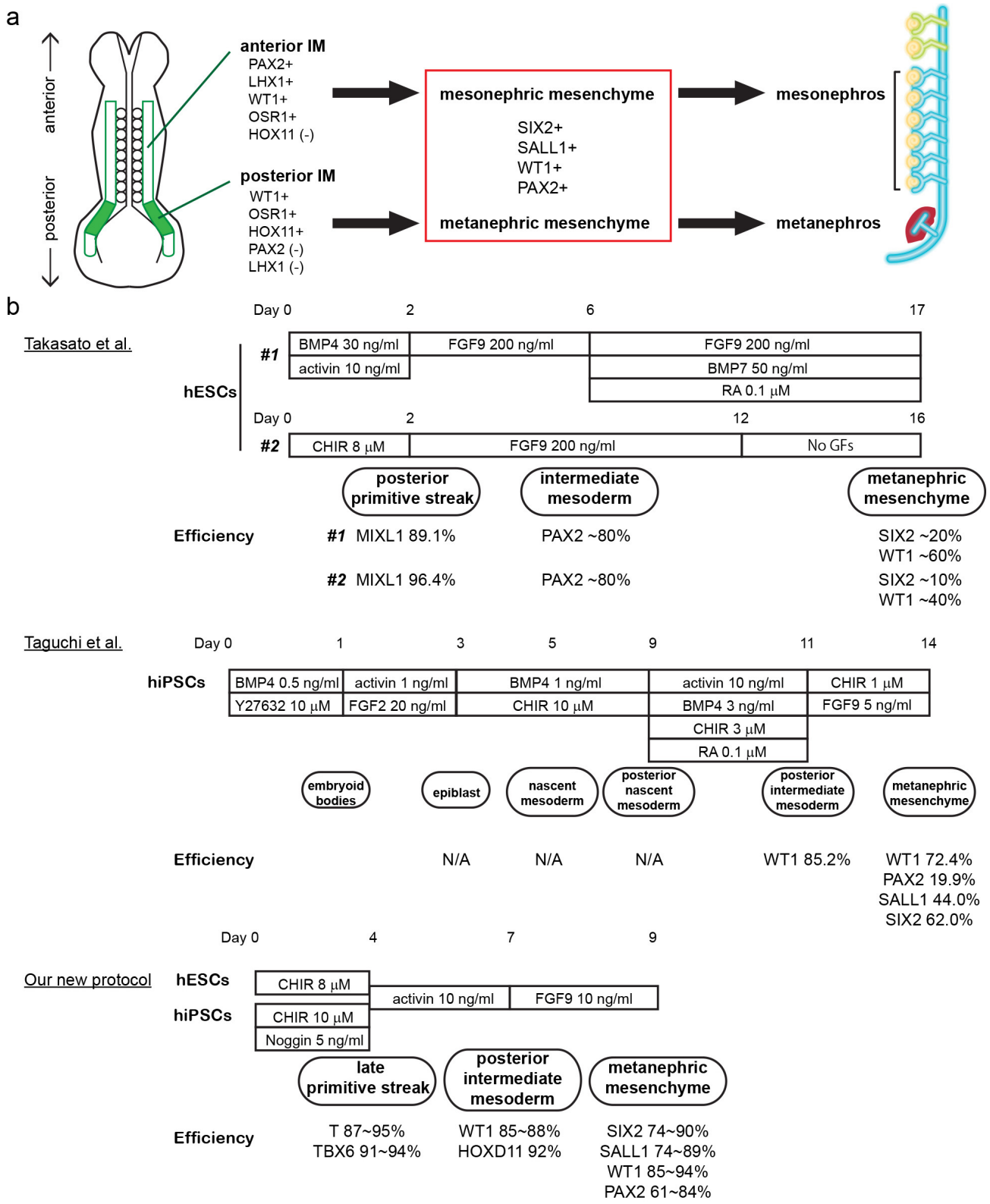
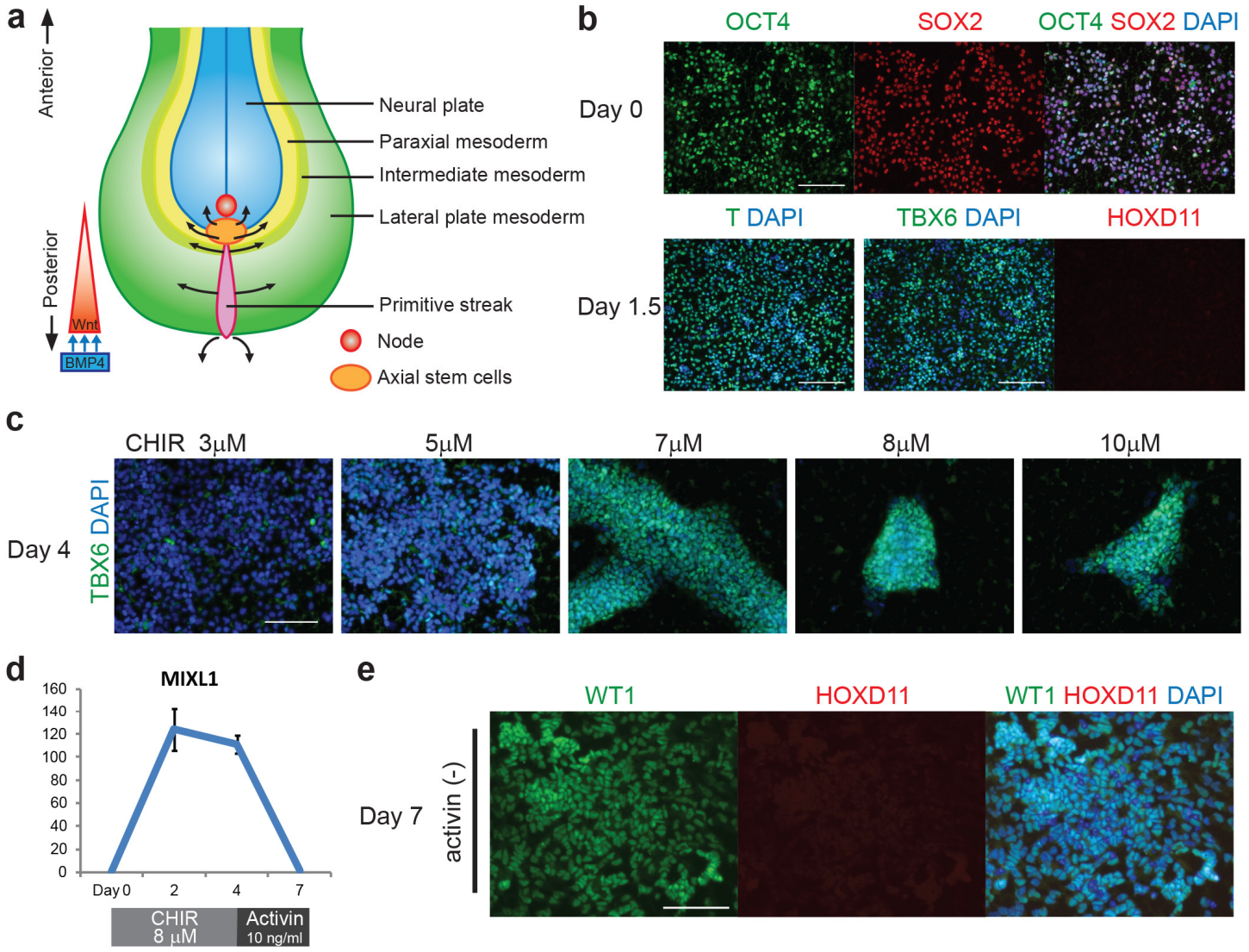


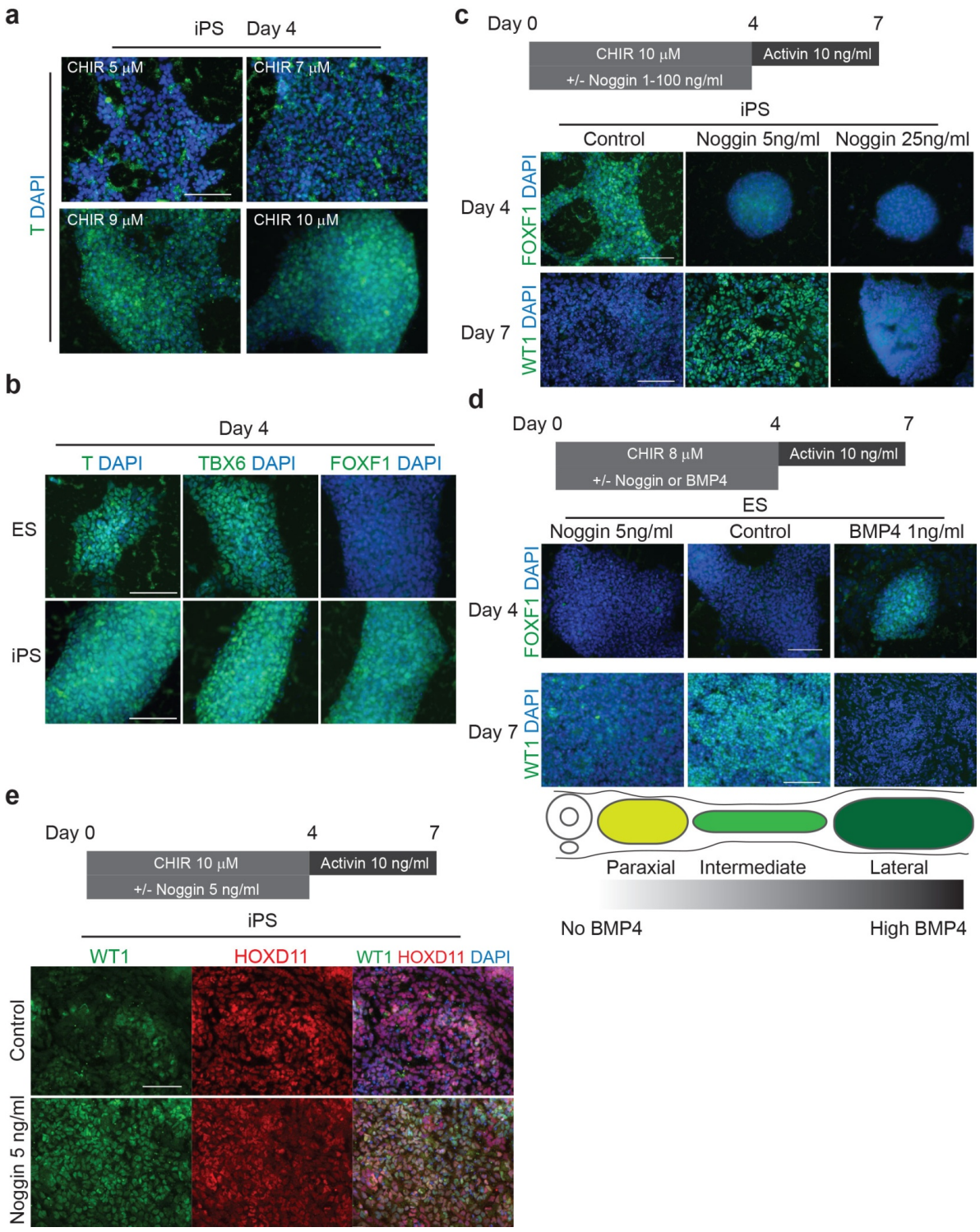
Supplementary Data Figure 1. Metanephric development and published protocols

(a) A schematic illustration of intermediate mesoderm and subsequent differentiation into mesonephros and metanephros. (b) The summary and comparison of published protocols and our new protocol. Takasato et al. Nat Cell Biol. 2014. Taguchi et al. Cell Stem Cell. 2014. RA: retinoic acid.



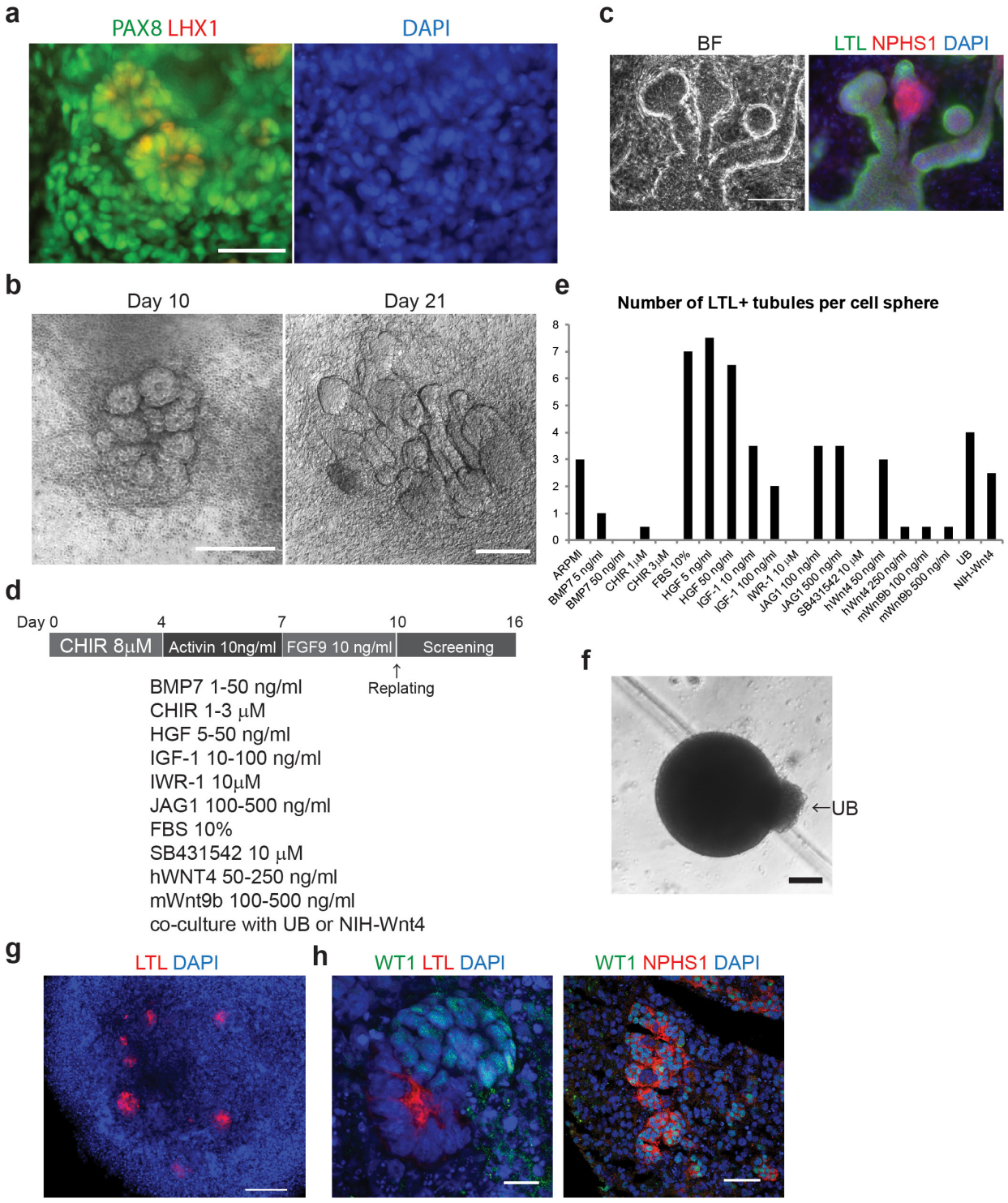
Supplementary Data Figure 2. Adjustment of the dose and CHIR treatment time

(a) A schematic illustration of primitive streak and subsequent differentiation into each mesoderm lineage. (b) Pluripotency was evaluated by staining with OCT4 and SOX2 before the differentiation. hESCs differentiated with CHIR 5 μM were positive for T and TBX6 on day 1.5 of differentiation, but cells did not stain for HOXD11. Scale bars: 200 μm. (c) Immunocytochemistry on day 4 of the differentiation with CHIR (3 to 10 μM). Sustained TBX6 expression was observed when the cells were differentiated with high doses of CHIR (7-10 μM). Scale bar: 100 μm. (d) Real-time PCR for MIXL1 in hESCs from day 0 to 7. hESCs were differentiated with CHIR 8 μM for 4 days, and activin 10 ng/ml for 3 days. MIXL1, another marker for primitive streak also showed sustained expression at least until day 4 of the differentiation. Expression returned to very low levels by day 7. n=2. (e) Staining with WT1 and HOXD11 on day 7 of the differentiation. hESCs were differentiated with CHIR 8 μM for 4 days and subsequently with the basic medium (ARPMI) for 3 days. Cells expressed WT1, but not HOXD11. Scale bar 100 μm.



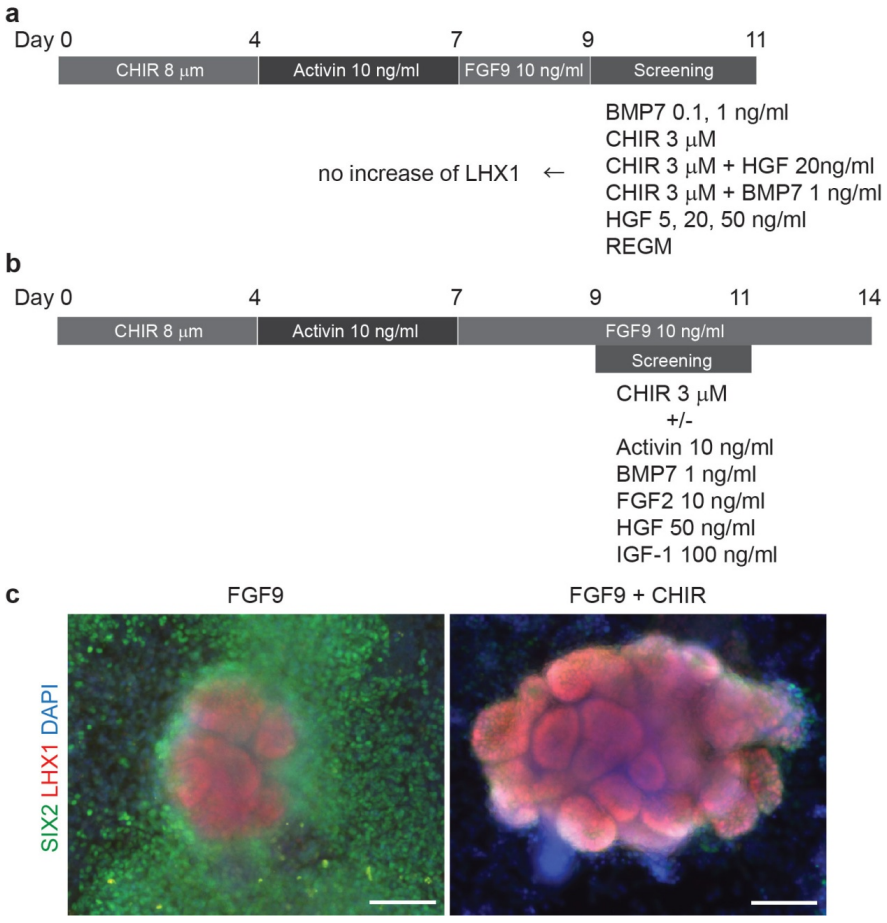
Supplementary Data Figure 3. Protocol adjustment in hiPSCs.

(a) Comparison of CHIR dose in T expression on day 4. A slightly higher dose of CHIR was required for sustained T expression in hiPSCs on day 4. (b) Immunocytochemistry for T, TBX6 or FOXF1 on day 4 of differentiation. hESCs were differentiated with CHIR 8 μM, and hiPSCs were differentiated with CHIR 10 μM. Notably, FOXF1 was negative in hESCs, but was positive in hiPSCs. (c) The tested protocol and representative immunocytochemistry in hiPSCs. Noggin at >5 ng/ml suppressed FOXF1 expression on day 4. To induce WT1 expression on day 7, Noggin 5 ng/ml was optimal. (d) The differentiation protocol and staining for FOXF1 on day 4, and for WT1 on day 7 in hESCs. Either additional Noggin or BMP4 significantly suppressed WT1 expression on day 7, and BMP4 induced FOXF1 expression on day 4, suggesting endogenous BMP4 signal is optimal in hESCs with CHIR treatment alone. (e) The protocol and staining with WT1 and HOXD11 on day 7 in hiPSCs. CHIR 10 μM + Noggin 5 ng/ml followed by activin 10 ng/ml showed the most efficient differentiation into WT1+HOXD11+ cells in hiPSCs. Scale bars: 100 μm.



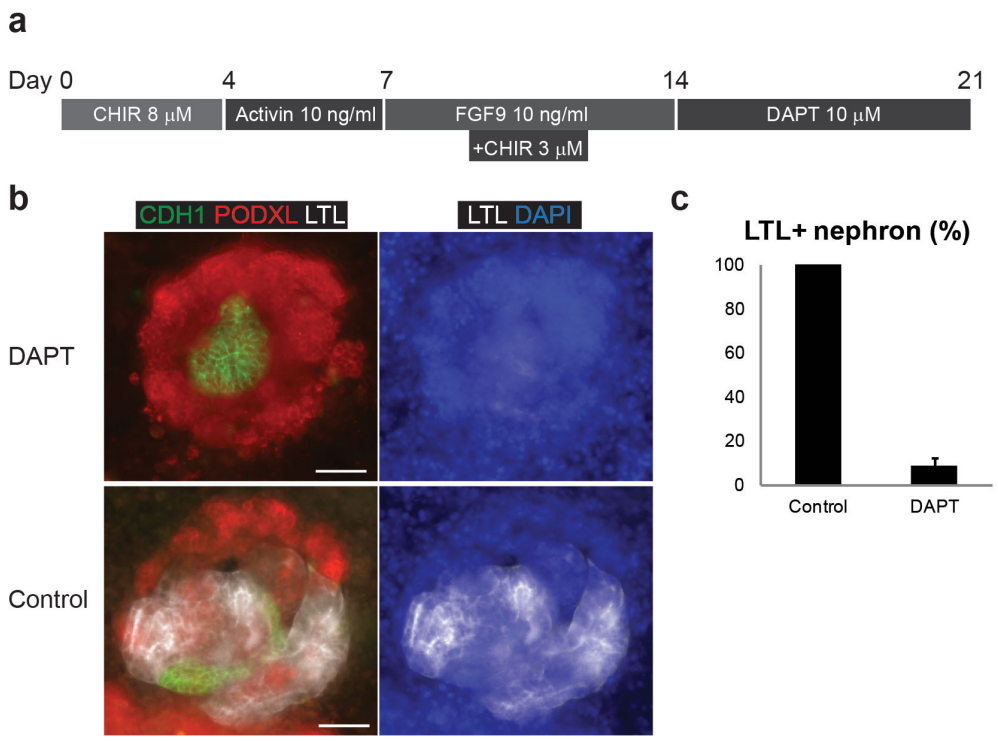
Supplementary Data Figure 4. Spontaneous differentiation of SIX2+ cells into nephrons and growth factor screening in 3D culture.

(a) Staining with PAX8 and LHX1 in hESCs on day 10 of the differentiation. hESCs were differentiated with CHIR 8 μM for 4 days, activn 10 ng/ml for 3 days, and FGF9 10ng/ml for 3 days. Sporadic expression of LHX1 was observed in PAX8+ cells. Scale bar: 50 μm. (b) Brighfield imaging for hESCs on day 10 and 21 of the differentiation. FGF9 was withdrawn on day 10, and cells were cultured in the basic medium by day 21. Scale bars: 50 μm. (c) Brightfield and immunocytochemistry for LTL and NPHS1 in hESCs on day 28 of the differentiation. Scale bar: 50 μm. (d) The protocol for growth factor screening. Cells were replated to 3D culture on day 10, and the listed growth factors and small molecules were tested. (e) The number of LTL+ tubules in the organoids. HGF showed a tendency to increase LTL+ tubules. n=2. (f) Brightfield imaging of 3D co-culture.with an ureteric bud cell sphere. Scale bar: 100 μm. (g) Whole-mountstaining for LTL in the organoids on day 16. Scale bar: 100 μm. (h) Immunohistochemistry of the organoids on day 16. Scale bars: 50 μm. LTL: lotus tetragonolobus lectin. NPHS1: Nephrin.



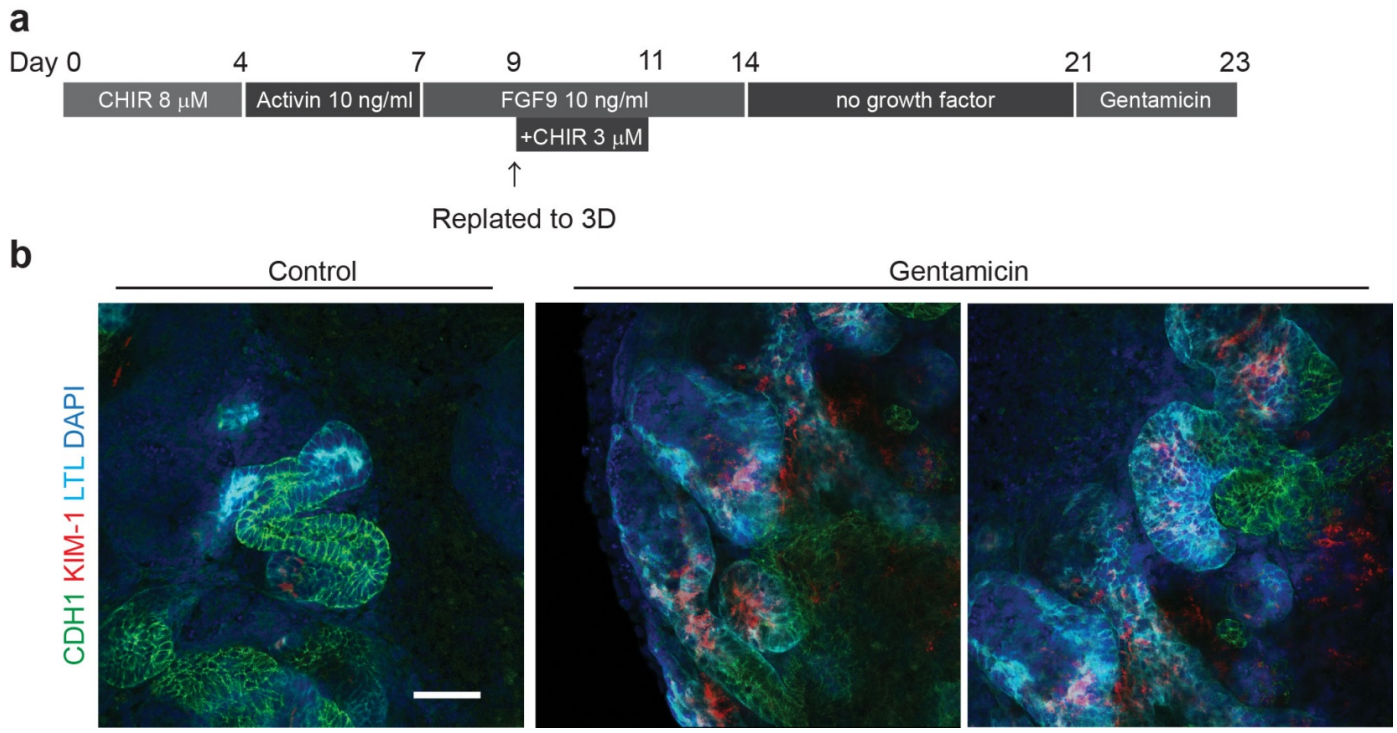
Supplementary Data Figure 5. Screening for growth factors and small molecules to induce renal vesicles.

(a, b) The tested protocols for renal vesicle induction. (c) Immunocytochemistry for SIX2 and LHX1 in structures on day 14 of differentiation. Transient treatment with CHIR 3 μM from day 9 to 11, in combination with FGF9 10 ng/ml from day 7 to 14, increased the number of LHX1+ cells and suppressed SIX2 expression, suggesting mesenchymal epithelial transition. Scale bars: 50 μm. REGM: renal cell growth medium (Lonza, #CC-3190).



Supplementary Data Figure 6. Kidney development analysis.

(a) DAPT was used to suppress Notch signaling from day 14 to 21. (b) CDH1, PODXL and LTL expression on day 21 in cells derived from hESCs in 2D culture. Scale bars: 50 μm. (c) Percentage of LTL+ nephron structures in control and DAPT-treated on day 21. The nephron number was counted as CDH1+ tubules from 10 fields (x20 magnification) of each sample (n=2). CDH1: Cadherin-1 (E-cadherin). PODXL: Podocalyxin-like (Podocalyxin).



Supplementary Data Figure 7. Nephrotoxic assay.

(A) The protocol for the nephrotoxic assay. Gentamicin 5 mg/ml was added from day 21 to 23. (B) Whole-mount staining on day 23 for CDH1, KIM-1 and LTL in kidney organoids derived from hESCs. Scale bars: 50 μm. CDH1: cadherin-1 (E-cadherin). PODXL: podocalyxin-like (Podocalyxin). LTL: lotus tetragonolobus lectin. KIM-1: kidney injury molecule-1.

Table S1. List of growth factors and small molecules

name	Source	Cat. #
Activin	R&D systems	338-AC-050
CHIR99021	TOCRIS	4423
DAPT	Stemgent	04-0041
human BMP-4	R&D systems	314-BP
human BMP-7	Peprotech	120-03B
human FGF2	Peprotech	100-18B
human FGF8	Peprotech	100-25
human FGF9	R&D systems	273-F9-025/CF
human HGF	R&D systems	294-HG-005/CF
human IGF-1	R&D systems	291-G1
human Jagged1	R&D systems	1277-JG-050
human Noggin	Peprotech	120-10C
human Wnt4	R&D systems	6076-WN
IDE-1	Stemgent	04-0026
IWR-1	Sigma	I0161
mouse Wnt-9b	R&D systems	3669-WN-025
Retinoic acid	Sigma	R2625
SB431542	Stemgent	04-0010
Y-27632 dihydrochloride	TOCRIS	1254

Table S2. List of primary antibodies

Antibody	Source	Catalog or clone number	Dilution
α-SMA	Sigma	F3777	1:500
AQP1	Milipore	AB2219	1:500
BRN-1	Santa cruz	sc-6028-R	1:200
DBA	Vector lab	RL-1032	1:200
E-cadherin	Abcam	ab11512	1:500
Endomucin	Abcam	Ab45771	1:500
Eya1	Santa cruz	sc-15094	1:50
FOXD1	LSBio	LS-B6453	1:200
FOXF1	R&D systems	AF4798	1:200
γH2AX	Cell signaling	2577	1:100
HNF-1β	Santa cruz	sc-7411	1:100
HOXD11	Sigma	SAB1403944	1:100
KIM-1	R&D systems	AF1750	1:200
Laminin	Sigma	L9393	1:500
LHX1	Developmental Studies Hybridoma Bank	4F2-c	1:50
LTL	Vector lab	B-1325	1:200
N-cadherin	Abcam	ab12221	1:100
Nephrin	Progen	GP-N2	1:100
PAX2	Covance	PRB-276P	1:500
PAX8	Proteintech	10336-1-AP	1:500
Podocalyxin	R&D systems	AF1658	1:500
SALL1	R&D systems	PP-K9814-00	1:100
SIX2	Proteintech	11562-1-AP	1:500
T	Santa cruz	sc-17745	1:100
TBX6	R&D systems	AF4744	1:100
Uromodulin	Biomedical Technologies Inc.	BT-590	1:150
WT-1	Santa cruz	sc192	1:50

Table S3. Primer sequences

Gene	Forward	Reverse
ACTB	CTCTTCCAGCCTTCCTTCCT	AGCACTGTGTTGGCGTACAG
BMP4	AAGCGTAGCCCTAAGCATCA	TGGTTGAGTTGAGGTGGTCA
KIM1	CGACAACGACTGTTCCAATG	AAAGGCATTGGAGGAACAAA
MIXL1	ACGTCTTTCAGCGCCGAACAG	TTGGTTCGGGCAGGCAGTTCA
OSR1	CCTTCCTTCAGGCAGTGAAC	CGGCACTTTGGAGAAAGAAG
PAX2	ACTCCATCAATGGGATCCTG	CCACACCACTCTGGGAATCT