Supplementary Materials and Methods

Isolation of cytosolic fractions

Kidney tissues were homogenized with a Dounce homogenizer in sucrose buffer (0.32 M sucrose and 10 mM Tris-Cl, pH 7.4) and the supernatants of each fraction were used to measure the enzymatic activities and other experimental parameters. Cultured cells were collected at 10,000 x *g* for 10 min at 4°C and were washed once with cold 1x PBS. Briefly, cells were homogenized with a Dounce homogenizer in sucrose buffer. Cell homogenates were centrifuged at 1,000 x *g* for 5 min, and the supernatants were further centrifuged at 15,000 x *g* for 30 min. The resulting supernatants were used as the cytosolic fractions to measure the activities of several cytosolic enzymes.

Enzyme assay

IDPc activity was determined from the production of NADPH, which was measured at 340 nm (Loverde and Lehrer, 1973). One unit of IDPc activity was defined as the amount of enzyme that catalyzed the production of 1 μmol of NADPH/min. Catalase activity was measured from the decomposition of hydrogen peroxide, which was determined by the decrease in absorbance at 240 nm (Beers and Sizer, 1952). Superoxide dismutase (SOD) activity was assayed spectrophotometrically using the pyrogallol assay (Marklund and Marklund, 1974), where one unit of activity was defined as the quantity of enzyme that reduces the superoxide-dependent color change by 50%. Glutathione reductase activity was quantified by the GSSG-dependent loss of NADPH (Pinto and Bartley, 1969) measured at 340 nm ($\varepsilon = 6.67\ mM^{-1}cm^{-1}$).

Measurement of intracellular ROS

Hydrogen peroxide oxidizes ferrous (Fe^{2+}) to ferric ions (Fe^{3+}) selectively in dilute acid and the resulting ferric ions can be detected using a ferric sensitive dye, such as xylenol orange, as an indirect measure of intracellular hydrogen peroxide concentration. FOX solution (0.1 mM xylenol orange, 2.5 mM ammonium ferrous sulfate, 100 mM sorbitol and 25 mM H_2SO_4) was added to cell extracts. After incubation at room temperature for 30 min, the absorbance was measured at 560 nm. Hydrogen peroxide was used to construct a standard curve as described (Jo et al., 2002). To visualize intracellular peroxide production, the oxidant-sensitive fluorescent probe DCFHDA (2',7'-dichlorofluoroscin diacetate, Molecular Probes, Eugene, OR, U.S,A.) was used. Cells (10^5 per well) were plated into 4-well plates containing glass slides coated with poly-L-lysine and incubated with 5.6 mM or 25 mM D-glucose for 24 h. Then 50 μM DCFHDA was added to the wells and incubated for 20 min at 37℃. The cells were washed twice with 1x PBS and DCF fluorescence (excitation, 488 nm; emission, 520 nm) was detected by fluorescence microscopy (Axio Imager Z1 HBO 100, Zeiss, Oberkochen, Germany) as described (Jo et al., 2002).

Lipid peroxidation assay

Thiobarbituric acid-reactive substance (TBARS) levels were determined as a measurement of lipid peroxidation. Cell extracts (500 μl) were mixed with 1 ml of thiobarbituric acid-trichloroacetic acid-HCl solution (0.375% thiobarbituric acid, 0.5% trichloroacetic acid in 0.25 N HCl, pH 2.0) and heated at 100°C for 15 min. After cooling at room temperature, the precipitate was removed by centrifugation at 1,000 x g for 10 min. Samples were evaluated for malondialdehyde (MDA) production using a Lipid Peroxidation Assay kit (Calbiochem, La Jolla, CA, U.S.A.).

Protein oxidation assay

Production of protein carbonyl groups in cells was visualized by an immunofluorescence assay as described (Pompella et al., 1996). Briefly, cells were cultured on 0.1% poly-lysine-coated glass slides. After washing with 1x PBS, cells were fixed with methanol:acetone (1:1, v/v) for 15 min. Cells on the glass slide were incubated with DNPH and then blocked with 1% fetal calf serum for 1 h. Reacted cells were hybridized with anti-DNP antibodies (Pompella et al., 1996).

Proline incorporation

Proline incorporation was measured as described by Ziyadeh et al. (Ziyadeh et al., 1994). Cells (10^5 per well) were plated into 24-well cell culture plates with MEM containing 5.6 mM glucose and 10% FBS. At confluence, they were incubated for 48 h in fresh media with 5.6 mM glucose in the absence of serum. Afterward, the medium was changed to serum-free media containing either 5.6 mM or 25 mM glucose. After an additional 12 h of culture, 1 μCi of ^{3}[H]-proline (L-(2,3,4,5)-^{3}[H]proline, 85.0 Ci/mmol; PerkinElmer Life Sciences Inc., Wellesley, MA, U.S.A.) was added to each well, and then the cells were incubated for 24 h, washed twice in ice-cold 10% TCA, redissolved in 500 μl of 0.5 N NaOH with 0.1% TritonX-100 and mixed with 2 ml of universal LSC-cocktail (PerkinElmer Life Sciences Inc.). β emission was measured in a PACKARD TRI-CARB 1600-TR liquid scintillation counter (Meriden, CT, U.S.A.). Additional cells plated in parallel were scraped off the plate with a policeman and then counted in a hemocytometer after trypsinization. Proline incorporation was expressed as counts per minute (cpm)/10^6 cells.

Cellular NADPH and GSH levels

Cellular NADPH and GSH levels were measured as previously described (Jo et al., 2002). Briefly, NADPH was assayed spectrophotometrically using the enzymatic cycling method.

$NADP^+$ and NADPH were assayed based on measurement of the reduced coenzyme at 340 nm. Total glutathione was determined in 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.2 mg of NADPH, 30 μg of 5,5'-dithiobis(2-nitrobenzoic acid) and 0.12 unit of glutathione reductase at 412 nm. The GSSG level was estimated by the DTNB-GSSG reductase recycling assay after treatment of 1 μl of 2-vinylpyridine and 3 μl of triethanolamine for 1 h to eliminate the GSH molecules.

References for Supplementary Materials and Methods

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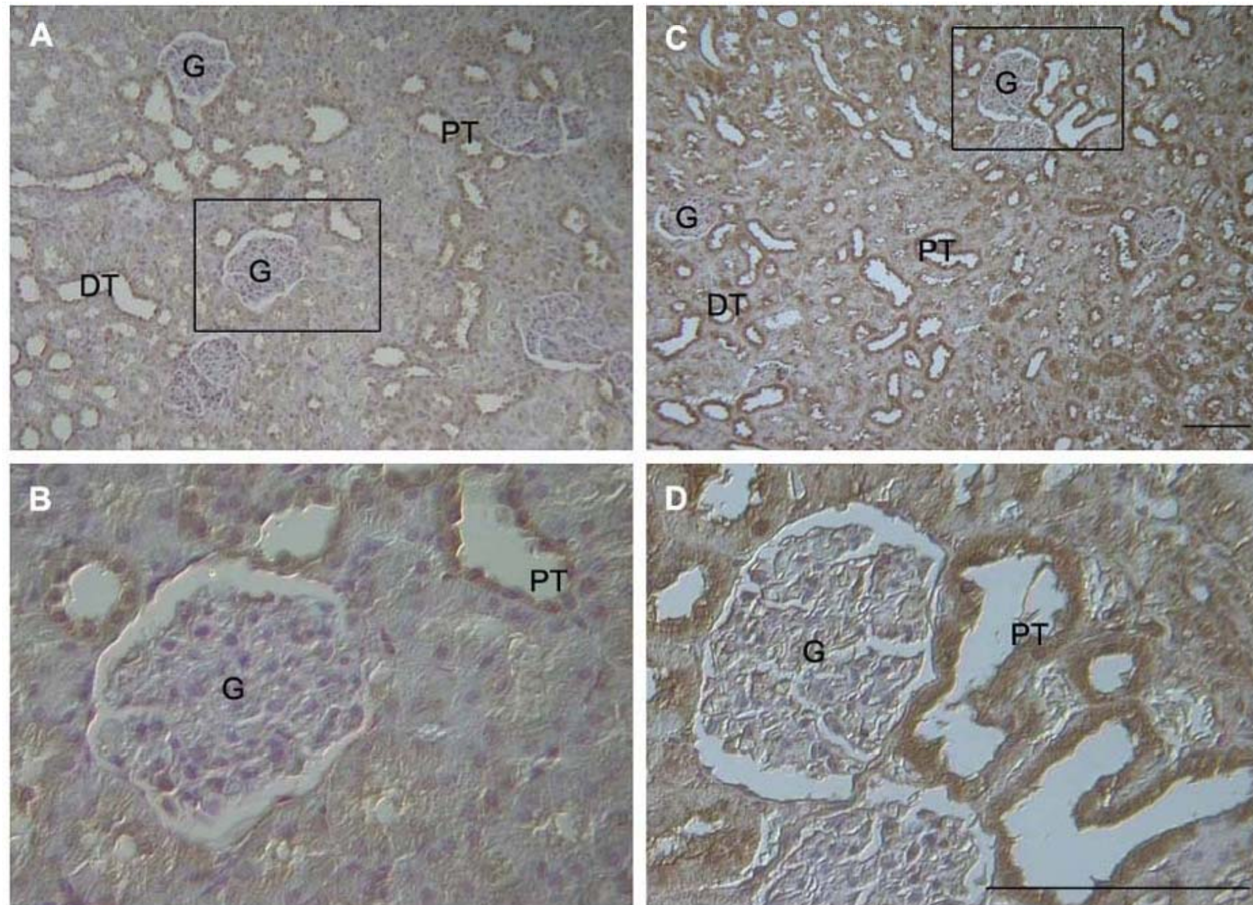
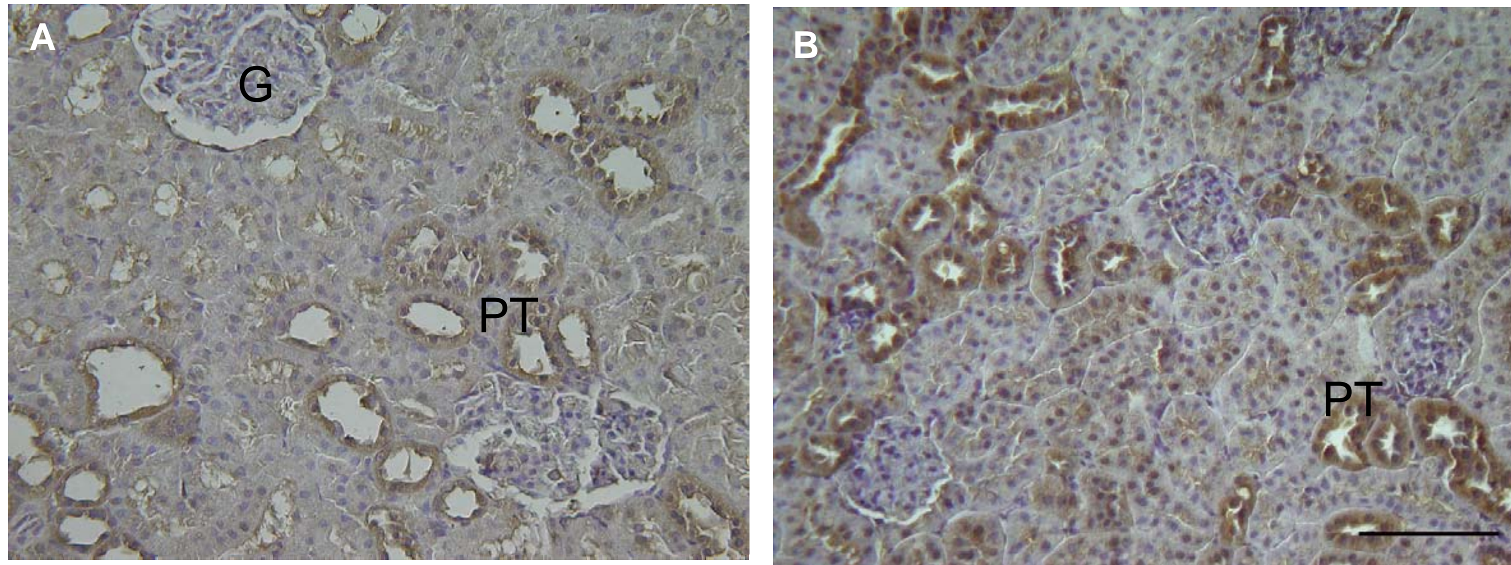
Lee et al., Supplementary Fig. 1



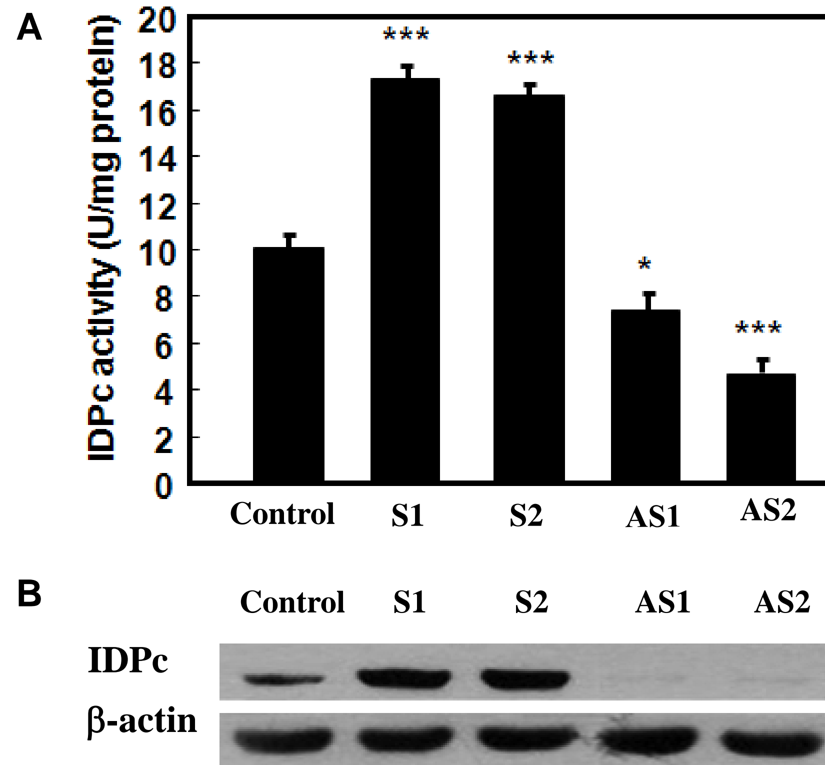
Supplementary Fig. 1. Induction of IDPc protein expression in STZ-induced diabetic rat kidneys.
(A-D) Immunohistochemical detection of the IDPc protein in the cortex of rat kidneys. (A) Control rat kidney and (B) enlarged image of inset from panel A; (C) rat kidney with diabetic nephropathy and (D) enlarged image of inset from panel C. Sections were 10 µm thick. Scale bar = 100 µm. G, glomerulus; PT, proximal convoluted tubule; DT, distal convoluted tubule.

Lee et al., Supplementary Fig. 2



Supplementary Fig. 2. Induction of IDPc protein expression in kidneys of type 2 diabetic mice.
(A, B) Immunohistochemical detection of the IDPc protein in the cortex of mice kidneys. (A) Kidney of a non-diabetic C57BL/6J mice. (B) Kidney of a type 2 diabetic Lepr^{db} (*db/db*) mice. Sections were 10 µm thick. Scale bar − 100 µm. G, glomerulus; PT, proximal convoluted tubule.

Lee et al., Supplementary Fig. 3



Supplementary Fig. 3. Enzyme activity and protein expression levels of IDPc in transfected OK cells.
(A) IDPc enzyme activity in cells transfected with LNCX-IDPc sense gene (S1 and S2) and LNCX-antisense IDPc gene (AS1, AS2). *** $p < 0.001$, * $p < 0.05$ compared to control cells transfected with the the LNCX vector alone.
(B) Immunoblot analysis of IDPc protein expression in cells transfected with the LNCX-IDPc sense gene (S1 and S2) and LNCX-antisense IDPc gene (AS1, AS2).

Supplementary Table 1. Characteristics of the STZ-induced diabetic rats.

Group	blood glucose (mg/dl)		body weight (g)		Kidney mass (g)	Kidney mass/ 100 g body weight
	Initial	Final	Initial	Final		
Control	80.4 ± 2.4	81.4 ± 1.0	211.8 ± 1.7	461.1 ± 9.0	2.92 ± 0.15	0.63 ± 0.03
STZ	81.2 ± 2.3	384.3 ± 24.3	220.3 ± 4.3	286.1 ± 15.3	3.46 ± 0.13	1.21 ± 0.09

Each value was measured in eight rats and is expressed as the mean ± S.E.

Supplementary Table 2. Antioxidative enzyme activities in IDPc transfected OK cells.

	glucose (mM)	Control	S1	AS2
SOD[1]	NG (5.6 mM)	47.0 ± 3.1	50.9 ± 2.4	46.5 ± 0.9
	HG (25 mM)	41.5 ± 3.7	47.2 ± 1.5	44.2 ± 1.9
CAT[1]	NG (5.6 mM)	9.6 ± 1.0	11.0 ± 0.5	11.4 ± 0.2
	HG (25 mM)	9.4 ± 0.3	10.2 ± 0.6	10.3 ± 0.1
GRd[2]	NG (5.6 mM)	21.7 ± 0.0	19.5 ± 0.1	21.7 ± 0.2
	HG (25 mM)	19.0 ± 0.1	17.9 ± 0.1	19.5 ± 0.1

Enzyme activities were measured from total cell lysates and each value represents the mean ± S.E. of three independent experiments.

[1]Enzyme activity represents Unit/mg protein.
[2]Enzyme activity represents Unit/g protein.
SOD, superoxide dismutase; CAT, catalase; GRd, glutathione reductase.