Supplemental information

Material and Methods

Culture conditions and implantation of mouse and chimeric organoids. Organoids were cultured in Advanced DMEM (12494; Gibco, Invitrogen Corporation, Grand Island, NY) supplemented with 2% Embryonic Stem cells Fetal Bovine Serum (ES-FBS, Gibco), 1% L-glutamine (Invitrogen Corporation, Carlsbad, CA) and 1% penicillin/streptomycin (Invitrogen). During the first 24 hours, 1.25 µmol/l Glycyl-H1152 dihydrochloride (Tocris), a Rho kinase inhibitor, was added to the culture medium.

Mouse or chimeric organoids were implanted under the kidney capsule of uninephrectomized athymic rats as previously described.[1] Briefly, male 6–8 week old athymic nude rats (Harlan Laboratories, Inc., Indianapolis, IN) received organoids preconditioned with 2 µg recombinant rat VEGF (Invitrogen) for 4 h and a local VEGF injection (1 µg) into the area of implantation, and intravenous VEGF injections (1 µg three times per week) into the tail vein until euthanasia. Recipients were euthanized by CO_2 inhalation 2 weeks after implantation.

Renal histology. Renal histology was performed as previously described.[1] Briefly, after euthanasia, the recovered rat kidneys and mouse renal organoids were fixed overnight in Duboscq-Brazil and embedded in paraffin. Three-µm sections were stained with hematoxylin-eosin (Bio-Optica, Milan, Italy) and observed using light microscopy (Olympus, BH2-RFCA, Melville, NY, USA). To make red blood cells readily visible using light microscopy, a modified protocol for hematoxylin-eosin staining was applied on PLP-fixed cryosections.[1]

Albumin uptake assay. Albumin uptake assays was performed as previously described[2, 3] with small modifications. Human AFSCs were incubated with serum free medium over night. After washing with Ringer's buffer pH 7.4, AFSCs were exposed to 50 µg/ml FITC-BSA (Sigma-

Aldrich) for 90 min at 37°C. The cells were then washed with Ringer's buffer and fixed in 2% PFA and 4% sucrose for 10 min at room temperature. Nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) and finally cells were mounted using Dako Fluorescence Mounting Medium (DAKO, Denmark) and examined using an inverted confocal laser scanning microscope (LS 510 Meta; Zeiss). Terminally differentiated human podocytes cultured as previously described[2] were used as positive control.

References

1. Xinaris C, Benedetti V, Rizzo P, Abbate M, Corna D, Azzollini N, Conti S, Unbekandt M, Davies JA, Morigi M, Benigni A, Remuzzi G: In vivo maturation of functional renal organoids formed from embryonic cell suspensions. J Am Soc Nephrol; 23: 1857-1868, 2012

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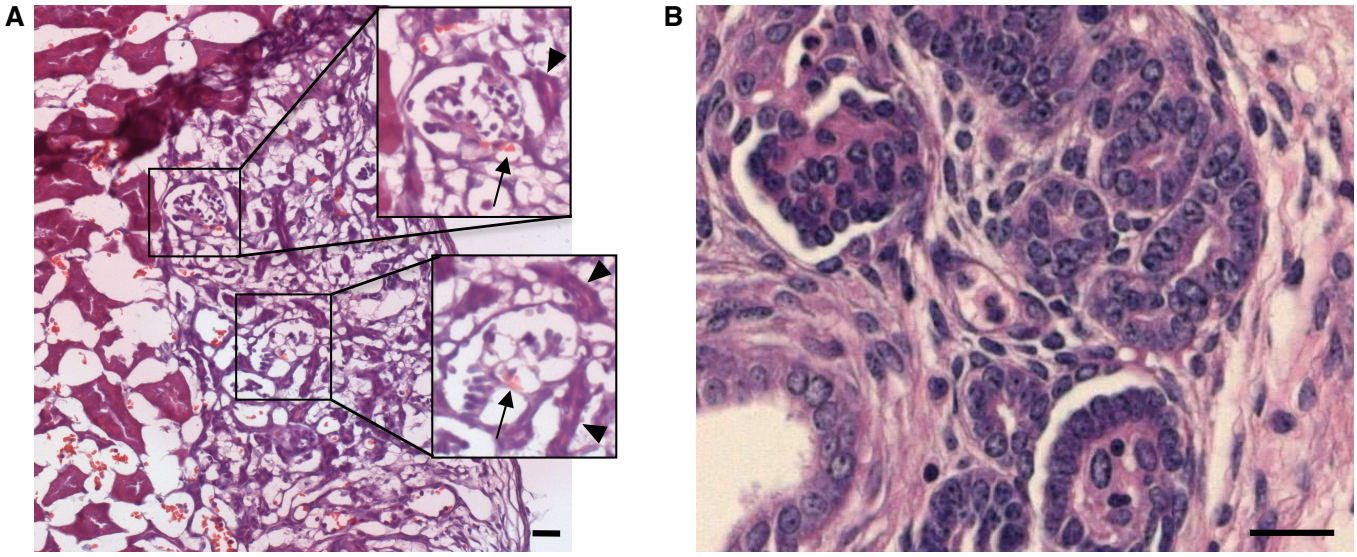
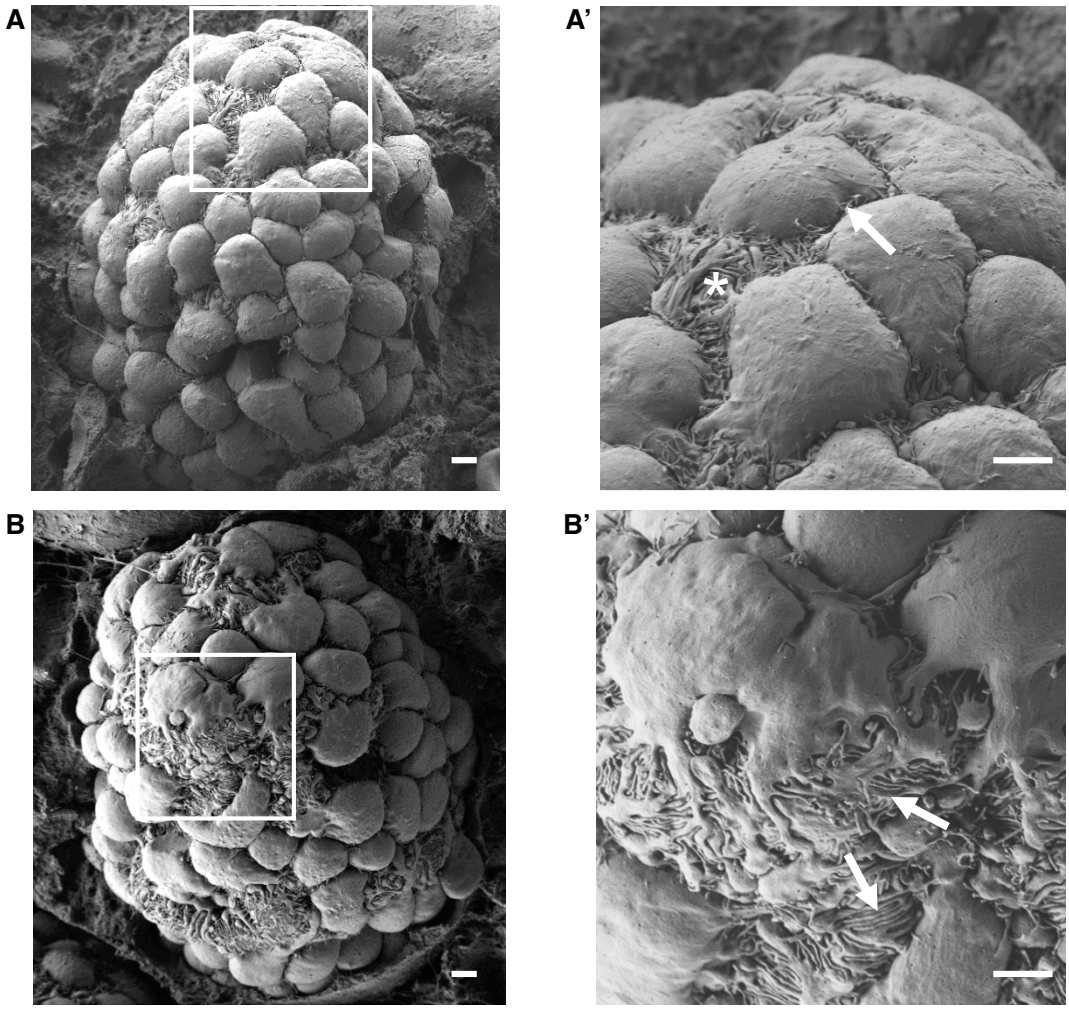
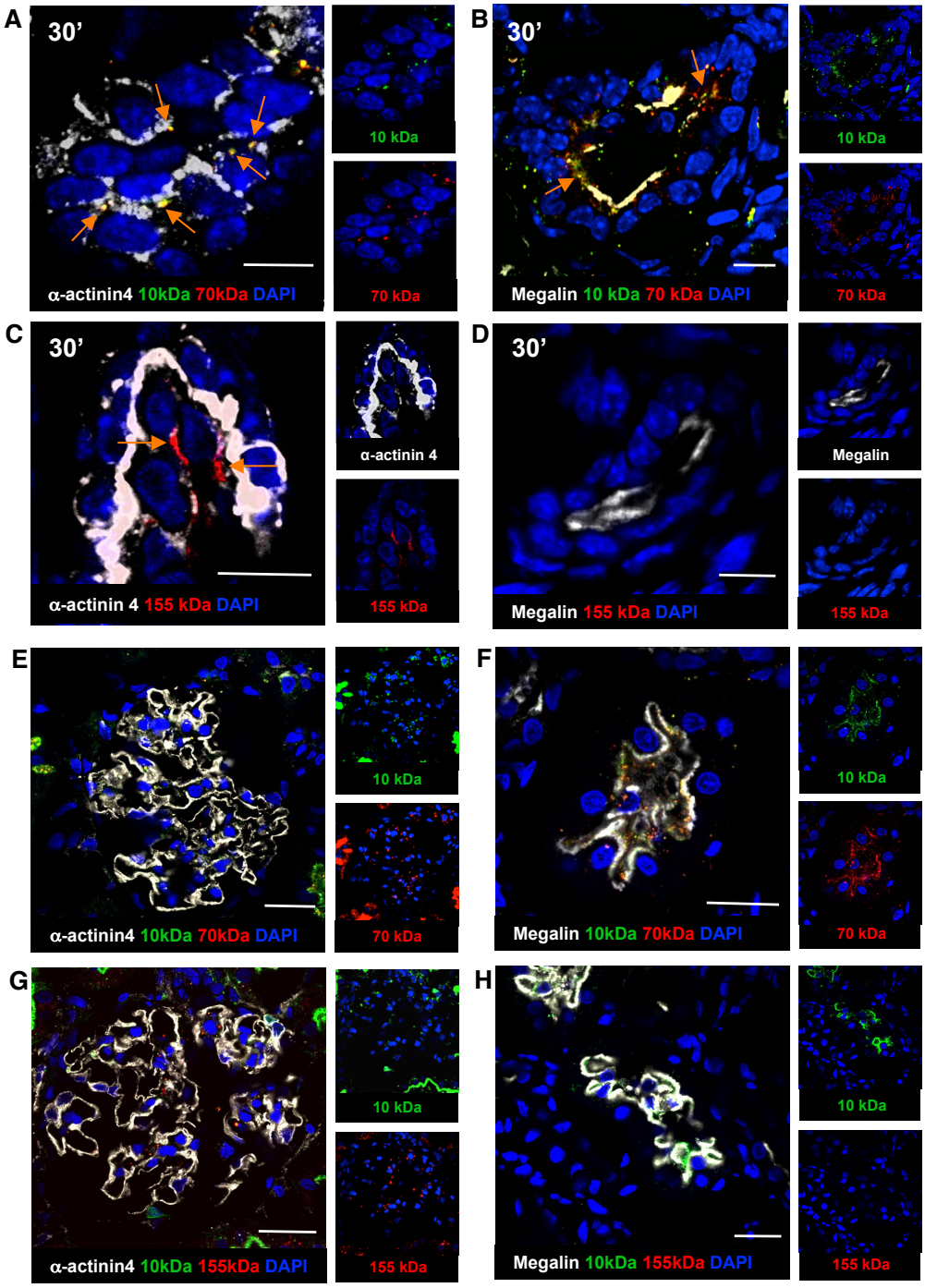


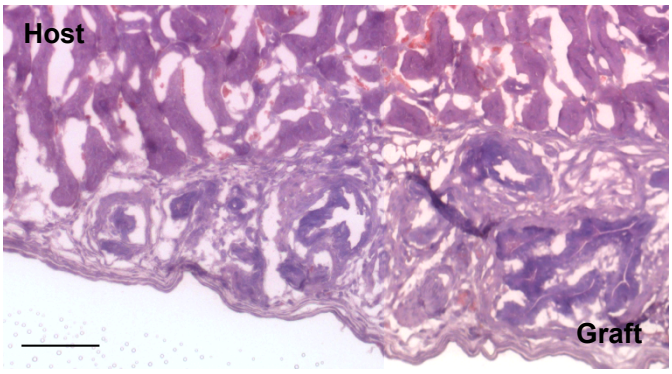
Supplementary Figure 1. Renal morphology of implanted organoids. (A) Histology of organoids showed tubular and glomerular structures (insets). Arrows: erythrocytes within vascularized glomerular structures (PLP-fixed tissue, hematoxylin and eosin stain). Arrowheads: tubular structures. **(B)** Hematoxylin and eosin staining in Duboscq Brazil-fixed paraffin-embedded renal tissue showing tubular and glomerular structures. Scale bars: 20 μm (A, B).



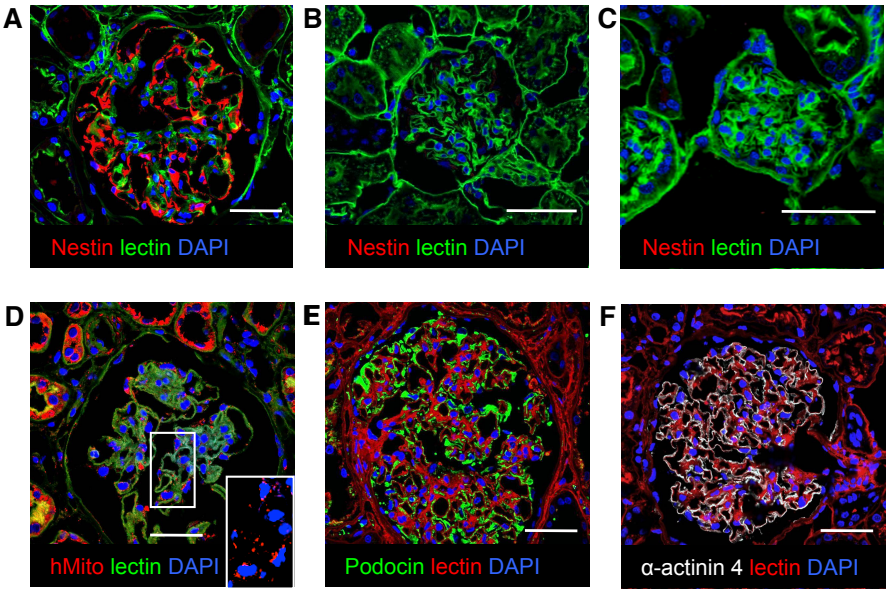
Supplementary Figure 2. Glomeruli of embryonic and neonatal kidneys. Scanning electron micrographs of metanephros (E18.5) **(A)** and neonatal control mouse **(B)** glomeruli with the corresponding high magnification insets **(A', B')**. **(A, A')** Metanephros glomerulus displaying both immature elongating primary processes (arrow) and, focally, more organized areas (asterisk). **(B, B')** Neonatal glomerulus showing more differentiated visceral epithelial cells with organized primary processes and interdigitating foot processes (arrows). Scale bars: 2 μm (A, A', B, B').



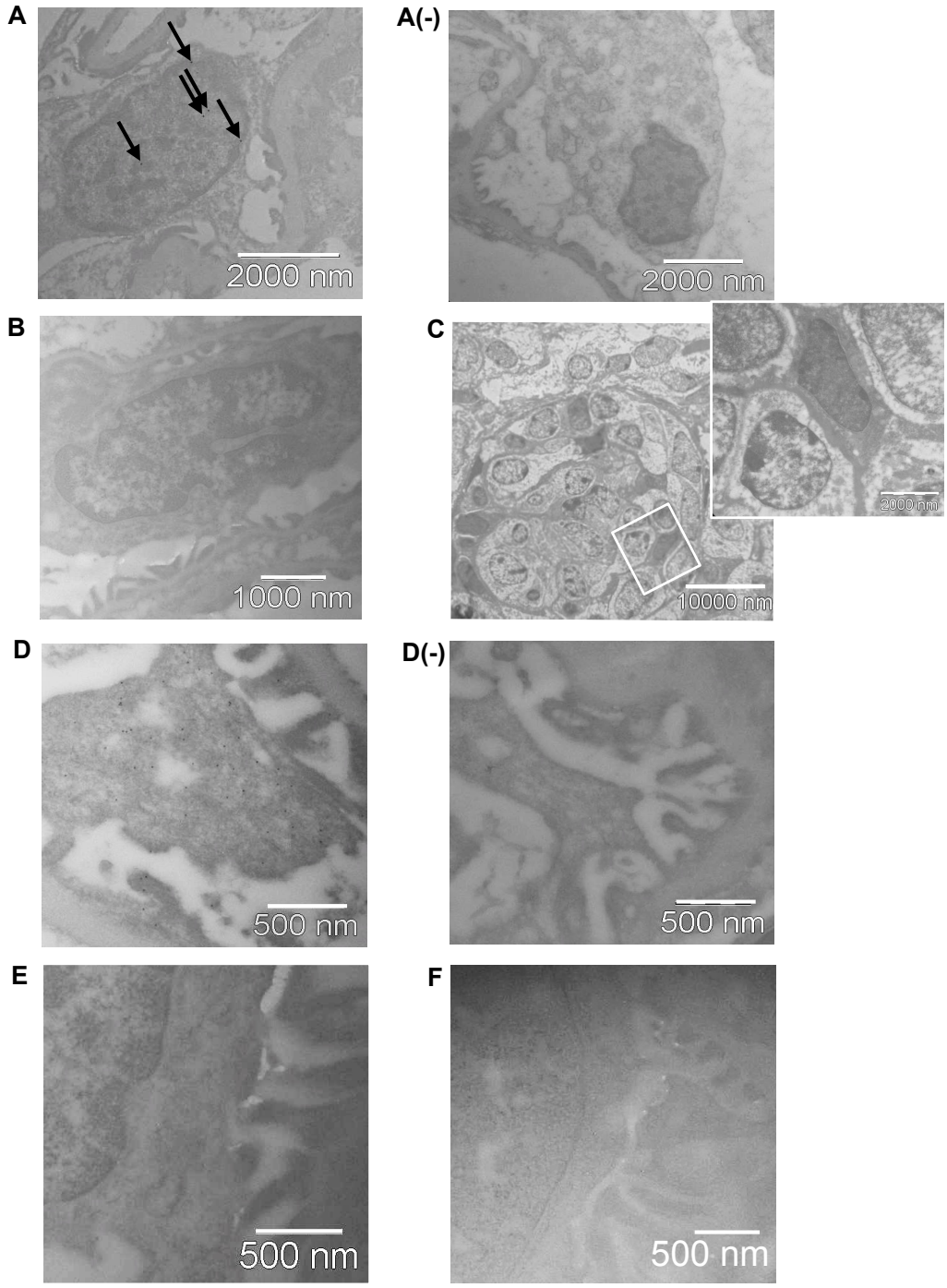
Supplementary Figure 3. Dextran uptake by implanted organoids and host rat tissue. **(A-D)** Labeled dextrans of different molecular weights injected into the tail vein of the recipient animal 30 minutes before euthanasia, in developing organoids. FITC- and RITC- **(A)** or TRICT- **(C)** conjugated dextrans (10 and 70 or 155 kDa, respectively) were found in α-actinin 4-positive glomeruli (white, arrows). **(B)** Low-molecular-weight dextrans (10 and 70 kDa) colocalized with megalin staining (white, arrows). **(D)** No TRITC-dextran was found in tubular lumina of the graft. Scale bars: 10 μm. **(E-H)** Labeled dextrans in host rat tissue. FITC- and RITC- **(E)**, or FITC- and TRICT- **(G)** conjugated dextrans in α-actinin 4-positive glomeruli (white). **(F)** Low-molecular-weight dextrans in megalin-positive tubules (white). **(H)** Absence of TRITC-conjugated tracer concomitant with the presence of FITC-conjugated tracer in megalin-positive tubules (white). DAPI (blue stained nuclei). Scale bars: 10 μm (A-D), 20 μm (E, H).



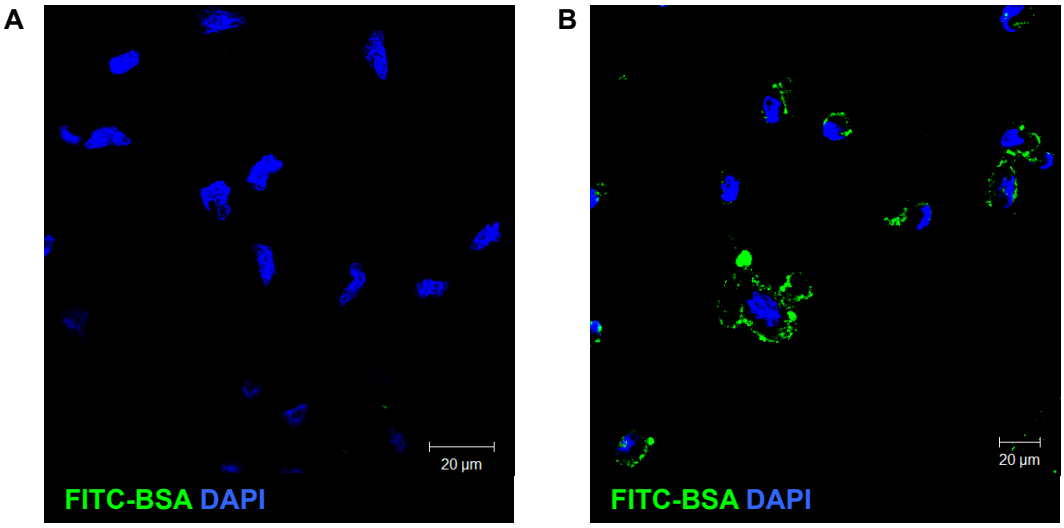
Supplementary Figure 4. Renal morphology of implanted chimeric organoids at 5 days. Histological examination revealed that the chimeric organoid implanted after 5 days of *in vitro* culture, and examined after 1 week in vivo, survived and increased in size. However, no recognizable tubular and glomerular structures developed within the graft. Scale bar: 100 μm.



Supplementary Figure 5. Expression of different markers in control tissues. (A-C) Nestin expression in control human **(A),** rat **(B)** and mouse **(C)** kidney revealing the specificity of the antibody for human antigen. **(D)** Expression of human mitochondrial marker in human tissue used as positive control (red, inset). **(D-E)** Expression of glomerular markers podocin (**E**, green) and α-actinin 4 (**F**, white) in human tissue. DAPI (blue) stained nuclei and renal structures are labeled with WGA (green) or LCA (red). Scale bars: 50 µm.



Supplementary Figure 6. Specificity of the anti-human nuclear antigen (HNA) and the anti-human nestin antibodies used to detect human AFSCs in the chimeric tissue by TEM. (A, arrows) HNA immunogold staining in a normal human podocyte. **(A(-))** No gold particles were seen when the primary antibody was omitted. **(B-C)** HNA staining was not detectable in host rat tissue **(B)** and in murine cells of chimeric tissue **(C)**. **(D)** Human-specific nestin immunogold staining was found in cytoplasm and foot processes of a normal human podocyte. By omitting the primary antibody, no signal was detectable **(D(-))**. Host rat **(E)** and murine **(F)** podocytes were negative for human-specific nestin staining. HNA and human nestin were detected through indirect immunogold technique using secondary antibodies conjugated with 25 nm or 12 nm-gold particles, respectively.



Supplementary Figure 7. In vitro uptake of BSA. (A) AFSCs or **(B)** differentiated podocytes were incubated with 50 µg/ml FITC-BSA for 90 minutes at 37°C.