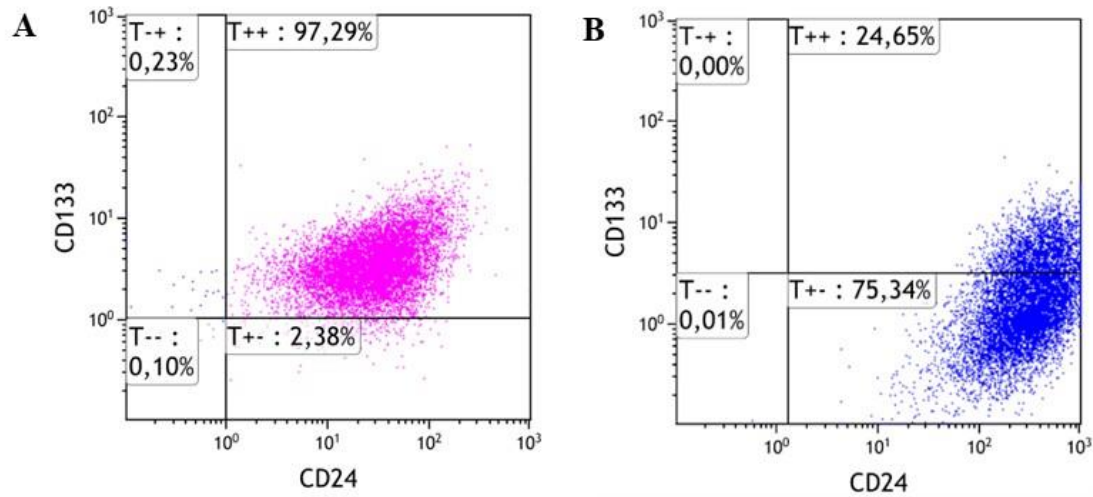
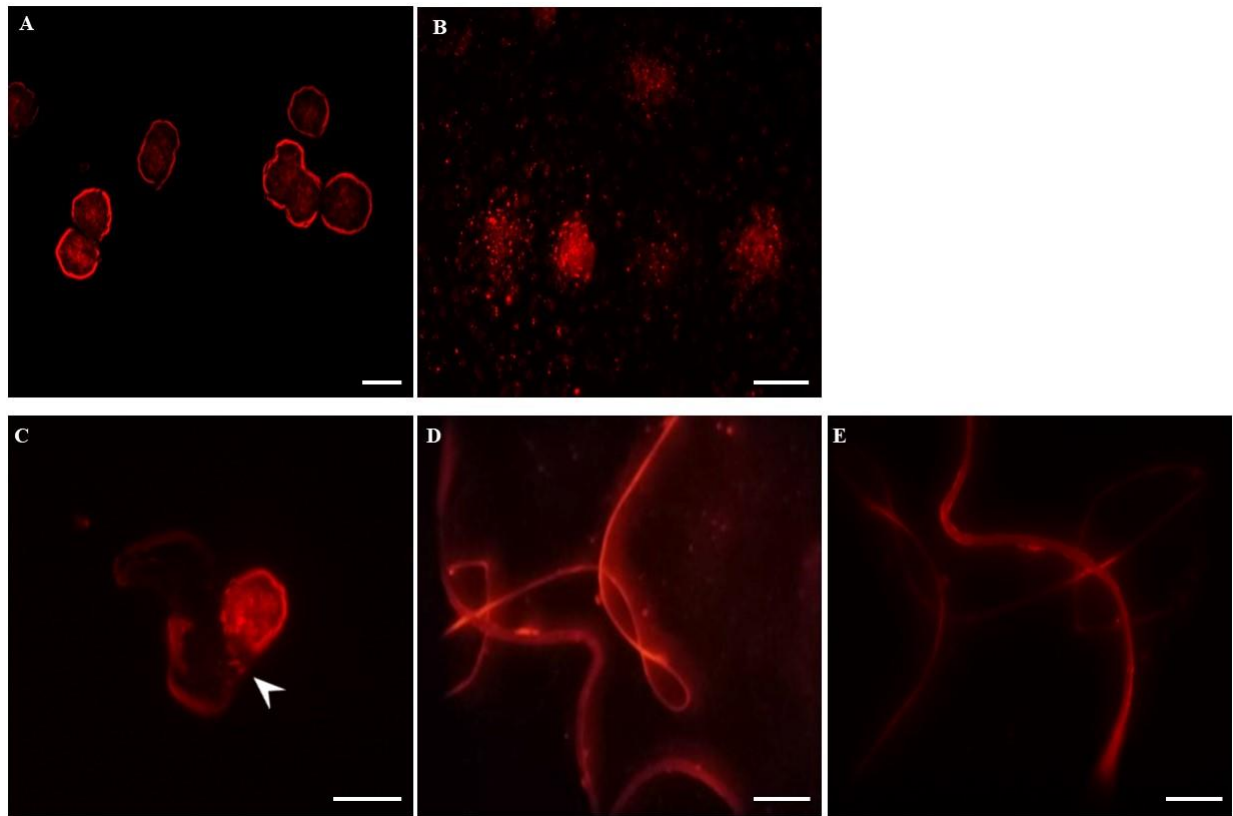


## Supplementary Material

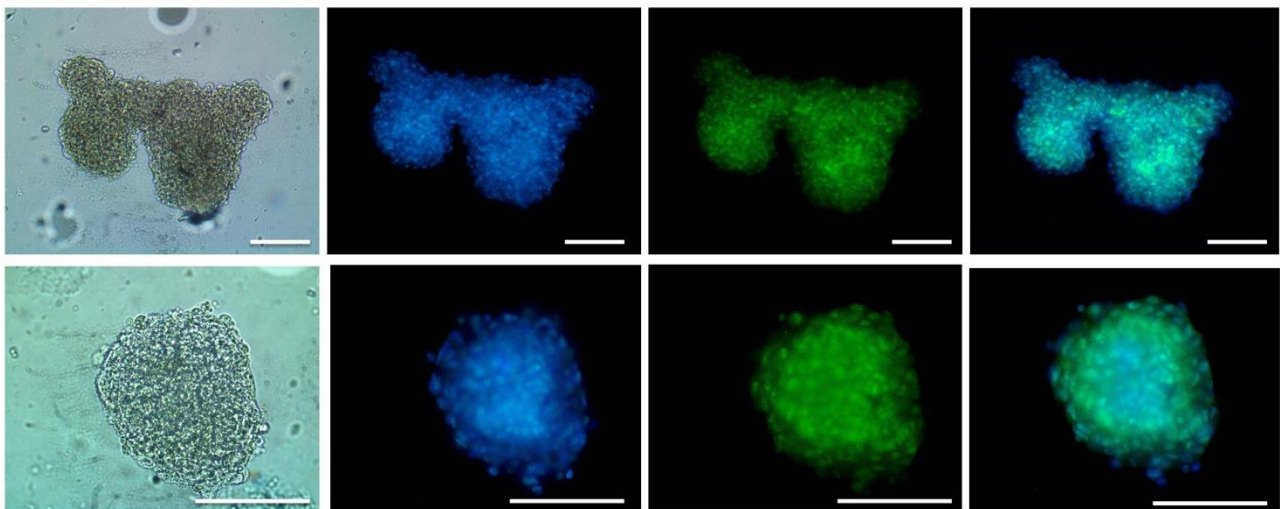


**Supplementary Figure 1. Cytofluorimetric analysis showing the expression of CD133 and CD24.**

- (A) ARPCs isolated from urine of an healthy subject co-expressing high levels of CD133 and CD24 markers.  
(B) uARPCs/CD133<sup>-</sup> mixed cell population used to generate renal spheroids.



**Supplementary Figure 2. Tracking of tubule-like structure sprouting from spheroids.** ARPCs were labeled with the PKH26 fluorescent cell tracking marker and were induced to form spheroids in the 3D plates. The spheroids generated from uARPCs/CD133<sup>-</sup> mixed cell population were dense with clear and defined thick edges (A), whereas spheroids generated from RPTECs appeared disaggregated (B). (C-E) The fluorescent tracker demonstrated the origin of long tubule-like structures from ARPCs generating spheroids. The scale bars represent 100  $\mu$ m.



**Supplementary Figure 3. Pax2 expression in ARPC spheroids.** (A) Renal spheroids formed by ARPCs observed at phase contrast microscope. (B) Whole-mounting immunofluorescence showing nuclei stained with DAPI (blue) Pax2 (green) in ARPC spheroids. The scale bars represent 50  $\mu$ m.

| Antibody                         | Clone  |
|----------------------------------|--------|
| CD133/2-PE human                 | REA820 |
| CD24-APC human                   | REA832 |
| Anti-SSEA 4-FITC human and mouse | REA101 |
| Anti-SOX2-FITC human and mouse   | REA320 |
| Anti-OCT3/4-PE human and mouse   | REA622 |
| GATA 3-APC human and mouse       | REA174 |
| Anti-NANOG-APC human             | REA314 |

**Supplementary Table 1. Antibodies used in Flow Cytometry Analysis.** All mAbs were purchased from Miltenyi Biotec (Bologna, Italy).

| <b>Primary Antibodies</b>        | <b>Clone</b>                             | <b>Company</b>                         |
|----------------------------------|------------------------------------------|----------------------------------------|
| ZO-1 Monoclonal Antibody         | R40.76-sc-33725, rat monoclonal antibody | Santa Cruz Biotechnology, USA)         |
| CD13 Monoclonal Antibody         | 1C7D7, Mouse/IgG1                        | Thermo Fisher Scientific, Waltham, USA |
| CD249 Monoclonal Antibody        | OTI4G8, Mouse/IgG2b                      | Thermo Fisher Scientific, Waltham, USA |
| UROMODULIN Polyclonal Antibody   | bs-2189R, rabbit IgG                     | Bioss Antibodies, Woburn, USA          |
| LOTUS TETRAGONOLOBUS fluorescein | FITC                                     | Thermo Fisher Scientific, Waltham, USA |

| <b>Secondary Antibodies</b>                      | <b>Company</b>                         |
|--------------------------------------------------|----------------------------------------|
| Alexa Fluor 488-conjugated goat anti-mouse IgG   | Thermo Fisher Scientific, Waltham, USA |
| Alexa Fluor 555- conjugated goat anti-mouse IgG  | Thermo Fisher Scientific, Waltham, USA |
| Alexa Fluor 488-conjugated goat anti-rabbit IgG  | Thermo Fisher Scientific, Waltham, USA |
| Alexa Fluor 555- conjugated goat anti-rabbit IgG | Thermo Fisher Scientific, Waltham, USA |

**Supplementary Tables 2. Antibodies used in Whole-mounting and frozen section immunostaining.**