Supplementary table 1. Clinical characteristics of donors of cells isolated from amniotic fluid and urine of preterm neonates that were used for characterization as KSPCs.

Sample Amniotic fluid	**Gestational age at amniocentesis and sample collection (w)**	**Indication for amniocentesis**	**Fetal abnormalities (ultrasound)**
1	26	Fetal abnormalities on ultrasound	Retrognathism, micrognathism and cleft palatum (Pierre-Robin sequence)
2	16	Maternal age (35 years old)	None
3	22	Fetal abnormality on ultrasound	Isolated cleft palate left
4	19	Maternal age (38 years old)	None
5	18	Pregnancy in twin-to-twin transfusion syndrome	None
Sample Preterm urine	**GA at birth and sample collection (w)**	**Reason for premature birth**	
1	34	Acute fetal distress	
2	32	Premature contractions	
3	34	Premature rupture of membranes	
4	36	Premature rupture of membranes	
5	34	Growth retardation of a twin	
6	34	Growth retardation of a twin	

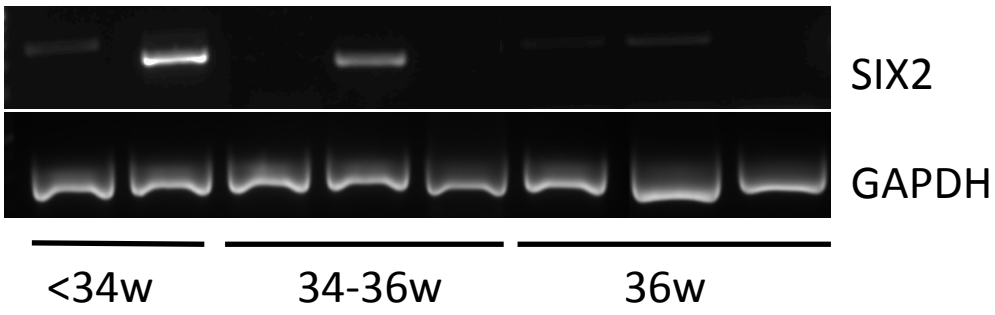
Supplementary table 2. Primers used for gene expression analyzes in quantitative PCR.

Target	Forward primer	Reverse primer
GAPDH	GAGTCAACGGATTTGGTCGTAT	CAGGAGGCATTGCTGATGATCT
SIX2	CTCAAGGCACACTACATCGAG	GTTGTGGCTGTTAGAATTGGA
CITED1	CTACTCCAACCTTGCGGTGAA	CCTATTGGAGATCCCGAGGAA
Vimentin	ACACCCTGCAATCTTTCAGACA	GATTCCACTTTGCGTTCAAGGT
OSR1	TTCAGCTAAAGCCCCAGAGA	CGGCACTTTGGAGAAAGAAG
FOXD1	TGGGGACTCTGCACCAAGGGACT	CGCGCCTGGAGGAGCGAACA
PAX2	AACGACAGAACCCGACTATG	ATCCCACTGGGTCATTGGAG
Lmx1b	AATGCAACCTGACCGAGAAG	ACATCATGCAGGTGAAGCAG
CD2AP	AGGCTGGTGGAGTGGAAC	CAGAGAAGGTATAGGTGAAGTAGG
Synaptopodin	AGCCCAAGGTGACCCCGAAT	CCCTGTCACGAGGTGCTGGC
Podocalyxin	CTTGAGACACAGACACAGAG	CCGTATGCCGCACTTATC
Nephrin	CGCAGGAGGAGGTGTCTTATTC	CGGGTTCCAGAGTGTCCAAG
CD13	GACAGCCAGTATGAGATGGACA	GGCCAGCAAGTACGTGGACATCTT
Megalin	ACAATGCATCCCCAACTCCT	AGGCCCCATTGTCACAAGTA
Cubilin	CCCCTTCTTGGGAAGTTCTG	CCACGTGGGTGAAGTTGATT
Pgp	TGACCCGCACTTCAGCTACATGAA	AAGGTCGGCTATGCTGTCAA

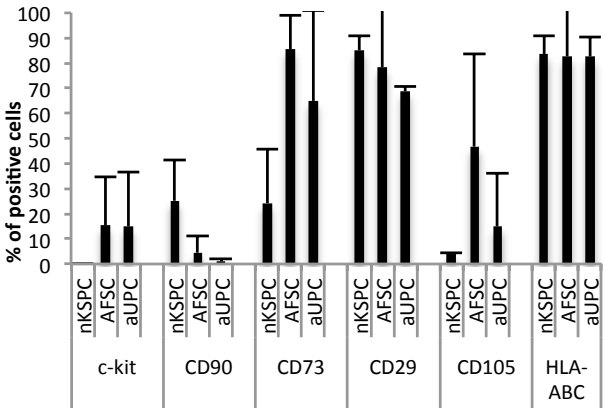
Supplementary table 3. Antibodies used in immunofluorescence staining.

Antibody	Supplier	Dilution
mouse anti-SIX2	H00010736-M01, monoclonal antibody 3D7, Abnova	1:100
goat anti-FOXD1	sc-47585, Santa Cruz	1:100
mouse anti-paxillin	610569, BD transduction laboratories	1:100
mouse anti-CD133/1 pure	W6B3C1, Miltenyi Biotec	1:100
mouse anti-synaptopodin	65294, Progen Biotechnik Heidelberg	1:100
mouse anti-podocalyxin	MAB430, Millipore	1:100
goat anti-podocin	EB12149, Everest Biotech	1:100
rabbit anti-WT1	SC-192, Santa Cruz Biotechnology	1:100
rabbit anti-TRPC6	Gift from Prof. J. Vriens	1:1000
Alexa Fluor® 488 donkey anti-mouse	A-21203, Life Technologies	1:1000
Alexa Fluor® 594 donkey anti-rabbit	A-21207, Life Technologies	1:1000
Alexa Fluor® 568 donkey anti-goat	A-11057, Life Technologies	1:1000
Alexa Fluor® 488 goat anti-rabbit	A-11008, Life Technologies	1:1000

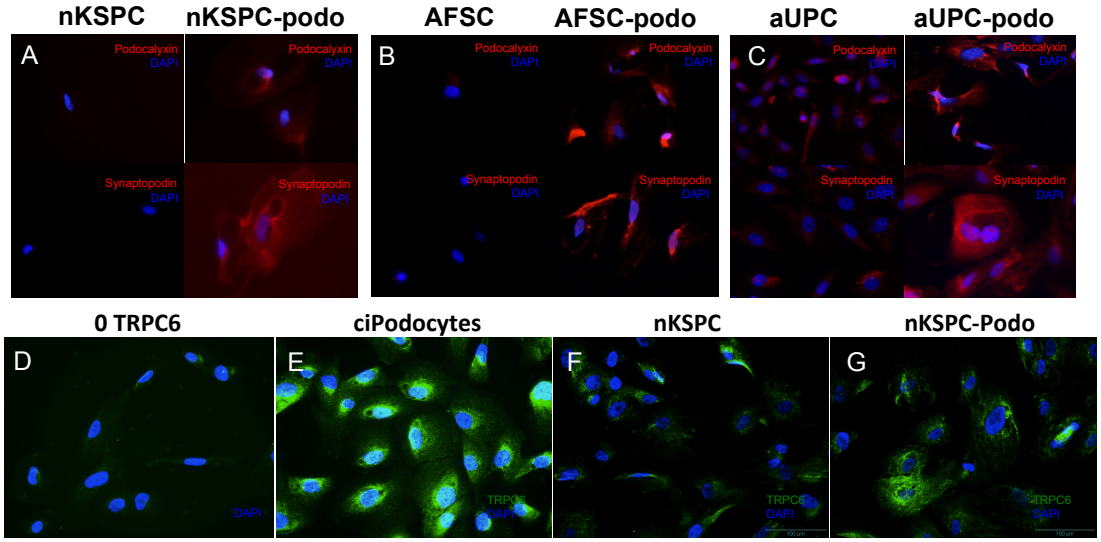
Supplementary Figure 1



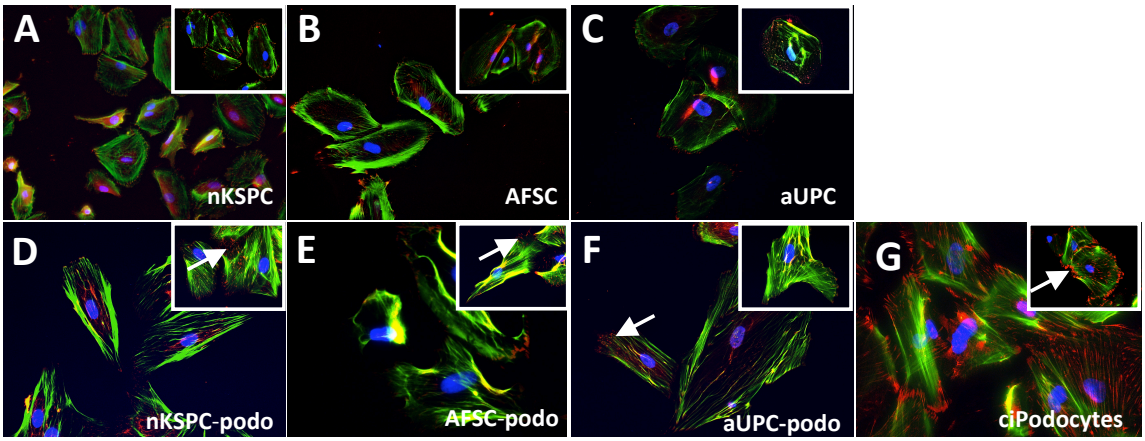
Supplementary Figure 2



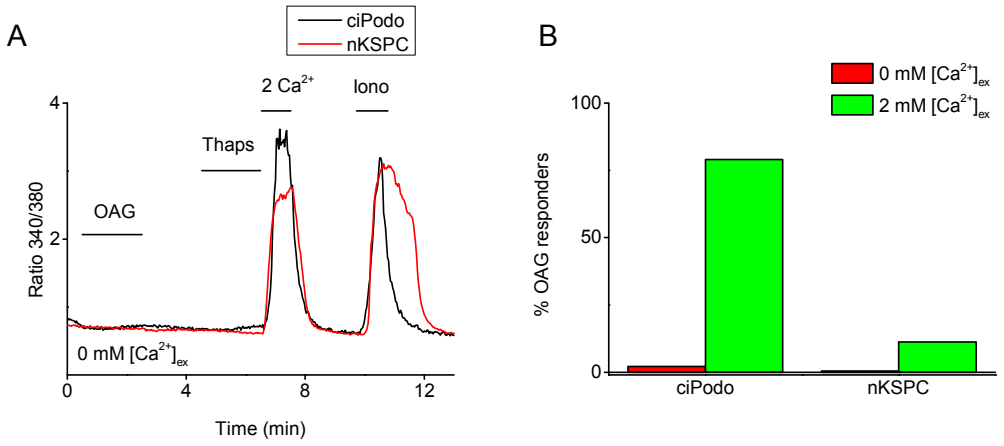
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Material and Methods

4.1 Isolation and culture of undifferentiated cells from urine and amniotic fluid

Freshly voided urine samples (about 5 ml) were collected from 12 preterm male neonates (born at 31-36 weeks GA) at 1 day after birth using a plastic bag attached to the penis (Urinocol Pediatrie, Braun Medical, Belgium); and from 10 healthy adults (about 100 ml) between 23 and 40 years old. Three to five milliliters of amniotic fluid (AF) samples were

obtained from 5 women who underwent amniocentesis between 15 to 22-weeks of pregnancy. Samples were centrifuged (5 minutes; 350g) and incubated in medium containing 1:1 ratio of keratinocyte-serum free medium and progenitor cell medium[33, 48]. Keratinocyte serum-free medium (Life technologies) was supplemented with 5 ng/ml epidermal growth factor (Peprotech), 50 ng/ml bovine pituitary extract (Life technologies), 30 ng/ml cholera toxin (Sigma), 100 U/ml penicillin and 1 mg/ml streptomycin. Progenitor cell medium contained ¾ Dulbecco's modified Eagle's medium (Lonza), ¼ Hamm's F12, 10% fetal bovine serum, 0.4 µg/ml hydrocortisone, 10^{-10}M cholera toxin, 5 ng/ml insulin, 1.8×10^{-4} M adenine, 5 µg/ml transferrin plus 2×10^{-9} M 3,39,5-triiodo-L-thyronine, 10 ng/ml epidermal growth factor and 1% penicillin-streptomycin. AF was centrifuged (5 minutes; 350g) and cells were plated and expanded in Chang C® medium (Irvine Scientific) as described by Zia *et al.*[49]. Shortly, colonies derived from a single cell were observed after 5-6 days, and when each separate colony had about 200-300 cells, they were mechanically isolated using fine-tipped pipettes. Colonies growing too close to each other were excluded. The cells of each colony were re-seeded into an individual well of a 24-well plate and sub-cultured upon 70% confluence by trypsinization and re-plating into a 12-well plate and thereafter into a six-well plate and finally moved in a $10cm^2$ culture dish. Culture medium was changed every second day.

4.2 Characterization of kidney-undifferentiated cells derived from urine and AF

4.2.1 FACS analysis

Clonal cell lines from preterm neonatal urine, amniotic fluid and adult urine were characterized at passage 4 using flow cytometry analysis. A total of 10^5 cells were trypsinized and incubated with either FITC, APC or PE-conjugated antibodies against CD24, CD105, CD73, human leukocyte antigen A-B-C, HLA-DR, CD14, CD34 and CD45 (BD bioscience, Erembodegem, Belgium), CD133 (130-080-801 Miltenyi Biotec, Bergish Gladbach, Germany) and CD29 (Acris, Herford, Germany) mouse anti-human monoclonal antibodies and appropriate isotype control. For the flow cytometry analysis of the transcription factors SIX2 and FOXD1 200000 cells were fixed using 2% PFA for 20 min, then permeabilization

with 0.1% triton for 15 minutes on ice, blocking with 1% BSA for 20 minutes and incubation with primary antibodies for 30 min and then secondary antibodies for 20 minutes. Stained cells were then analyzed using a BD FACSCANTO II (BD biosciences, San Jose, CA, USA) with the BD FACSDiva™ software, and data were computed using the FlowJo software (Tree Star, Ashland, OR, USA).

4.2.2 Quantitative rt-PCR

The expression of the specific markers SIX2, CITED1 and Vimentin was analyzed by quantitative rt-PCR in the clonal cell lines. *GAPDH* was used as housekeeping gene. Briefly, RNA was isolated using RNeasy Micro Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. 500 ng of RNA was used to synthesize cDNA using a mix of Oligo (dT) 12-18 Primer (Invitrogen: 1206193), Random primers (Invitrogen: 1208582), dNTP mix (100 mM Invitrogen: AM8228G) and SuperScript® III, plus 5X first-strand reaction mix (Invitrogen).

Quantitative rt-PCR was performed using a CFX96 Real-Time detection system (Bio-Rad) with Platinum SyberGreen qPCR Supermix (Invitrogen, 11733-046), 10 µM of primers (Supplementary table 2) and 1,5 µl of cDNA. Results were analyzed using CFX Manager™ software.

4.2.3 Immunofluorescence staining

Cells were seeded on a Falcon®™ 8-well Lab-Tek® Chamber Slide™ System (Thomas Scientific, USA) at a density of 20000 cells/chamber. Cells were fixed with 4% paraformaldehyde for 15 minutes. They were permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) for 10 minutes on ice and blocked in blocking buffer containing 2% BSA, 2% FSB and 0.2% gelatin in PBS for 1 hour. Cells were incubated with primary antibodies (Supplementary table 3) overnight at 4°C and further with AlexaFluor secondary antibodies for 1 hour in dark at room temperature (Supplementary table 3) and TRPC6 was an in-house antibody (1:1000 dilution) and kindly offered by Prof. Joris Vriens. 4'4', 6-diamidino-2-

phenylindole (DAPI) was diluted to 1:1000 in mounting medium (Dako, S3023) for nuclear staining. The cells were washed 5 times with PBS between steps. Slides were visualized with a fluorescence microscope (Olympus BX41, Olympus Belgium NV).

4.3 Single cell cDNA synthesis

Single cell cDNA was prepared according to the protocol in Picelli *et al*. up to PCR purification (step 26) and 18 PCR cycles were used in step 14. Individual 40 cells were manually picked and washed in 1x phosphate-buffered saline (PBS) (Sigma-Aldrich) supplemented with 0.01% polyvinylpyrrolidone (PVP) (Sigma-Aldrich). Single cells (volume: 0.3µl) were then transferred to separate 0.2 ml PCR tubes containing 2µl cell lysis buffer and kept on ice. The single cell manipulations were conducted using a STRIPPER (Origio) pipet and MXL3-75 tips (Origio). Picking medium was used in "no cell" controls.

4.5 Differentiation of KSPCs towards podocytes and proximal tubule cells

A representative clonal subpopulation of kidney-undifferentiated cells of each source, preterm neonatal urine, AF and adult urine was used for differentiation. For podocyte-differentiation, kidney undifferentiated cells were incubated for 7 days at 37°C in 5% CO2 in VRAD medium[39] containing DMEM-F12 (Lonza) supplemented with 10% FBS (Lonza), 100 nM vitamin D3 (Sigma), 100 µM all-trans retinoic acid (Sigma) and 1% penicillin-streptomycin, freshly prepared and changed every 3 days. All the results of differentiation of cells towards podocytes were compared to conditionally immortalized podocytes (ciPodocytes), a well established podocyte cell line[50] after 7 days in culture at 37°C. For PTEC-differentiation, kidney undifferentiated cells were incubated for 4 days at 37°C in PTEC medium composed of DMEM-F12 (Lonza), 10% FBS, ITS (Sigma), hydrocortisone (Sigma), EGF (Sigma), tri-iodothyronine (Sigma) and 1% Penicillin/Streptomycin. Results of differentiation of cells towards PTECs were compared to conditionally immortalized proximal tubule cells (ciPTECs), after 10 days in culture at 37°C.

Gene expression was analyzed by quantitative rt-PCR for the podocyte-specific genes: *LXM1b*, *CD2AP*, synaptopodin, podocalyxin and nephrin or PTEC-specific genes: *CD13*, megalin, cubilin and Pgp (Supplementary table 2) as described above, and the fold increase in gene expression was presented as relative to housekeeping gene *GAPDH* expression and normalized to the expression in ciPodocytes[50] or ciPTECs.

Statistical analysis was performed for the means using OneWay ANOVA with Bonferroni correction for multiple analyses in the SPSS Statistics Software. Statistical significance was considered at $p<0.05$.

Immunofluorescence staining was performed as described above for the podocyte specific proteins synaptopodin, podocalyxin, podocin and WT-1 (Supplementary table 3). For the actin filaments staining, the toxin phalloidin 488 (A12370, Life Technologies) was used in a dilution of 1:500.

4.4.1 Western blotting

Cells were harvested in 80 µl of RIPA buffer containing 10 µl/ml of PMSF, 30 µl/ml aprotinin and 10 µl/ml of sodium orthovanadate. 25 µg of protein were loaded in pre-cast gels (NU-PAGE Bis-Tris - Invitrogen) and incubated with anti-Pgp antibody overnight and goat anti-rabbit HRP-conjugated secondary antibody for 2 hours.

4.5.2 Scanning electron microscopy

nKSPCs were seeded on glass cover slips and induced to differentiate into podocytes for 7 days. Cells were fixed in 2.5% glutaraldehyde (buffered with 0.1 M sodium cacodylate buffer, pH of 7.4) for 2 hour; post fixed in 1.25% osmium tetroxide in cacodylate buffer for 90 minutes, and dehydrated through an increasing ethanol series. Cell monolayers were then dried with pure hexamethyldisilazane (Fluka Chemie AG, Buchs, Switzerland), sputter-coated with gold, and observed at scanning electron microscope (JEOL 7401F field emission SEM).

4.6 Functional Assays

4.6.1 Albumin endocytosis

To analyze the ability of derived-podocytes to endocytose albumin, cells were seeded on a Falcon®™ 8-well Lab-Tek® Chamber Slide™ System (Thomas Scientific, USA) at a density of 20000 cells/chamber and differentiated towards podocytes as described above. After 7 days, cells were incubated with DMEM-F12 medium (Lonza, Breda, The Netherlands) for serum starvation for 2 hours and then, incubated in medium supplemented with 50 μg/ml of Alexa Fluor 555-conjugated BSA (A-34786, Life Technologies), well-known for the analyzes of the rate of endocytosis and exocytosis by cells. As endocytosis is an energy-dependent process incubations were performed at 37°C for 30 minutes and also at 4°C for inhibition while loading and for the duration of the experiment. After incubation, cells were washed 5 times with ice-cold PBS and fixed in 4% paraformaldehyde; the slides were mounted with mounting medium (Dako, S3023) plus 1:1000 dilution of DAPI and analyzed with a fluorescence microscope (Olympus BX41, Olympus Belgium NV). ImageJ Software was used for quantification of fluorescent particles applying the following formula for corrected total fluorescence: Integrated Density – (Total Area of image X Mean fluorescence of background readings).

4.6.2 Calcium influx

The urinary and AF kidney undifferentiated cells were seeded on glass cover slips in 12-well plates in a density of 30000 cells/well and differentiated towards podocytes in VRAD medium as described. After 7 days, the derived-podocytes were incubated with 2 μM Fura-2 acetoxymethyl ester (Molecular Probes, Invitrogen) for 20 minutes at 37°C. The standard imaging solution contained (in mM): 150 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4 with NaOH). Bath solutions were perfused by gravity via a multi-barreled pipette tip with a single outlet of 0.8 mm inner diameter. The intracellular Ca^{2+} concentration was monitored through the ratio of fluorescence measured upon alternating illumination at 340 and 380 nm excitation filters using a MT-10 illumination system and the Xcellence pro software (Olympus, Planegg,

Germany). Calcium influx was stimulated by addition of 150 mM 1-oleoyl-2-acetyl-sn-glycerol (OAG) an agonist of TRPC6 [51] and cells were considered as responders when upon stimulation, the increases in the fluorescence ratio were higher than 0.05 of baseline. ciPodocytes incubated for 7 days in 37°C were used as positive control. Nonresponsive cells that did also not respond to application of 2 μM ionomycin (Sigma I0634) were excluded from the analysis.

4.6.3 Electrophysiology

In order to confirm that increases in intracellular concentration of calcium happened due to influx of cations via activation of TRPC6 channels in ciPodocytes and derived-podocytes, we performed whole-cell patch clamp analysis. Whole-cell membrane currents were measured with an EPC-10 amplifier and the PatchMasterPro Software (HEKA Elektronik, Lambrecht, Germany). Current measurements were performed at a sampling rate of 20 kHz and currents were digitally filtered at 2.9 kHz. The standard extracellular solution contained (in mM): 150 NaCl, 1 $MgCl_2$, 10 glucose and 10 HEPES (pH 7.4 with NaOH) and the standard internal solution contained (in mM): 150 NaCl, 5 MgCl2, 10 HEPES, 5 EGTA (pH 7.4 with NaOH). The standard patch pipette resistance was between 2 MΩ and 5 MΩ when filled with pipette solution. Currents were measured applying a 400ms ramp protocol from -120 mV to +120 mV every 2s.

4.6.4 Calcein efflux assay

In order to confirm the potential of nKSPC-PTECs to efflux drugs via p-glycoprotein (PgP), we incubated cells with calcein-AM (Invitrogen C1430) and Pgp-inhibitor PSC833 (Tocris 4042). 75000/well nKSPC, nKSPC-PTEC and ciPTECs were seed in triplicates in 24-well plates and incubated with 1uM calcein-AM in DMSO or 1μM Calcein-AM in DMSO + 5 μM PSC833 for 1 hour at 37°C in dark. Cells were washed with HBSS/HEPES and incubated for

20 minutes with 0.1% Triton in HBSS/HEPES, then 200µl of lysates were tranferred to white 96-well MTP plate and fluorescence was determined at ex485/em535 nm.