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Supplementary materials and methods

In vivo studies. Wild-type mice on a mixed genetic background (C57BL/6 x 129sv) were injected with streptozotocin (STZ) for 3 consecutive days (100 mg/kg/day). Three weeks after the onset of hyperglycemia, urine was collected to mimic diabetic conditions of Irs2−/− mice. Anesthesia and perioperative analgesia were used to maintain animal well-being throughout the experiments. All animal husbandry was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Experimental procedures were performed with the appropriate governmental and institutional ethical and legal approval.

Supplementary Figure Legends

Supplementary Figure 1. Generation and characterization of immortalized podocytes from wild-type and Irs2−/− mice. Conditionally immortalized murine podocyte cell lines were generated from the kidneys of wild-type (WT) and Irs2−/− mice by transfection with the temperature-sensitive (ts) SV40-LTag gene (mPodWT and mPodIRS2KO, respectively). A) Phase contrast photographs of cells growing at 33ºC (upper panels) and cells cultured for 14 days in a non-permissive temperature (37ºC) (lower panels). B) (Left panel) PCR analysis of genomic DNA from mouse podocytes showing a 530 pb band corresponding to wild-type (mPodWT) cells and a 660 pb band corresponding to Irs2−/− (mPodIRS2KO) cells. (Right panel) PCR analysis of mouse tail DNA as a PCR control. C) Western blot analysis from total protein extracts and quantification of mPodWT and mPodIRS2KO podocytes 33ºC and 37ºC. Representative images of p27 and β actin are shown.

Supplementary Figure 2. Podocytes from WT and Irs2−/− mice were serum-starved and stimulated with insulin for 15 minutes. Quantification of A) phospho tyrosine IR levels and B) phospho tyrosine IRS1 levels. Mean ± SD of 3 independent experiments. *p < 0.05 vs. respective non-stimulated controls. C) Podocyte cell lines (mpodWT, mPodIRS2KO, mPodIRS2KO/PTP1BKO) were stimulated with insulin and phospho AKT473 levels were quantified. Mean ± SD of 3 different experiments. *p<0.05. insulin-stimulated mPodWT vs control D)
PTEN protein expression was quantified in the different cell lines stimulated with insulin (mPodWT, mPodIRS2KO, mPodIRS2KO + IRS2 adenovirus for 36 h). Mean ± SD of 3 different experiments. Note mPodIRS2KO maintain PTEN levels even after IRS2 reconstitution.

Supplementary Figure 3. Diabetic Irs2⁻/⁻ mice present albuminuria A) Representative image of an SDS-PAGE gel of urine from 12-week-old wild-type (Irs2⁺/⁺) and Irs2⁻/⁻ mice. Note that band at 66 kDa corresponding to albumin was detected only in Irs2⁻/⁻ mice (2 μl urine were loaded for each lane). B) Quantification of urine albumin/creatinine ratio in wild-type and Irs2⁻/⁻ mice at 5 and 12 weeks. C) Quantification of urine albumin/creatinine ratio in wild-type and Irs2⁻/⁻ mice (12 weeks) and in wild-type mice injected with citrate or STZ D) Representative image of an SDS-PAGE gel of urine from wild-type mice injected with citrate or STZ and from 12-week-old wild-type (Irs2⁺/⁺) and Irs2⁻/⁻ mice with comparable hyperglycaemia during a similar period of time. Each symbol indicates a different animal. At least 6 different mice were analyzed per group.