Supplemental Methods

Animal studies

Generation of M-Sec knockout mice. M-Sec knockout (KO) mice were generated at the Riken Centre for Integrative Medical Sciences (Prof. H. Ohno, Japan). The target vector, containing a 4-kb genomic fragment (promoter), neomycin resistance (NeoR) gene, floxed at both ends, a 3.5-kb genomic fragment (intron5 to exon10) and the HSV-tk gene, was constructed with pBluescript II SK (+). The linearized targeting vector was introduced into B6;129 hybrid ES cells. The correctly targeted cells were screened by long-range PCR and analysed by Southern blotting. Chimeric mice were bred with C57BL/6 mice to obtain germline-transmitted animals. Heterozygous mice were crossed with transgenic animals expressing Cre under the control of the CAG promoter to remove the NeoR gene and then backcrossed to C57BL/6 mice for more than 8 generations before inter-crossing to obtain homozygous. To genotype animals, genomic DNA was isolated from mouse-tail tissue and genotyping performed by traditional PCR using the following primers (M-Sec WT: 5'TCTCCTTCTTCTTCGCAGACTC3'; M-Sec KO: 5'TGAAGCTACAAACTGCTCTGCC3'; M-Sec common: 5'GGTCCTGGTGTTTTTACTGGAC3'). After an initial denaturation at 94°C for 2 minutes, the DNA was amplified for 35 cycles with the following setting: denaturation at 98°C for 10 seconds, annealing at 60°C for 30 seconds, and elongation at 68°C for 1 minute. PCR products were resolved in a 2% agarose gel containing ethidium bromide and digital images of the gels captured using the Gel Doc XR system (Bio-Rad, Milan, Italy). M-Sec protein was undetectable in podocytes isolated from M-Sec KO mice. In addition, M-Sec mRNA was absent in spleen, heart, liver, lung, and kidney from M-Sec KO animals.

M-Sec-deletion-induced nephropathy. Male wild type (WT) BALB/c (Jackson Laboratories, Bar Harbor, ME) and M-Sec KO mice were maintained on a normal diet under standard animal housing conditions. Systolic blood pressure was assessed by tail-cuff plethysmography. Blood samples were taken via saphenous vein puncture on alert 4-h–fasted animals. Glucose levels were measured using a glucometer (Accuchek; Roche, Milan, Italy) and total cholesterol, triglycerides, and HDL

cholesterol levels on a clinical blood chemistry analyser. After sacrifice, the kidneys were rapidly dissected and weighed. The right kidney was snap-frozen in N_2 and stored at -80°C for both mRNA and protein analysis. Half of the left kidney was fixed in 10% PBS-formalin then paraffin-embedded for light microscopy; the remaining tissue was embedded in optimal cutting temperature compound and snap-frozen in N_2. In subgroups of mice, glomeruli were isolated using a modified Dynabead method.

Adriamycin-induced nephropathy. Eighty week-old male BALB/c mice were injected with a single dose of either adriamycin (10.2 mg/kg) or vehicle (*n*=6-7 per group) into the tail vein. Twenty days after injection, urines were collected, animals sacrificed, and renal tissues harvested and processed as described above.

Bone marrow (BM) transplantation. Eight-week-old male M-Sec KO and WT mice were used as BM donors. BM cells were flushed from tibial and femoral cavities under sterile conditions, filtered through 70 μm nylon meshes (BD Biosciences, Milan, Italy), and then transplanted without further purification or in vitro expansion. Before transplantation, recipient male mice, aged 8 weeks, underwent whole body-irradiation with 7 Gy. After 24 h, post-irradiated mice were injected with 2.0 x 10^6 BM cells via the tail vein. M-Sec KO mice received a BM transplant from either WT (KO-c^{wt}; *n*=6) or KO (KO-c^{ko}; *n*=6) animals and WT recipients from either KO (WT-c^{ko}; *n*=6) or WT (WT-c^{wt}; *n*=6) animals. At 24 weeks of age mice were sacrificed, DNA was isolated from blood cells (DNeasy Blood and Tissue Kit, Qiagen, Milan, Italy) and chimerism confirmed by PCR.

Glomerular isolation. Anesthetized mice were perfused with 8 X 10^7 surface-inactivated Dynabeads (Invitrogen, Milan, Italy). The kidneys, removed and minced, were digested in a collagenase A solution containing 100 units/ml DNase I (Roche Diagnostics, Milan, Italy), then passed twice through a cell strainer. The cell suspension was collected by centrifugation, then glomeruli containing Dynabeads were gathered by the magnetic particle concentrator and washed. The procedure of isolation and washing was repeated (6-8 times) until no tubular contamination was found as assessed under light microscopy.

Microscopy

Light Microscopy. Paraffin-embedded renal sections were stained using Periodic acid–Schiff (PAS). The extent of sclerosis in 50 sequential glomeruli for each animal (x400) was scored from grade 0 to 4, where 0=none, 1=1%–10%, 2=11%–20%, 3=21%–30%, and 4=>31% and results averaged (glomerular sclerosis index).

Electron Microscopy. Renal cortex pieces (1 mm^3) were fixed in 2% glutaraldehyde, 4% paraformaldehyde in phosphate buffer 0.12 mol/l for 4 h at room temperature, postfixed in 1% osmium tetroxide for 2 h, dehydrated in graded ethanol, and embedded in Epon 812. Ultrathin sections (70 nm thickness) were obtained with a Leica EM UC6 ultramicrotome, counterstained with uranyl acetate and lead citrate, and examined with an Energy Filter Trasmission Electron Microscope (EFTEM, ZEISS LIBRA® 120) equipped with an yttrium aluminium garnet (YAG) scintillator slow-scan charge-coupled device (CCD) camera (Sharp eye, TRS, Moorenweis, Germany). Analysis was performed blindly in 20 glomeruli for each animal.

Fluorescent and DIC microscopy. A Zeiss APOTOME 2 system equipped with an incubator for live-cell imaging/time-lapse microscopy and Nomarski optics for differential interference contrast (DIC) microscopy was used in TNT studies. DIC microscopy was used to avoid TNT phototoxic damage. Sequences of optical planes (Z-stack) were acquired for reconstruction in the x-z plane.

In vitro studies

Cells. Primary murine podocytes and TECs were isolated from WT and M-Sec-KO mice at 4,6, and 10 weeks of age. Decapsulated kidneys were diced and digested in 1 mg/ml collagenase A for 30 min at 37 °C. Glomeruli were harvested using the Dynabead method and plated on collagen type IV-coated dishes at 37°C in DMEM medium with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 mM HEPES, 1 mM sodium bicarbonate, and 1 mM sodium pyruvate. Subculture of primary podocytes was performed by detaching the glomerular cells with 0.25% trypsin-EDTA, followed by sieving through a 40-μm cell strainer (Falcon; BD Biosciences), and plating on

collagen type IV-coated dishes. After glomeruli isolation, the remaining renal suspension was passed through a 70-µm cell strainer to retain most of the tubular fragments and avoid contamination of other nephron segments. The tubular fragments were resuspended by flushing the sieve in the reverse direction, centrifuged and plated on collagen type I-coated dishes at 37°C in DMEM/F12 (1:1) medium with 1% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 15 mM HEPES, 50 nmol/l hydrocortisone, 5 g/ml insulin, 5 g/ml transferrin, 50 nmol/l selenium. After 7 days, TEC cultures were organized as a confluent monolayer. Cells were used before the third passage in all experiments. Podocyte cell lines were routinely tested for mycoplasma and demonstrated to be mycoplasma free before use. Cells were exposed to TNF-α (50 ng/ml) for 3 and 6h, AD (0.8 µg/ml) for 24h, rotenone (50 nM) for 24h, serum deprivation (0.1%) for 24h, and human/murine sera (4%) for 24h. Archived sera from patients with non-recurrent primary FSGS and healthy controls (*n*=4 per group) was provided by Prof. Ghiggeri GM. Sera from M-Sec-KO and WT mice was collected at sacrifice.

TNTs. To visualize plasma membrane and TNTs, podocytes were labelled with 5 µg/ml of WGA conjugated with Alexa Fluor®-488 (Thermo Fisher Scientific, Milan, Italy) in HBSS for 10 minutes at 37°C. The number of cells connected by straight WGA-labeled structures that did not adhere the substrate, as assessed by Z-stack, and were smaller than 1 µm in diameter was counted in blind using a Apotome II Zeiss Microscope controlled by the Axiovision software. Results were expressed as the percentage of total counted cells (at least 150 cells). To inhibit TNT formation, podocytes were treated with the actin-depolymerising compound, latrunculin-B, for 1 hours at the dose of 200 nM.

Mitochondrial transfer. CellTracker Blue CMFDA (Thermo Fisher Scientific, Milan Italy), a cytoplasmic dye suitable for long-term cell tracing, was used at 3µM concentration to label recipients cells. Donor cells were transfected with a modified insect virus (Baculovirus) expressing a fusion construct of a green fluorescent protein with mitochondrial E1α pyruvate dehydrogenase (CellLight™ Mitochondria-GFP, BacMam 2.0, Thermo Fisher Scientific) or labelled with

Mitotracker Red dye (Thermo Fisher Scientific). In a subset of experiments donor podocytes were pre-treated with either Rotenone (50 nM) or vehicle for 24h. Cells were co-cultured at a ratio of 1:1 in ibiTreated µ-dish for 24 hours. Both fluorescent and DIC microscopy (α-plan Apochromat 63×1.46 oil Korr objective) were used to visually assess mitochondrial transfer. To quantify transfer efficiency, cells were fixed in 2% PFA, passed through a cell strainer, then analyzed by flow cytometry (Citoflex, Beckman Coulter). Single positive cells (donor and recipient cells alone) were used to set up the instrument. Data analysis was performed using the Cytexpert 2.3 software. To control for TNT-independent transfer, recipient cells were plated in the bottom compartment of a transwell system (Corning, New York, USA) and donor cells on a 0.4 µm pore-sized insert. As additional control, the two cell populations were separately seeded into a 2 Well Ibidi Culture-Insert. After cell attachment, the insert was removed (0.5 µm cell free gap) and the two populations co-cultured for 24 hours under gentle shaking.

Apoptosis. Apoptosis was detected by transferase-mediated dUTP nick end-labeling (TUNEL) assay (ApopTag In Situ Apoptosis Detection Kit, Millipore, Billerica, MA). Results were expressed as the number of positive cells per glomerulus (at least 20 random glomeruli) or percentage of positive cultured cells.

sh-RNA. Podocytes ($3x10^5$) were transfected using Lipofectamine 3000 Transfection Reagent (Thermo Fisher Scientific) with a plasmid construct encoding M-Sec-specific sh-RNA (shRNA: 5'-GATCCGACTGCTGGAGGCCACATTCCTGT-3', scramble negative control: 5'-GCACTACCAGAGCTAACTCAGATAGTACT-3') cloned in a pGFP-V-RS vector (ExactHuSH, OriGene Technologies Inc). Knockdown efficiency was assessed by western blotting.

Oxygen consumption rate

Real-time measurements of oxygen consumption rate (OCR) were performed using the Seahorse Bioscience XF Cell Mito Stress Test assay kit on the XFe96 Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA). Cells were plated at a density of 12,000 cells/well in 96-well assay plates (Seahorse Bioscience) and cultured in standard medium. After 24 h, the medium was

replaced by XF Base Medium (Seahorse Bioscience) with the addition of 10 mM glucose, 1 mM pyruvic acid, and 2 mM L-glutamine. OCR was measured at baseline and after sequential addition of 1 μM oligomycin, 1 μM carbonylcyanide-4- (trifluoromethoxy)-phenylhydrazone (FCCP), and 0.5 μM rotenone/antimycin A. Data were analysed using the WAVE software (Agilent) and normalised using total protein concentration. The metabolic parameters were calculated using the XFMito Stress Test Report Generator (Agilent).

mRNA analyses and mtDNA copy number

mtDNA copy number. Glomerular DNA was isolated using the Pure Link Genomic DNA kit (Thermo Fisher Scientific). Equal amounts of DNA (0.3 ng/μl) were amplified by real-time PCR using specific primers for mitochondrial- (cytochrome b) and nuclear- (actin) encoded genes in a SYBR Green PCR Master Mix. The CT values from triplicate reactions were averaged and mtDNA copy number calculated following the manufacturer's protocol (Mouse Mitochondrial DNA Copy Number Assay Kit Detroit R&D, Detroit, USA).

mRNA. Total RNA was extracted from both isolated glomeruli and podocytes using the TRIZOL reagent (Thermo Fisher Scientific, Milan, Italy). One μg of total RNA was reverse transcribed into cDNA using the high capacity reverse transcription kit from Thermo Fisher Scientific (Milan, Italy). mRNA expression was analysed by real-time PCR using pre-developed TaqMan reagents: M-Sec (Mm00447578, Hs00969305), CCL2 (Mm00441242), TNF-α (Mm00443258), nephrin (Mm00497828), TFAM (Mm00447485), COX5A (Mm00432638) NDUFB9 (Mm00612543) ATP5G3 (Mm00558162), COX1 (Mm04225243), ND4L (Mm04225306), PINK1 (Mm00550827), PARK2 (Mm01323528), OPA1 (Mm00453879), MFN1 (Mm00612599), DNM1 (Mm01342903), PPARGC1(Mm01208835) (Thermo Fisher Scientific). Fluorescence for each cycle was analysed quantitatively and gene expression normalized relative to the expression of either hypoxanthine-guanine phosphoribosyltransferase (HPRT) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Protein Analysis.

Immunohistochemistry. Immunohistochemistry was performed on 4-μm paraffin sections of formalin-fixed tissue. After antigen retrieval in the citrate buffer, sections were exposed to 3% H_2O_2 to neutralize endogenous peroxidase activity and blocked with avidin-biotin and bovine serum albumin (BSA). Kidney sections were then incubated overnight at 4°C with primary antibodies directed against M-Sec (Catalog No. sc-30138/sc-48418, Santa Cruz, Glostrup, Denmark), podocin (Catalog No. P-0372), MAC-2 (Catalog No. CL8942AP, Cederlane, Biozol), p57 (Catalog No. sc-8298, Santa Cruz, Glostrup, Denmark) and CD44 (Catalog No. 550538, BD, Milan, Italy). After washing in PBS, sections were exposed to secondary biotinylated-labelled antibodies (Catalog No. 112-065-003, Jackson ImmunoResearch Laboratories, West Grove, USA; Catalog No. E0353, E0413, DAKO, Glostrup, Denmark) for 1 h, followed by incubation with streptavidin-HRP (Catalog No. P0397, DAKO, Glostrup, Denmark) for 1h. Diaminobenzidine was used as a chromogen substrate for HRP. Sections were visualized with an Olympus-BX4I microscope and digitized with a high-resolution camera (Carl Zeiss, Oberkochen, Germany). On average 30 randomly selected glomeruli from both outer and inner cortex were assessed per mouse. Results are expressed either as the number of positive cells/glomerular area or as percentage area of positive staining per glomerulus. Two investigators performed evaluations in a blinded fashion.

Immunofluorescence.

Renal Cortex. Sections were fixed in cold acetone for 5 min and blocked in 3% BSA. Subsequently, sections were incubated for 1 hour with primary antibodies: rabbit anti-podocin (Catalog No. P-0372), anti-synaptopodin (Catalog No. BS-3633R, Bioss, Massachusetts, USA), anti-Ki-67 (Catalog No. ab9260) and mouse anti-desmin (Catalog No. N1526, Dako, Glostrup, Denmark). Following washing, FITC-conjugated goat-anti mouse (Catalog No. 62-6511, Thermo Fisher Scientific, Milan, Italy) and swine-anti rabbit (Catalog No. F0205, DAKO, Glostrup, Denmark) secondary antibodies were added. A FITC-conjugated anti-mouse complement-3 antibody (Catalog No. ICN55510, Thermo Fisher Scientific, Milan, Italy) was used in direct immunofluorescence. Sections were examined using a Zeiss APOTOME 2 system equipped with a high-resolution camera (Carl Zeiss,

Oberkochen, Germany) and quantitated using the Axiovision 4.7 image analysis software (Zeiss). Results were expressed as percentage positively stained tissue within the glomerular tuft. On average 30 randomly selected glomeruli from both outer and inner cortex were assessed per mouse.

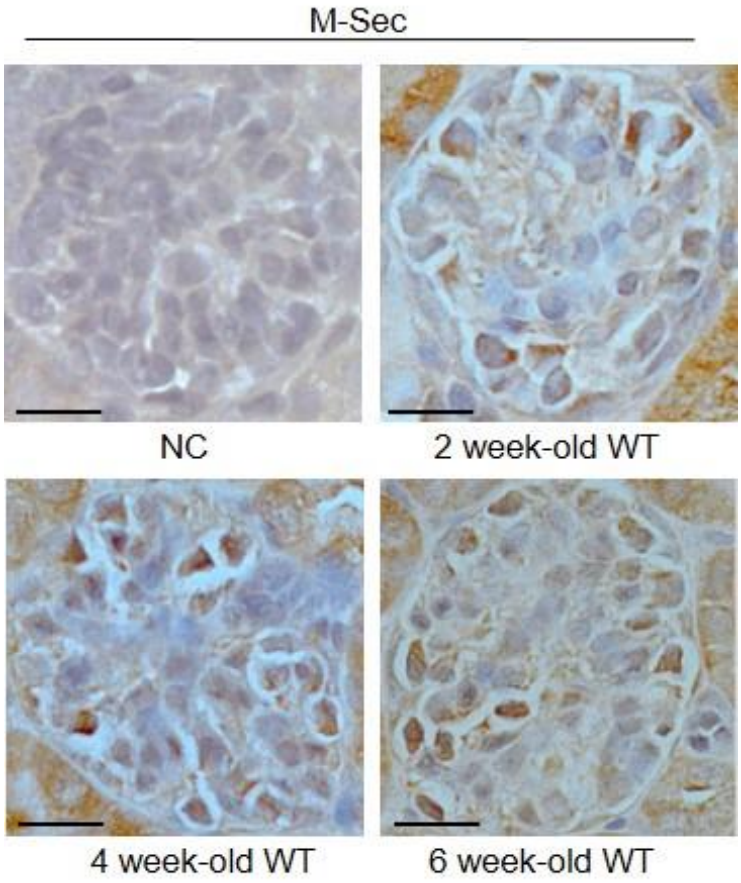
Cultured cells: to prevent disruption of existing TNTs during fixation, 16% w/v paraformaldehyde was added along the sides of the dishes, keeping the overlying culture medium intact, to a final w/v concentration of 4%. F-actin was fluorescently labelled with Alexa Fluor® 488 phalloidin (Catalog No. A12379, Thermo Fisher Scientific).

Double immunofluorescence Staining was performed on 4-μm paraffin sections of formalin-fixed tissue. After antigen retrieval in citrate buffer, sections were blocked with avidin-biotin and BSA and then incubated overnight at 4°C with primary antibodies anti M-Sec (human sections) and anti-CD44 (mouse sections). After washing in PBS, sections were exposed to secondary biotinylated-labelled antibodies (Jackson ImmunoResearch Laboratories, West Grove, USA and DAKO, Glostrup, Denmark) for 1 h, followed by incubation with Alexa 488-conjugated streptavidin (Catalog No. A21370; Thermo Fisher Scientific, Milan, Italy) or streptavidin-RPE (Catalog No. R0438, Dako, Glostrup, Denmark). After further blocking in BSA, sections were incubated with a primary antibody direct against synaptopodin (Catalog No. 61094, Progen, Heidelberg, Germany) or CD68 (Catalog No. M0876, Dako, Glostrup, Denmark) (human sections), Annexin 3A (Catalog No. HPA013398; mouse sections) for 18 h a 4°C, followed by a FITC–conjugated swine anti-rabbit or RPE-conjugated goat anti-rat antibody (Catalog No. 31680, Thermo Fisher Scientific, Milan, Italy). Digitalised images were colour-combined and assembled into photomontages by using Adobe Photoshop (Universal Imaging Corporation, West Chester, PA).

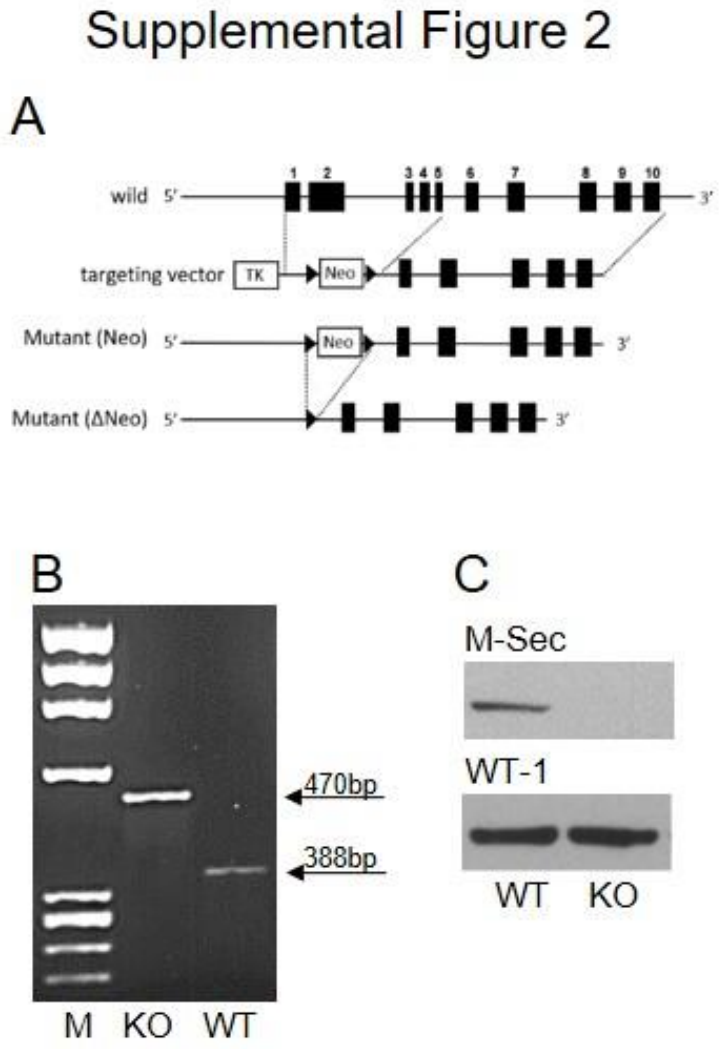
Immunoblotting. Podocytes were homogenised in RIPA buffer containing 0.5% NP40 (vol./vol.), 0.5% sodium deoxycholate (wt/vol), 0.1% SDS (wt/vol), 10 mmol/l EDTA and proteases inhibitors. Protein extracts were obtained by centrifugation at 14,000×g for 20 min at 4°C, preceded by a 45 min incubation period on ice. Total protein concentration was determined using the DC Protein Assay Kit (Bio-Rad, Milan, Italy). Equal amounts of protein samples were separated on SDS

polyacrylamide gel electrophoresis and electrotransferred to nitrocellulose membranes. Following blocking in 5% non-fat-milk in TBS (pH 7.6), membranes were incubated with a primary antibody directed against either M-Sec or nephrin (Catalog No. GP-N2, Progen, Heidelberg, Germany) overnight at 4°C. After washing, secondary HRP-linked antibodies (Catalog No. sc-2903, Santa Cruz, Glostrup, Denmark; Catalog No #7070, Cell Signalling, Leinden, Netherland) was added for 1 hour. Detection was performed using Super signal PICO (Euroclone, Milan, Italy) and visualised on a Gel-Doc system (Bio-Rad, Milan, Italy). Band intensities were quantified by densitometry. Tubulin was used as internal control.

Supplemental Figure 1

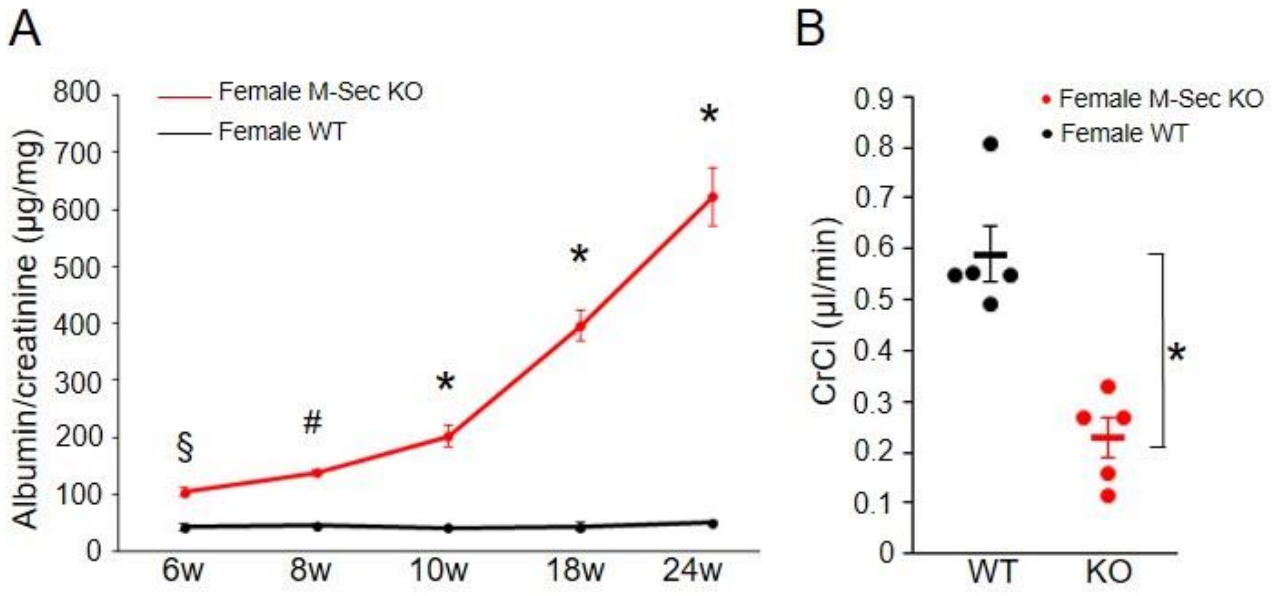


Supplemental Figure 1. M-Sec expression. Representative immunohistochemistry images of glomerular M-Sec staining in M-Sec WT mice at 2-4-6 weeks of age (magnification 400X, bar=50 µm). NC: negative control.

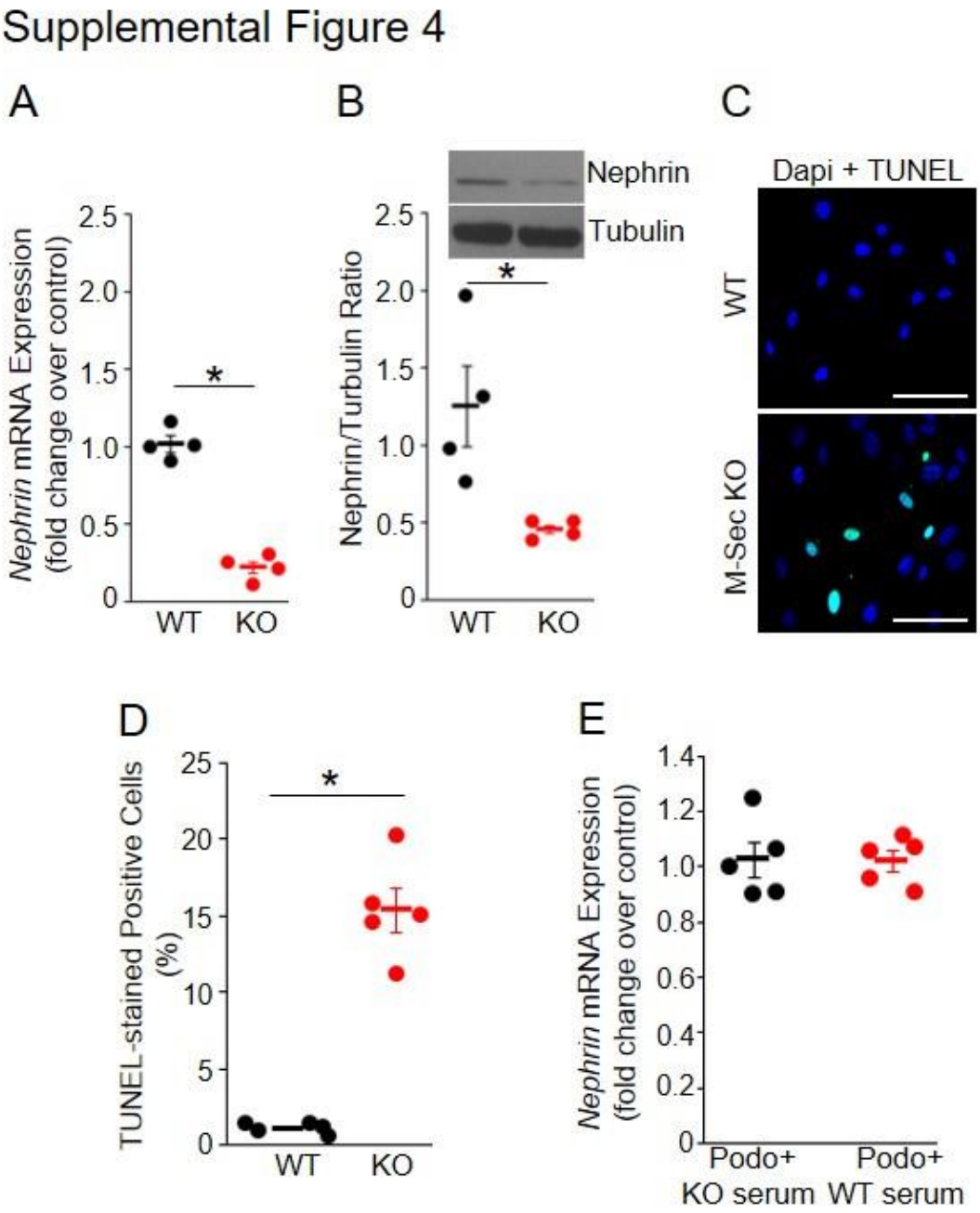


Supplemental Figure 2. Generation of M-Sec KO mice. (A) Schematic illustration of the strategy for generating M-Sec-KO mice by deletion of gene exons 1-5. **(B)** Representative agarose gel showing genotyping results for both M-Sec-KO (470 bp) and WT mice (388 bp) (P7=postnatal day 7). M: molecular weight marker. **(C)** M-Sec protein expression in purified WT podocytes and lack of M-Sec expression in podocytes harvested from four week-old M-Sec KO mice, as detected by Western blotting. WT-1 was used as internal control.

Supplemental Figure 3

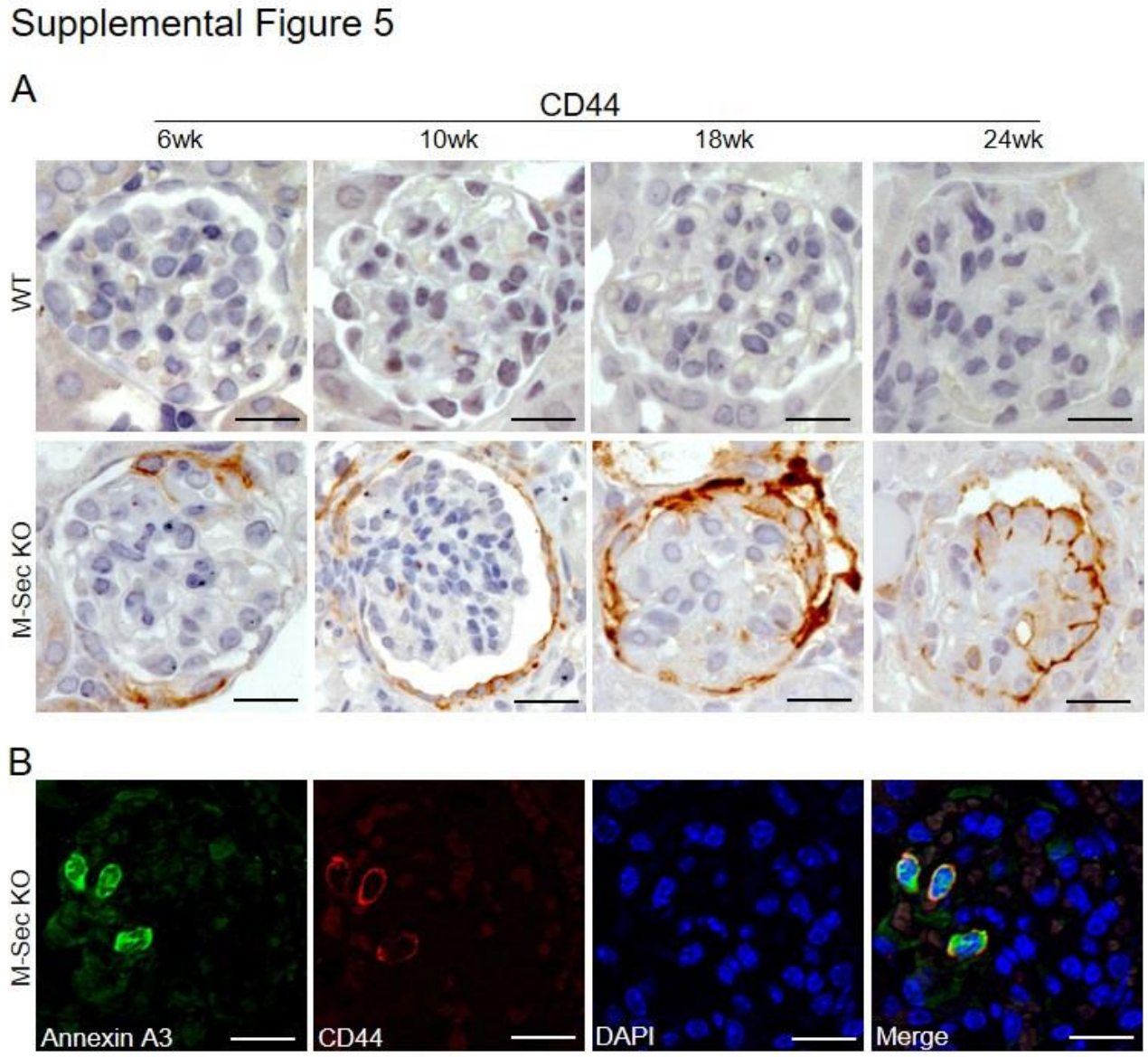


Supplemental Figure 3. Renal function in female M-Sec KO mice. (A) Urinary albumin concentration was measured in 18-hours urine collections by ELISA and normalised to urinary creatinine levels. Results for female both WT and M-Sec KO mice at 6,8,10,18, and 24 weeks of age are shown (n=5 per group; *p<0.001, #p<0.01, §p<0.05 M-Sec KO vs. WT). **(B)** Creatinine clearance in 24 week-old female M-Sec-KO and WT mice (n=5 per group; *p<0.001 M-Sec-KO vs. WT).



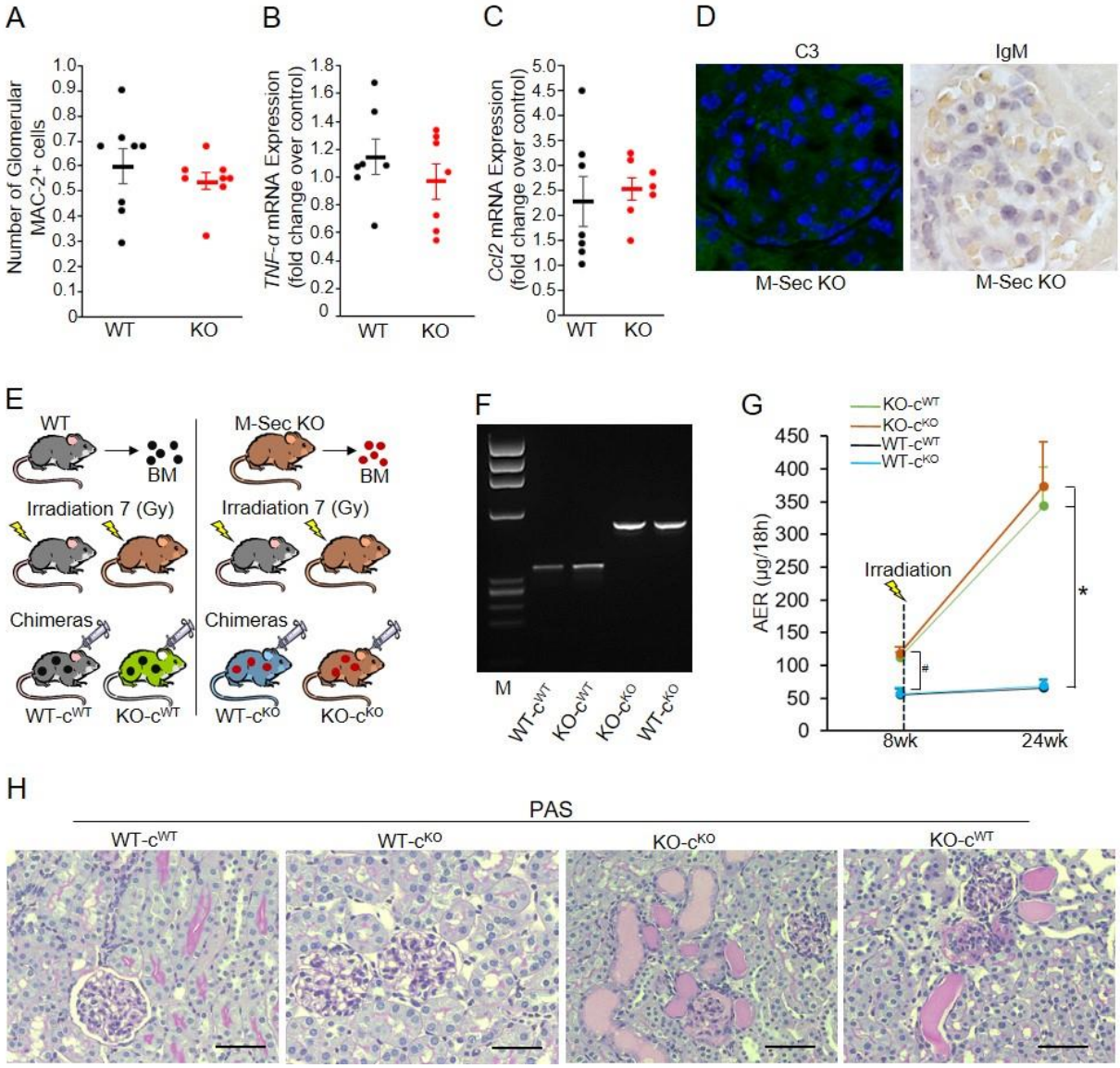
Supplemental Figure 4. Effect of M-Sec deletion on nephrin expression and apoptosis in cultured podocytes. (A,B) Nephrin both mRNA and protein expression was assessed in primary podocytes isolated from WT and M-Sec-KO mice by real time-PCR (GAPDH internal control) and immunoblotting (tubulin loading control), respectively. Results are reported in the graphs and a representative immunoblot is shown (*n*=4; *p<0.001 KO vs. WT). **(C,D)** Apoptosis was assessed in primary podocytes isolated from WT and M-Sec-KO mice by TUNEL assay (green) and nuclei counterstained with DAPI. The percentage of apoptotic cells is shown in the graph (magnification x200, bar=100μm) (*n*=5; *p<0.001 KO vs. WT). **(E)** WT podocytes were exposed to 4% serum

from either WT or M-Sec-KO mice (10-weeks of age) for 24 hours and nephrin mRNA levels measured by real-time PCR (*n*=5; p=ns).



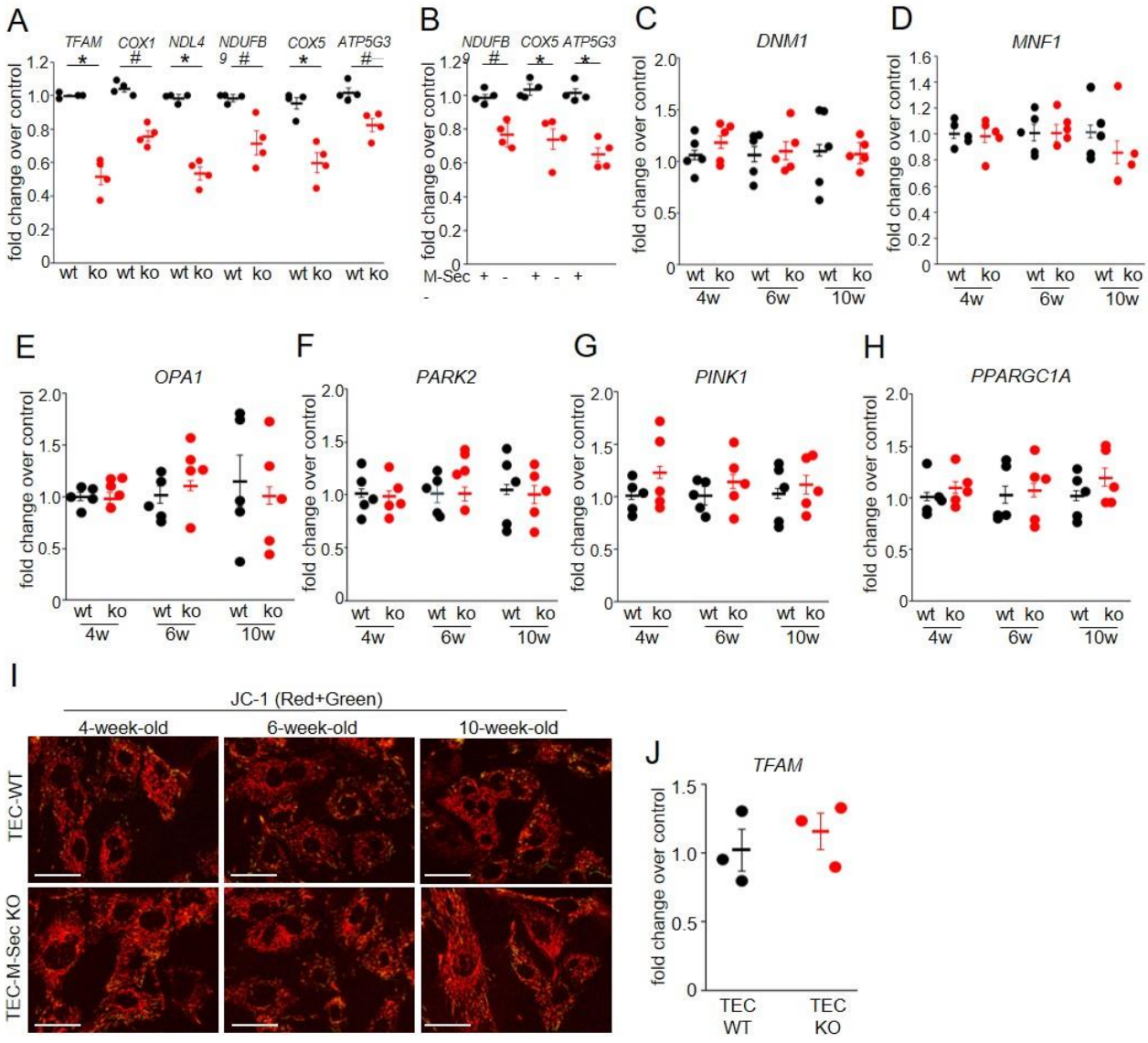
Supplemental Figure 5. Effect of M-Sec deletion on PEC activation. (A) Activated PECs were identified by immunostaining for CD44 in the glomeruli from 6-10-18-24 week-old WT and M-Sec KO mice (*n*=6-7 per group). **(B)** Double immunofluorescence for the PEC marker Annexin A3 and CD44 was performed in 18-week-old M-Sec-KO mice and the merged image shows colocalization (magnification 400X, bar=50µm).

Supplemental Figure 6



Supplemental Figure 6. Markers of inflammation and chimeric animals. (A) Glomerular macrophage accrual was evaluated in both WT and M-Sec-KO mice (*n*=8 per group) at 18 week of age by counting the number of MAC-2 positive cells per glomerulus (p=ns). **(B,C)** TNF-α and CCL2 mRNA levels were measured in isolated glomeruli obtained from both 6 week-old WT and M-Sec-KO mice (*n*=7 per group) by real time-PCR and corrected for the expression of the housekeeping gene HPRT (p=ns). **(D)** There was no glomerular staining for IgM and C3 within the glomerular tuft of M-Sec-KO mice (n=8 mice/per group, 30 glomeruli). **(E)** Schematic illustration of the protocol used for generating chimeric mice. Recipient WT and M-Sec-KO mice were lethally irradiated, and then reconstituted with bone marrow (BM) from either WT (WT-c^{WT}; KO-c^{WT}) or KO (WT-c^{KO}; KO-c^{KO}) mice. **(F)** PCR genotyping of peripheral blood cells collected 15 weeks after BM transplantation. M: marker. **(G)** Albumin excretion rate was measured at baseline (8-weeks of age) and 15 weeks after BM transplantation (24-weeks of age) (*p<0.001; #p<0.05 recipient M-Sec-KO vs. recipient WT*;* *n*=6 per group). **(H)** Representative images of PAS staining of renal cortex sections from transplanted animals (magnification 200X, bar=100µm).

Supplemental Figure 7



Supplemental Figure 7. Mitochondrial abnormalities in isolated glomeruli and glomerular cells lacking M-Sec. (A) Expression of TFAM, mtDNA encoded genes (COX1, ND4L), and nuclear genes encoding for mitochondrial proteins (COX5A, NDUFB9, ATP5G3) in primary podocytes from WT and M-Sec-KO mice (*p<0.001; #p<0.01 KO vs. WT; *n*=4). **(B)** Expression of COX5A, NDUFB9, ATP5G3 in podocytes transfected with either M-Sec shRNA (M-Sec^{+}) or a mock (M-Sec^{-}) plasmid (GAPDH housekeeping gene) (*p<0.001; #p<0.01 M-Sec^{+} vs. M-Sec^{-}; *n*=4). **(C-H)** mRNA expression of key regulators of mitochondrial fusion (OPA-1, MNF1), fission (DNM1), biogenesis (PGC-1a), and mitophagy (PARK2, PINK1) were assessed in the glomeruli isolated from both WT and M-Sec KO mice at 4-6-10 weeks of age (*n*=5 per group; p=ns). **(I)** Mitochondrial membrane potential (MMP) was assessed using the fluorescent probe JC-1 in TECs from both WT and M-Sec-KO mice (4-6-10 weeks of age). **(J)** mRNA expression of TFAM in primary podocytes from WT and M-Sec-KO mice. (GAPDH housekeeping gene) (*n*=3; p=ns).