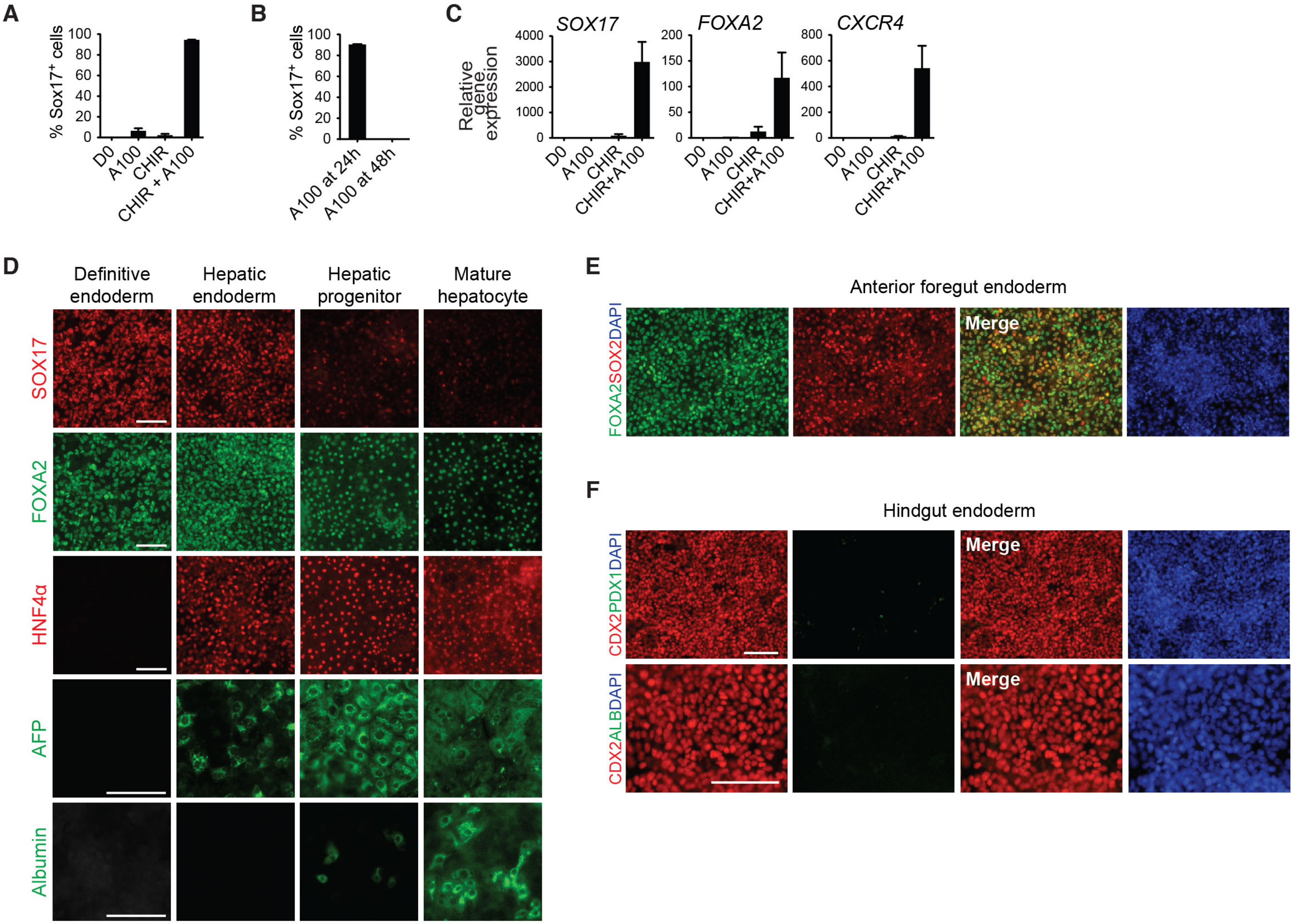
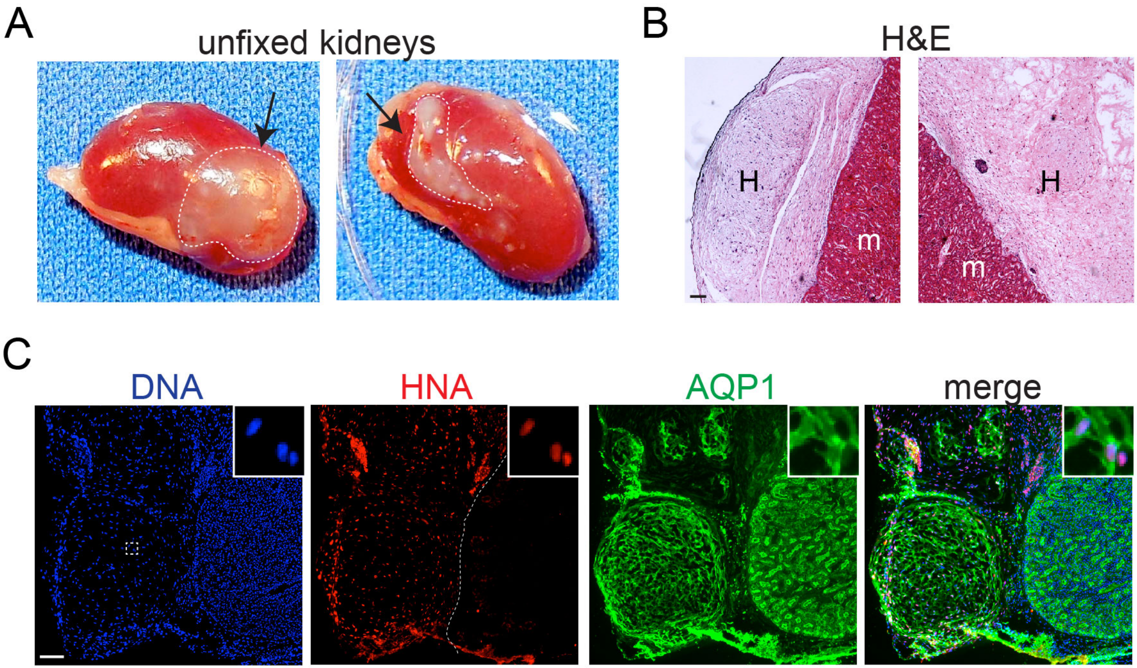
Supplemental Figure 1



Supplemental Figure 1. Efficient differentiation of definitive endoderm and endodermal derivatives from CHIR-induced mesendodermal cells. (A) Quantification of SOX17+ cells at day 4 of differentiation. Undifferentiated hPSCs (Day 0) serve as the negative control. (B) Quantification of SOX17+ cells at day 4 of differentiation of hPSCs treated with activin A at either 24 or 48 hours after treatment with CHIR. (C) Quantitative RT-PCR of definitive endoderm genes in hPSCs treated with CHIR or CHIR + activin A. Gene expression normalized to Day 0. (D) hPSCs differentiated into definitive endoderm with CHIR + activin A were further differentiated into hepatocytes. Immunostaining for stage-specific markers at each step of differentiation is shown. (E) Cells at the definitive endoderm stage were differentiated with SB431542 and Noggin for 3 days into anterior foregut endoderm. (F) Cells at the definitive endoderm stage were further differentiated with FGF4 and CHIR for 4 days into hindgut endoderm expressing CDX2 without expression of foregut differentiation markers PDX1 or albumin. A100, activin A 100 ng/mL; ALB, albumin; C-PEP, pro-insulin C-peptide; INS, insulin; SS, somatostatin. Data represent means ± s.e.m. (n = 3). Scale bar, 100 μm.

Supplemental Figure 2



Supplemental Figure 2. Growth of PSC-derived kidney progenitor cells *in vivo*. (A) NOD-SCID mouse kidneys four weeks after subcapsular injection with human iPSC-derived tubule cultures. Macroscopic growths are indicated by arrows and surrounded by white dashed lines. (B) Hematoxylin and eosin (H&E) stained sections in these growths showing interface between mouse kidney (m) and human growth (H). (C) Colocalization of human nuclear antigen (HNA) with aquaporin-1 (AQP1). Insets show higher magnification of white boxed region (DNA panel). White dashed line in HNA panel indicates the boundary between human and mouse tissue.

Supplemental Table 1: List of Primary Antibodies

Antibody	Source	Dilution
Acetylated Tubulin	Sigma	1:500
AFP	Dako	1:200
Albumin	Dako	1:200
Aquaporin-1	Millipore	1:100
BRACHURY	Santa Cruz	1:100
CDX2	Biogenex	1:100
CK19	Dako	1:50
E-cadherin	Abcam	1:1000
FOXA2	EMD Millipore	1:200
FOXF1	R&D	1:500
HNF4A	Santa Cruz	1:100
Human Nuclear Antigen	Millipore	1:250
Insulin	Dako	1:1000
Kidney-specific protein	Gift from Dr. Hiroshi Itoh and Dr. Toshiaki Monkawa	1:100
Laminin	Sigma	1:500
LHX1	Developmental Studies Hybridoma Bank	1:50
Lotus Tetragonolobus Lectin (LTL), biotinylated	Vector Labs	1:200
MIXL1	Gift from Dr. Andrew Elefanty	1:100
N-cadherin	Abcam	1:100
OCT4	Santa Cruz	1:100
PAX2	Covance	1:100
PAX6	Stemgent	1:100
PDX1	R&D	1:100
Pro-insulin C-peptide	EMD Millipore	1:500
Polycystin-2	Santa Cruz	1:100
Somatostatin	Dako	1:1000
SALL1	Cosmo Bio	1:200
SIX2	Abnova	1:200
SIX2	Proteintech	1:200
SOX2	Santa Cruz	1:100
SOX17	R&D	1:100
TBX6	R&D	1:100
WT1	Santa Cruz	1:50

Supplemental Table 2: Primer Sequences

Gene	Forward	Reverse
AQP1	ATTAACCCTGCTCGGTCCTT	ACCCTGGAGTTGATGTCGTC
AQP2	ACGCCTTCACGTGTGTGTAT	TTGTTTTCTGCGCCGAAGTG
B-ACTIN	CCAACCGCGAGAGAGTGA	TCCATCACGATGCCAGTG
BMP-4	AAGCGTAGCCCTAAGCATCA	TGGTTGAGTTGAGGTGGTCA

BRACHYURY	GTGCTGTCCCAGGTGGCTTACAGATG	CCTTAACAGCTCAACTCTAACTACTTG
CXCR4	CACCGCATCTGGAGAACCA	GCCCATTTCCTCGGTGTAGTT
EOMES	ATCATTACGAAACAGGGCAGGC	CGGGGTTGGTATTTGTGTAAGG
EYA1	TGCATATGGGCAAACACAGT	CCAGGTTGAGGGGTACTGAA
EYA2	CCGGTCTAAGAGGAGCAGTG	CTGGTCACAATCCTCCAGGT
FOXA2	CCATTGCTGTTGTTGCAGGGAAGT	CACCGTGTCAGGATTGGGAATGCT
FOXF1	CAGCCTCACATCACGCAAGG	AGCCGAGCTGCAAGGCATC
FOXI3	CCACCCCTTGTCTCAACACT	TTGCTCAGTTGCAAGGTGTC
GSC	GAGGAGAAAGTGGAGGTCTGGTT	CTCTGATGAGGACCGCTTCTG
KDR	TTTTTGCCCTTGTTCTGTCC	TCATTGTTCCCAGCATTTCA
LHX1	ATCCTGGACCGCTTTCTCTT	GTACCGAAACACCGGAAGAA
MEGALIN	TGTGATGCAGCCATCGAACT	TGCATTTGGGGAGGTCAGTC
MEOX1	AAAGTGTCCCCTGCATTCTG	CACTCCAGGGTTCCACATCT
MIXL1	ACGTCTTTCAGCGCCGAACAG	TTGGTTCGGGCAGGCAGTTCA
NANOG	TGCTTATTCAGGACAGCCCT	TCTGGTCTTCTGTTTCTTGACT
NEPHRIN	GACCCAGCTTCCCATCACTA	GCATTGGAGAGGAGCAGAAG
OCT4	CAGTGCCCGAAACCCACAC	GGAGACCCAGCAGCCTCAAA
OSR1	CCTTCCTTCAGGCAGTGAAC	CGGCACTTTGGAGAAAGAAG
OTX2	GCAGAGGTCCTATCCCATGA	CTGGGTGGAAAGAGAAGCTG
PAX2	CAAAGTTCAGCAGCCTTTCC	CCACACCACTCTGGGAATCT
PAX6	ACCCATTATCCAGATGTGTTTGCCCGAG	ATGGTGAAGCTGGGCATAGGCGGCAG
PAX8	GCAACCATTCAACCTCCCTA	CTGCTGCTGCTCTGTGAGTC
SIX2	CTGGAGAGCCACCAGTTCTC	GCTGCGACTCTTTTCCTTGA
SIX4	CCCAAGATGGAGGGTCTGTA	TGTGCTTCCATCTGAAGTGC
SOX1	CAATGCGGGGAGGAGAAGTC	CTCTGGACCAAACTGTGGCG
SOX3	AGACCAGGACCGTGTGAAAC	GTCGATGAATGGTCGCTTCT
SNAI1	CCCACATCCTTCTCACTGC	GTCAGCCTTTGTCCTGTAGC
SOX17	CGCACGGAATTTGAACAGTA	GGATCAGGGACCTGTCACAC
SYNAPTOPODIN	GCCGCAAATCCATGTTTACT	CTCATCCGCTGTCTGTACCA
TBX6	AAGTACCAACCCCGCATACA	TAGGCTGTCACGGAGATGAA
UMOD	AAACCCATGCCACTTACAGC	CGGTCTTCAGGCTGACTTTC
WT1	GGGTACGAGAGCGATAACCA	TCTCACCAGTGTGCTTCCTG