Supplemental Fig. S1. Streptozotocin causes kidney hypertrophy. Weights of Sprague-Dawley rats were determined prior to injection of 65 mg/ml streptozotocin as described in the Methods in the text. Four days after streptozotocin administration, the kidneys were harvested. Hypertrophy is expressed as ratio of both kidney weights and pre-streptozotocin body weight. Control, n = 6; Streptozotocin, n = 4. *p < 0.01 vs control by ANOVA.
**Supplemental Fig. S2.** 24 hours serum-deprived mesangial cells were incubated with 5 mM glucose plus 20 mM mannitol (LG) or 25 mM glucose (HG) for 48 hours. Total RNAs were isolated. 2 µg of RNA was used in RT-PCR with a PTEN-specific primers (Forward-CAGAGGCCTATGTATATT; Reverse-TCCTGGTATGAAGAACGTAT). The glyceraldehyde 3-phosphate dehydrogenase primers (Forward-ACCACAGTCCATGCCATCA; Reverse-TCCACCAACCTGTTGCTGTA) was used in the same reaction for internal control. Bottom panel shows ratio of PTEN and GAPDH signals. *p < 0.05 vs. low glucose by ANOVA; n = 4.
Supplemental Fig. S3. Serum-deprived mesangial cells were incubated with 5 mM glucose plus 20 mM mannitol (LG) or 25 mM glucose for 48 hours. (A) Cells were trypsinized and counted using a hemocytometer. Total protein content in the lysed cells was measured. Hypertrophy was expressed as the ratio of total protein to number of cells. (B) During the last 2 hours of incubation with glucose, the cells were labeled with 1 µCi/ml 35S-methionine. Protein synthesis was measured as described in the Methods. Mean of triplicate measurements are shown. *p < 0.05 vs. control by ANOVA.
**Supplemental Fig. S4.** Expression of PTEN inhibits high glucose-induced protein synthesis without any effect on DNA synthesis. Serum-deprived mesangial cells were infected with Ad GFP or Ad PTEN (50 moi) for 24 hours followed by incubation with with 5 mM glucose plus 20 mM mannitol (LG) or 25 mM glucose (HG) for 48 hours. The cells were then incubated with $^3$H-Thymidine and $^{35}$S-Methionine and the incorporation into DNA and protein was determined as described in the Methods in the text. Means of triplicate measurements are shown. p < 0.01 by ANOVA.
Supplemental Fig. S5. Expression of dominant negative PTEN increases proteins synthesis without any significant effect on DNA synthesis. Quiescent mesangial cells were infected with GFP or Ad PTEN C/S (50 moi) for 72 hours. In the case of high glucose, the cells were infected with Ad GFP for 24 hours before incubation with 25 mM glucose for 48 hours (HG). The cells were then incubated with \(^{3}\text{H}\)-Thymidine and \(^{35}\text{S}\)-methionine and the incorporation into DNA and protein was determined as described in the Methods in the text. Means of triplicate measurements are shown. *p < 0.05 by ANOVA.
Supplemental Fig. S6. Expression of PTEN inhibits TGFβ-induced protein synthesis without any significant effect on DNA synthesis. Mesangial cells were incubated with Ad GFP or Ad PTEN (moi 50) for 24 hours before incubation with 2 ng/ml TGFβ for 48 hours. The cells were then incubated with $^3$H-Thymidine and $^{35}$S-methionine and the incorporation into DNA and protein was determined as described in the Methods in the text. Means of triplicate measurements are shown. *p < 0.01 and **p < 0.001 by ANOVA.