Supplementary methods

Isolation and culture of proximal tubular cells from brain-dead donor (bddPTC)

a. Acquisition of human donor kidneys

Human kidneys were provided by the regional organ procurement organization. Kidneys recovered from brain-dead donors but were subsequently judged unsuitable for transplant and designated for research were used in this study. The exclusion criteria of the donor were the age under 18 years old, no research authorization, positivity for antibody against hepatitis C virus, and positivity for the antigen test for SARS-CoV-2. Other donor information was not identified on the laboratory side. Kidneys were processed in our laboratory within 24 h of organ recovery.

b. Preparation of reagents and instruments for kidney dissection.
   1. Prepare the instruments and reagents listed in **supplementary table** S1, including cell culture medium and digestion medium.
   2. Pipette collagen solution into ten T-75 flasks (5 mL/flask). Gently shake the flasks to make the solution cover the bottom surface of the flask.
   3. Incubate the flasks for 1 h at room temperature.
   4. Remove the collagen solution. Open the lid and leave the flask in the biosafety hood for more than 1 h for drying.
   5. Close the lid and keep the flasks in the incubator at 37°C.
   6. Warm Digestion medium, culture medium-10% fetal bovine serum (FBS), and culture medium-1%FBS at 37°C in water bath.
   7. Prepare the instruments, including a sterilized tray, beaker, blade, and forceps. Place tray on ice, and place other instruments in the tray for cooling.

c. Dissection/cell suspension.

Perform all kidney dissection and cell isolation procedures in a biosafety hood. Operators must wear personal protective equipment and use double gloves.

   1. Place the donor kidneys on the tray on ice.
   2. Remove the perirenal fat and renal capsule using a blade and forceps.
   3. Divide the kidneys coronally in half and expose the renal papilla.

4. Dissect the cortex, about 10 mm from the surface, and cut into 1.5 cm pieces.
5. Put the cut kidney pieces in the beaker cooled on the tray, rinse with 20 mL of cold phosphate buffered saline (PBS) at p.h. 7.2.
6. If desired, after rinsing, place several pieces in 4% paraformaldehyde (PFA) for fixation and histological assessment.
7. Mince remaining pieces with forceps until homozygous.
8. Transfer the minced/homozygous tissue to ten 50 mL falcon tubes and fill each tube to 30 mL with cold PBS.
9. Wait for the tissue to naturally settle down (for 1 or 2 min without centrifugation) and then remove the supernatant.
10. Repeat the step (rinsing with cold PBS and removing supernatant) 1-3 times until the supernatant becomes transparent.
11. Add 20 mL digestion medium to each tube and incubate at 37°C for 2h while gently shaken.
12. Add 20 mL of culture medium-10%FBS in each tube to stop tissue digestion.
13. Set 250 µm cell strainer on a new sterilized dish. Transfer the tissue in all tubes on the strainer and push the tissue using syringe plunger while adding 50 mL of cold PBS.
14. Set 100 µm cell strainers on five new 50 mL falcon tubes. Put 250 µm filtered-tissue solution on the strainer while adding 20 mL of cold PBS to each tube. Grind the tissue on the strainer using syringe plunger if the strainer gets clotted.
15. Centrifuge the filtered solution at 500 G for 5 min.
16. Remove the supernatant and resuspend it with 20 mL of cold PBS to each tube.
17. Set 70 µm cell strainer on five new 50 mL falcon tubes and pipette tissue suspension through strainer while adding 20 mL of cold PBS to each tube. Do not grind the tissue on 70 µm strainer.
18. Centrifuge the filtered solution at 500 G for 5 min.
19. Remove the supernatant and resuspend the pellet in each tube with 15 mL of Culture medium-10%FBS.
20. Put the resulting kidney cell (bddKC) single cell suspension in each tube to a collagen coated flask. Incubate overnight.

d. Primary culture and cell sorting of bddKC.

1. Replace the medium to Culture medium-1%FBS on the first full day of culture.
2. Change the medium every 2 or 3 days.
3. Cells should become confluent on day 5 or 6 of culture. When confluent, passage the cells using Trypsin-EDTA and continue culturing.
4. When bddKC become confluent at passage 1, trypsinize cells and transfer to round bottom-96 well cell culture plate. Cells should be transferred to at least 5 wells (for cell sorting and controls).
5. Centrifuge 5 min at 500G at 4°C. Remove supernatant and reconstitute cell pellet with anti-CD10 antibody (1:100 dilution, Cat. No. 50-166-673, Thermo Fisher Scientific, Waltham, MA) and anti-CD13 antibody (1:200 dilution, Cat. No. 50-165-739, Thermo Fisher Scientific) in PBS.
6. Incubate at 4°C for 30 min. Avoid light from this point onward.
7. Centrifuge at 500G at 4°C for 5 min. Remove supernatant.
8. Reconstitute the pellet with 150 µL of cold culture medium-1%FBS.
9. Centrifuge at 500 G at 4°C for 5 min. Remove supernatant.
10. Reconstitute the pellet with 150 µL of cold Culture media-1%FBS.
11. Add 2 µL of 4',6-diamidino-2-phenylindole (DAPI) solution (1:75 dilution, Cat. No. BDB564907, Thermo Fisher Scientific).
12. Sort CD10/CD13 double positive bddKC cells.
13. Seed sorted cells (now "bddPTC" cells) on new T-75 flask at 10^7cells/ flask with culture medium-1%FBS.
14. Culture bddPTC and use for experiments at passage 4 or less.

Supplementary table S1: The list of instruments and reagents

Instruments
▪ Scalpel
▪ Scissors
▪ Forceps
▪ 100 mL beaker
▪ Small or middle size petri dish (for mincing the kidney tissue at c-7)
▪ Large size petri dish (for cell solution filtered through 250 µm strainer at c-13)
▪ 70, 100, and 250 µm cell strainers.

- Syringe plunger
- 50 mL tubes

Reagents

- Rat Collagen Type 1 (Cat. No. C3867, Sigma)
- ITS Premix Universal Culture Supplement (Cat. No. 354351, Corning)
- Fetal Bovine Serum (FBS) (Cat. No. F-0500-A, Atlas Biologicals)
- Liberase™ (Cat. No. 5401119001, Sigma)
- Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) (Cat. No. D8437, Sigma)
- Penicillin-Streptomycin 10,000 U/mL (P/S) (Cat. No. 15140-122, Thermo Fisher Scientific)

Mixture of reagents	Amount
<u>Collagen solution</u>	
▪ ddH2O	50 mL
▪ Rat Collagen Type 1	50 µL
<u>DMEM/F12+1%P/S</u>	
▪ DMEM/F12	500 mL
▪ Penicillin /Streptomycin	5 mL
<u>Digestion medium</u>	
▪ Liberase (26U/mL, dissolved in ddH2O)	2 mL
▪ DMEM/F12+1%P/S	200 mL
<u>Culture medium-10%FBS</u>	
▪ DMEM/F12+1%P/S	630 mL
▪ FBS	70 mL
<u>Culture medium-1%FBS</u>	
▪ DMEM/F12+1%P/S	300 mL
▪ FBS	3 mL
▪ ITS	300 µL