Revised Materials and Methods

Western blotting

For the nephrin immunoprecipitation assay, cells were extracted in ice-cold NP40 (Nonidet P40) extraction buffer (50 mM Tris/HCl, pH 7.5, containing 1 mM EDTA, 120 mM NaCl, 50 mM NaF, 1 mM benzamidine, 1% NP40, 1 mM microcystin, 7.2 mM 2-mercaptoethanol, 5 mM orthovanadate and 1 mg/ml each of pepstatin, leupeptin and antipain). Cell extracts were centrifuged at 10000*g* for 10 min at 4 °C prior to Nephrin being immunoprecipitated with mouse anti-nephrin antibody and Protein G –Sepharose at 4oC. Subsequently the beads were isolated by centrifugation and washed four times with NP40 extraction buffer. The bound proteins were separated by SDS PAGE followed by electrophoretic transfer to Immobilion-P membrane (Millipore, Watford, U.K.). The membranes were blocked in 10% (w/v) BSA dissolved in TBS-T (Tris-buffered saline with 0.1% Tween 20; 20 mM Tris/HCl, 137 mM NaCl and 0.1% Tween 20) and subsequently incubated with anti nephrin antibody followed by (HRP)-conjugated donkey anti-rabbit IgG. The signal was detected by enhanced chemiluminescence (Amersham Biosciences).