SUPPLEMENTARY DATA

METHODS

Extraction and quantification of endocannabinoids. Frozen kidney tissue samples from diabetic (n=3) and non-diabetic (n=5) mice were homogenized in chloroform/methanol/Tris-HCl 50 mM pH 7.4 (2:1:1, v/v) containing 10 pmol of [2H]8-AEA, [2H]4-PEA and [2H]4-OEA, and 50 pmol of [2H]5-2-AG as internal deuterated standards (Cayman Chemicals, Ann Arbor, MI). The extract was purified by silica gel mini-columns and the eluted fraction analysed by liquid chromatography-atmospheric pressure-mass spectrometry (LC-APCI-MS). Analyses were carried out in the selected ion-monitoring mode using m/z values of 356 and 348 (molecular ions +1 for deuterated and undeuterated AEA), 304 and 300 (molecular ions +1 for deuterated and undeuterated PEA), 330 and 326 (molecular ions +1 for deuterated and undeuterated OEA), and 384.35 and 379.35 (molecular ions +1 for deuterated and undeuterated 2-AG). AEA, OEA, PEA and 2-AG concentrations were calculated by isotope dilution and expressed as pmol per milligrams (2-AG, PEA, OEA) or grams (AEA) of wet tissue weight. The concentrations of 2-AG were obtained by adding up to the amounts of the 2-isomer also those of the 1(3)-isomer, which mostly originates from the isomerization of the former during work-up.

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.

Glomerular Volume. Glomerular cross-sectional area (A_G) was measured in 20 glomerular profiles per mouse by using a computerized image analysis system (Axiovision 4.7, Zeiss). The glomerular volume (V_G) was then calculated as: $V_G=\beta/K[A_G\ I^{3/2}$, where $\beta=1.38$ is the size distributor coefficient and K=1.01 is the shape coefficient for glomeruli idealized as a sphere.

Immunofluorescence. Sections were fixed in cold acetone for 5 min and blocked in 3% BSA. Subsequently, sections were incubated for an hour with guinea pig anti-nephrin (Progen Biotechnik, Maaßstraße, Germany), rabbit anti-ZO-1 (Zymed Laboratories, San Francisco, CA), anti-synaptopodin (Synaptic System, Gottingen, Germany), or anti-fibronectin primary antibodies. Following washing, FITC-conjugated donkey anti-guinea pig (Santa Cruz, Glostrup, Denmark) and swine-anti rabbit (DAKO, Glostrup, Denmark) secondary antibodies were added. Sections were examined using an Olympus epifluorescence microscope (Olympus Bx4I), digitised with a high resolution camera (Carl Zeiss, Oberkochen, Germany) and quantitated using an image analysis software (Axiovision 4.7, Zeiss). Results were calculated as percentage positively stained tissue within the glomerular tuft. On average 20 randomly selected hilar glomerular tuft cross-sections were assessed per mouse.

Immunoblotting. Renal cortex and 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). Proteins were separated by SDS-PAGE and electrotransferred to

nitrocellulose membrane. Following blocking in 5% non-fat milk in TBS (pH 7.6), membranes were incubated with primary antibodies against nephrin, ZO-1, DAGL (Cayman, Ann Arbor, MI), MGL (Cayman, Ann Arbor, MI), CB1 (Cayman, Ann Arbor, MI), CB2, CCR2 (Epitomics, Burlingame, CA), phospho-ERK (Euroclone, Milan, Italy), phospho-Akt (Santa Cruz, Glostrup, Denmark), phospho-p38 (Cell Signalling, Milan, Italy) or TGF-β1 (R&D System, Milan, Italy) overnight at 4°C. After washing, a secondary HRP-linked (Santa Cruz, Glostrup, Denmark) antibody was added for 1 hour. Detection was performed using either the ECL chemiluminescence substrate (Amersham) or Super signal PICO (Euroclone, Milan, Italy) and visualised on a Gel-Doc system (Bio-Rad, Milan, Italy). After detection, membranes were stripped and reprobed for both total Akt, ERK, and p38. Band intensities were quantified by densitometry. Tubulin was used as internal control.

mRNA Analysis. Total RNA was extracted from the renal cortex and isolated glomeruli using the TRIZOL reagent (Invitrogen, Milan, Italy). One μg of total RNA was reverse transcribed into cDNA using the high capacity reverse transcription kit from Applied Biosystems (Monza, Italy). Nephrin, ZO-1, TGF-β1, fibronectin, collagen type I, MCP-1, CCR2, GR1+ mRNA expression was analysed by TaqMan real-time PCR using pre-developed TaqMan reagents (Applied Biosystems, Monza, Italy; catalogue number nephrin: Mm00497828; ZO-1: Mm01320637; TGF-β1: Mm00441724; fibronectin: Mm01256744; collagen I: Mm00483937; MCP-1: Mm00441242; CCR2: Mm00438270; $GR1^+$: Mm00841873). Fluorescence for each cycle was analysed quantitatively and gene expression normalized relative to the expression of hypoxanthine-guanine phosphoribosyltransferase (HPRT). Because housekeeping genes ubiquitously expressed in the renal cortex do not control for variations in the glomerular number per specimen or changes in podocyte number, WT-1, a podocyte specific gene, was used as endogenous reference for the evaluation of nephrin and ZO-1 expression.

Total RNA was extracted from cultured podocytes using RNeasy Mini spin columns, freed from contaminant DNA by treatment with DNase I, and reverse transcribed. Traditional PCR was performed with primers; specifically designed to amplify murine CB2 (forward: 5'TCATTGCCATCCTCTTTTCC3'; reverse: 5'GAACCAGCATATGAGCAGCA3') and CB1 (forward: 5'CTGGTTCTGATCCTGGTGGT3'; reverse: 5'TGTCTCAGGTCCTTGCTCCT3'). After an initial denaturation at 94°C for 9 minutes, the cDNA was amplified for 32 cycles with the following setting: denaturation at 95°C for 45 seconds, annealing at 64°C for 1 minute, and elongation at 72°C for 1 minute with a final elongation at 72°C for 10 minutes. PCR products were resolved in a 2% agarose gel containing ethidium bromide, and digital images of the gels were captured using the Gel Doc XR system (Bio-Rad, Milan, Italy).