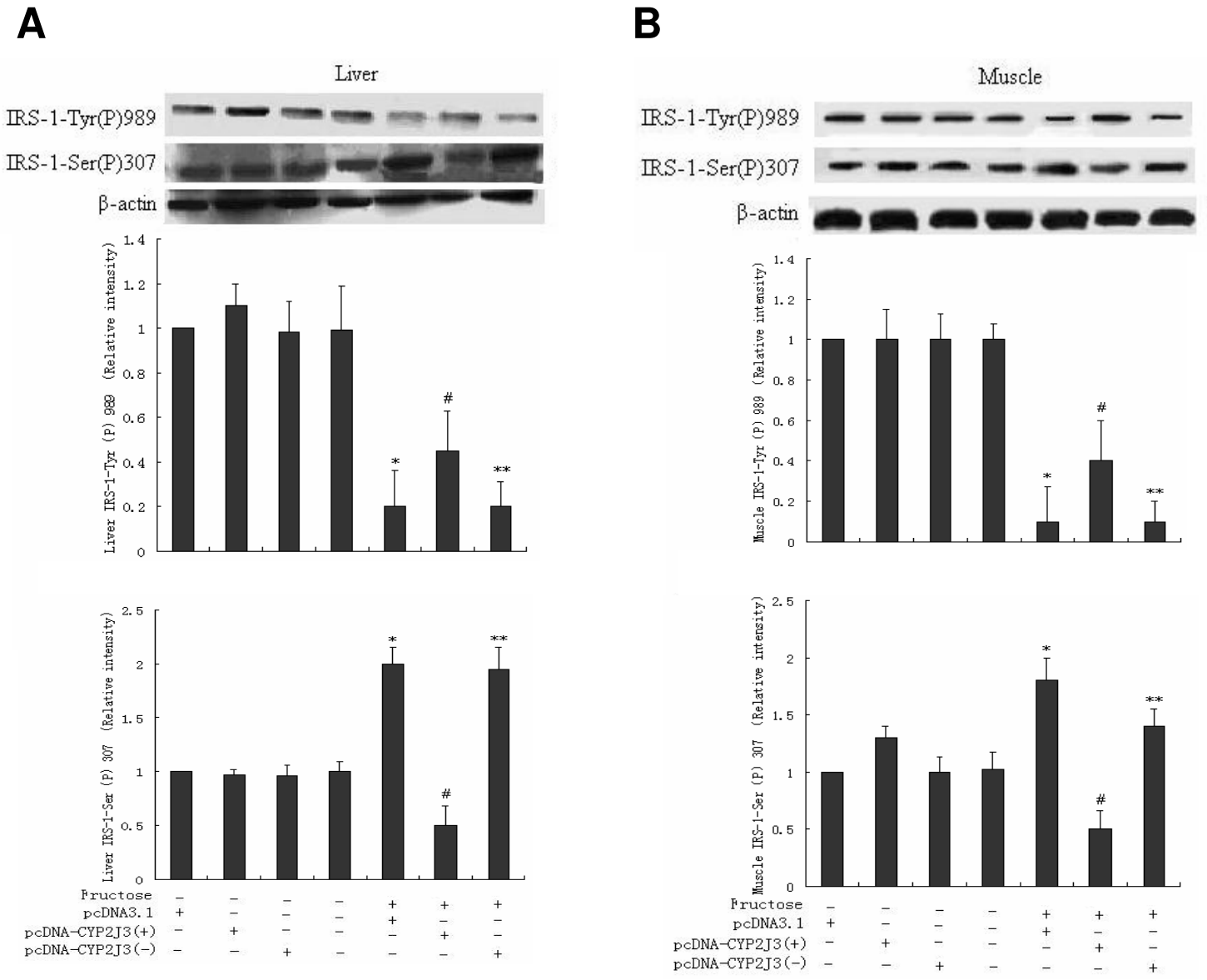
Supplementary information for Xu et al.

EXPANDED MATERIALS AND METHODS

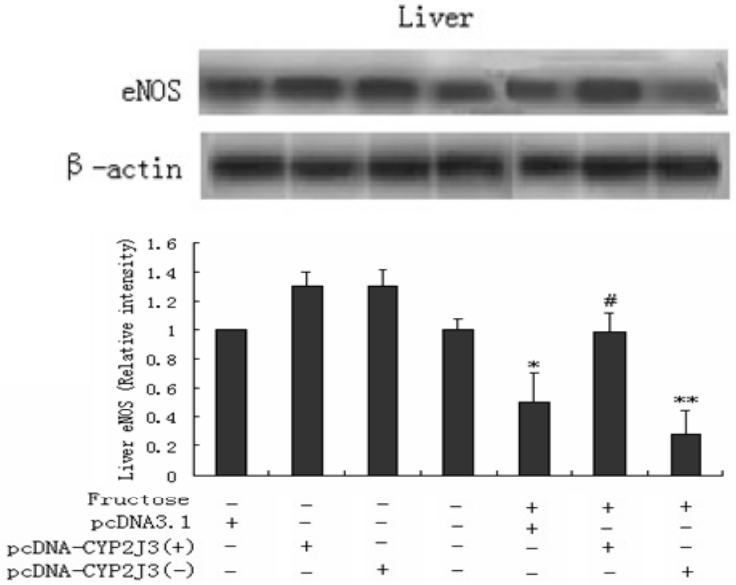
Rat serum and urine analyses

Just prior to gene delivery at week 3 of the study, approximately 1 ml of blood was drawn from the tail vein of each rat. After coagulation, serum was collected by centrifugation and stored at -80°C. Urine samples were collected over a 24-hour period prior to gene delivery, as described previously (19). Serum glucose was determined by the glucose oxidase/phosphohydrolase method, serum cholesterol by the cholesterol oxidase/phosphohydrolase method, and serum triglyceride by the glycerol phosphate oxidase/phosphohydrolase method as described elsewhere (19). Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) method. Serum insulin levels were measured with a magnetic solid phase enzyme immunoassay kit from BioChem Immunosystems (Rome, Italy). Two weeks after gene delivery (i.e. at week 5 of the study), serum and urine samples were collected and analyzed in a similar manner from 8 rats from each of the above groups. Serum sodium, serum potassium, serum magnesium, serum LDL-C, serum HDL-C, urine sodium, urine potassium and urine magnesium were measured on an AEROSET Clinical Chemistry System (Abbott Laboratories, Chicago, IL). Urinary osmolarity was measured on a model 3D3 single-sample osmometer (Advanced Instruments, Inc, Norwood, MA).

Figure S1. CYP2J3 gene delivery attenuates changes in IRS phosphorylation and eNOS protein levels in liver and skeletal muscle of fructose-treated rats. IRS-1-Ser(P)307, IRS-1-Tyr(P)989 and β-actin (A, B) or eNOS and β-actin (C, D) levels were assessed by western blotting in liver (A, C) and skeletal muscle (B, D) lysates from 3 rats per treatment group at week 5 of the study. Representative western blots and corresponding densitometric quantification of three experiments are shown. Values shown are mean ± SEM. **P*<0.05 vs. N+pcDNA3.1; #*P*<0.05 vs. F+pcDNA3.1; ***P*<0.05 vs. F+pcDNA-CYP2J3(+).



C



D

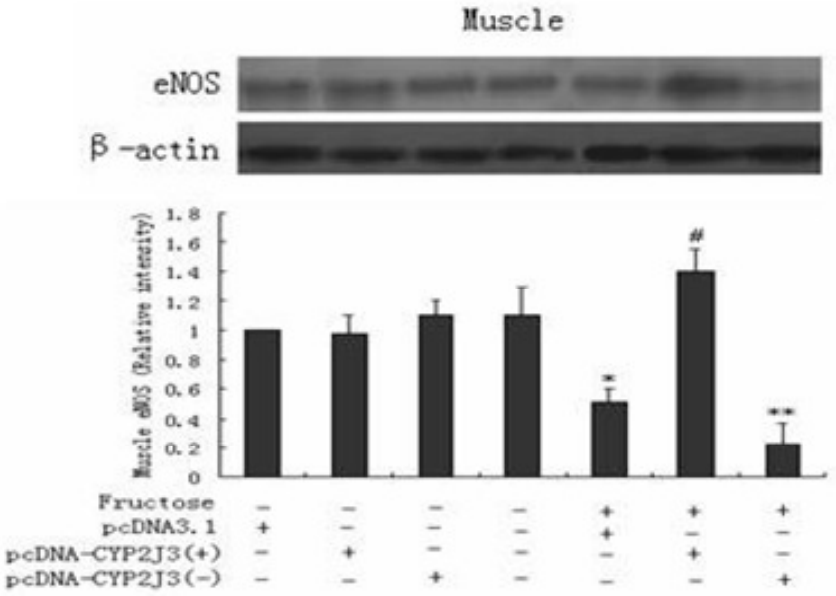


Figure S2. CYP2J3 gene delivery attenuates changes in IRS-1 and eNOS phosphorylation levels in skeletal muscle of db/db mice. IRS-1-Ser(P)307, IRS-1-Tyr(P)989 and IRS-1 (A) or, eNOS-Ser(P)1177, eNOS-Thr(P)495 and eNOS (B) levels were assessed by western blotting in skeletal muscle lysates from 3 mice per treatment group two weeks after gene delivery. Representative western blots and corresponding densitometric quantification are shown. Values shown are mean ± SEM. **P*<0.05 vs. pcDNA3.1; #*P*<0.05 vs. db/db pcDNA3.1.

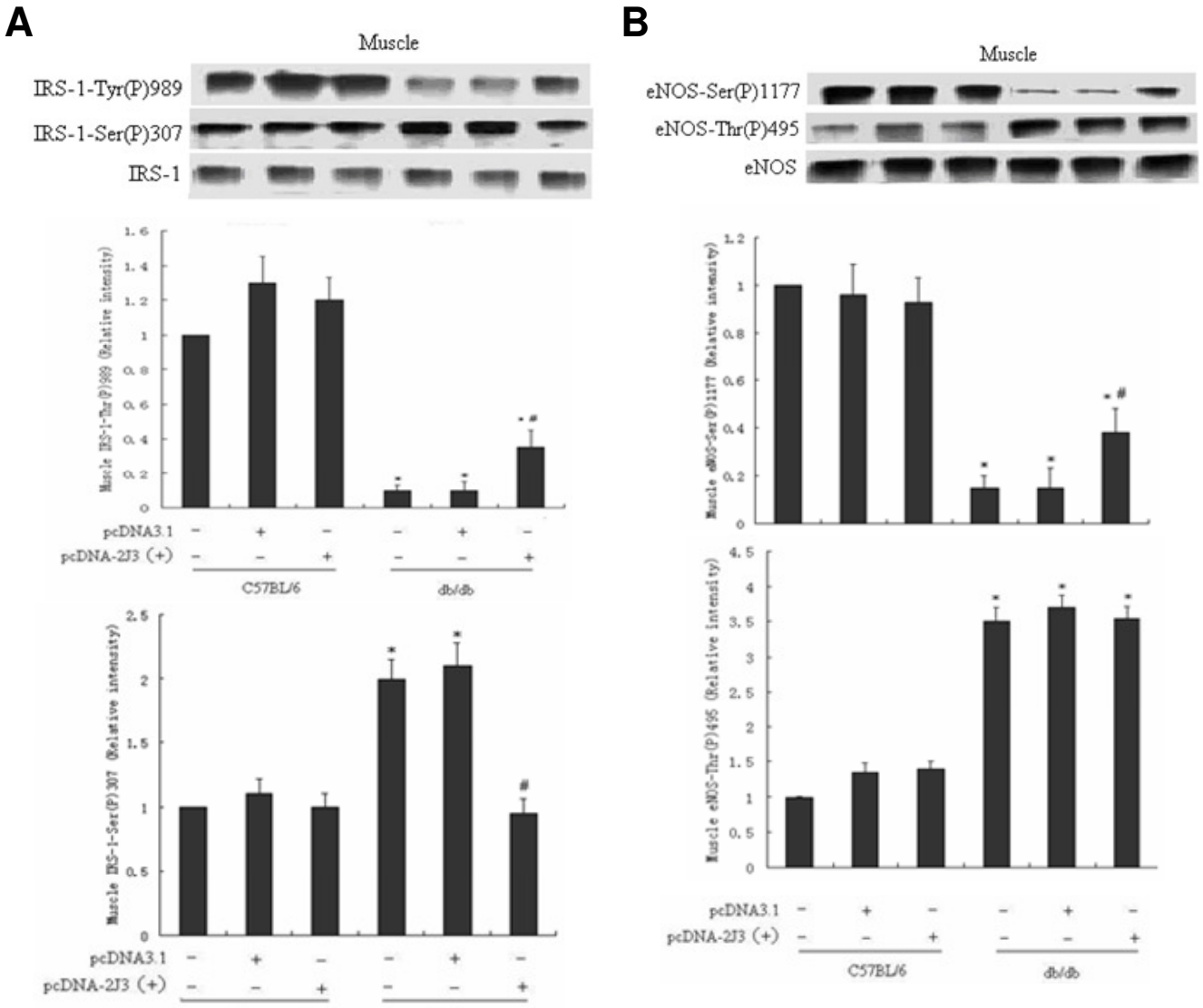
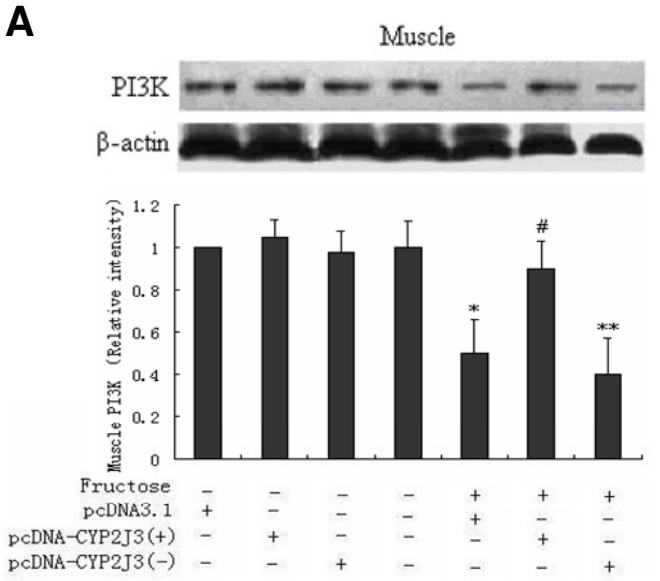


Figure S3. CYP2J3 gene delivery prevents the decrease in PI3K (P110)expression in skeletal muscles of fructose-treated rats or db/db mice. PI3K and β-actin levels were assessed by western blotting in skeletal muscle from 3 rats (A) or mice (B) per indicated treatment group two weeks after gene delivery. Representative western blots and densitometric quantification PI3K relative to β-actin protein levels are shown. Values shown are mean ± SEM. **P*<0.05 vs. N+pcDNA3.1 or C57BL/6; #*P*<0.05 vs. F+pcDNA3.1 or db/db+pcDNA3.1; ***P*<0.05 vs. F+pcDNA-CYP2J3(+).



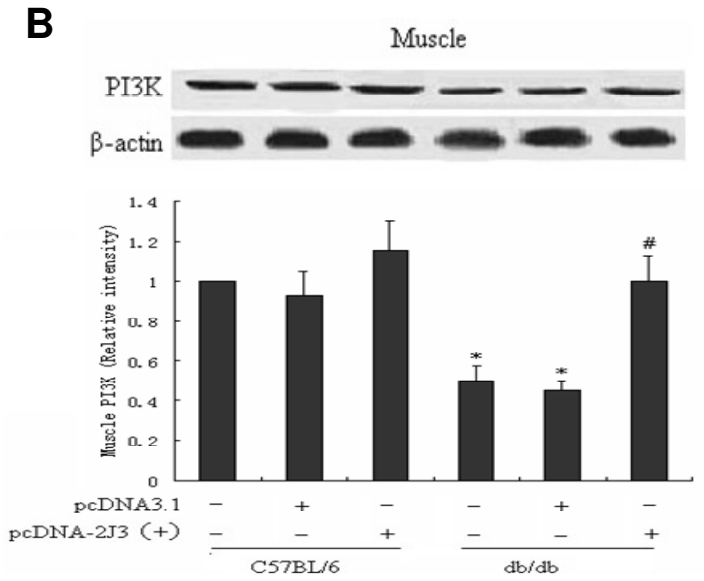


Figure S4. CYP2J3 gene delivery prevents the decrease in phospho-AKT (T308) detected in liver and skeletal muscles of fructose-treated rats or db/db mice. Phospho-AKT (P-AKT) and AKT levels were assessed by western blotting in liver (A, C) and skeletal muscle (B, D) from 3 rats (A, B) or mice (C, D) per indicated treatment group two weeks after gene delivery. Representative western blots and densitometric quantification of P-AKT relative to AKT protein level are shown. Values shown are mean ± SEM. **P*<0.05 vs. N+pcDNA3.1 or C57BL/6; #*P*<0.05 vs. F+pcDNA3.1 or db/db+pcDNA3.1; ***P*<0.05 vs. F+pcDNA-CYP2J3(+).

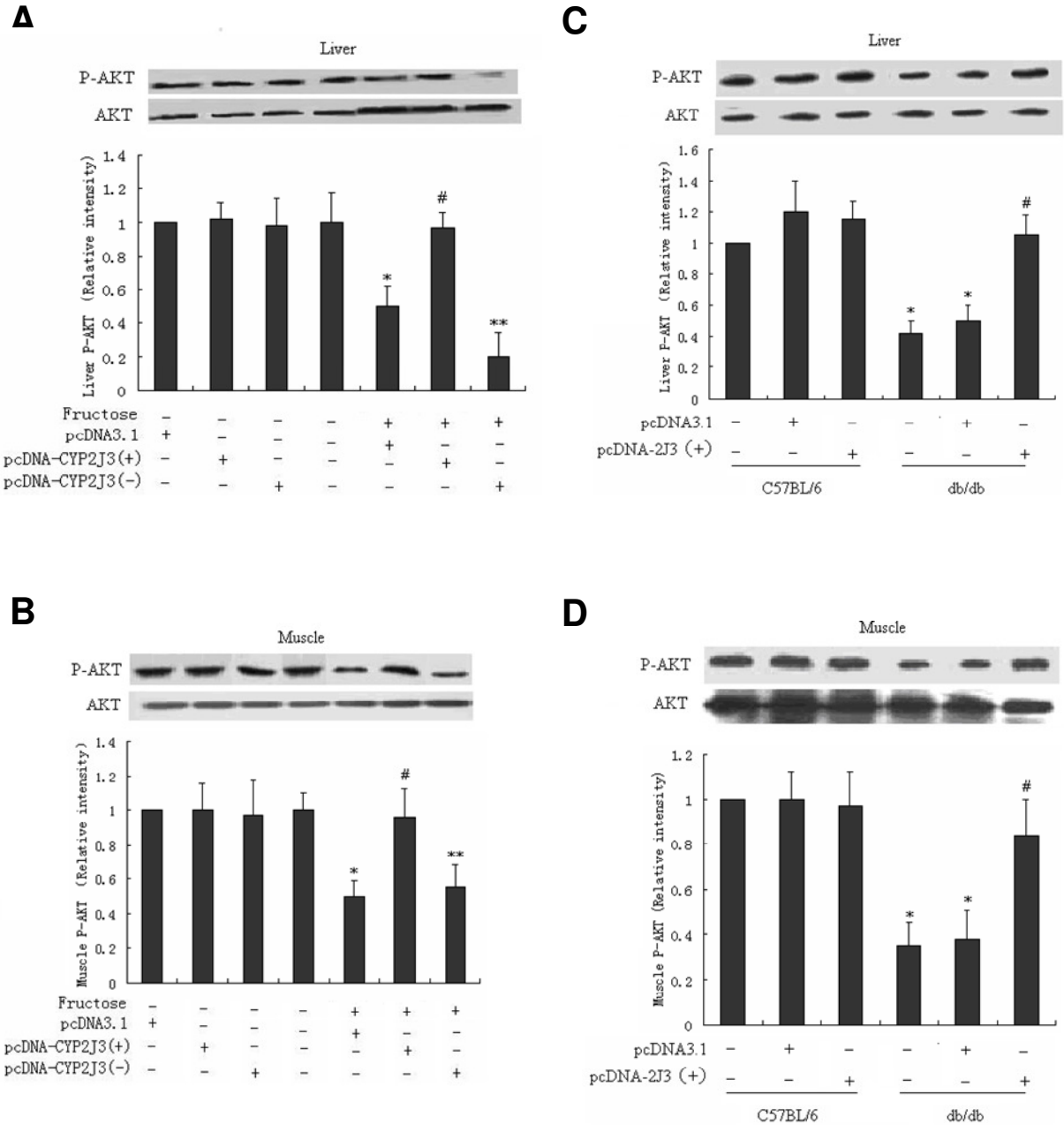
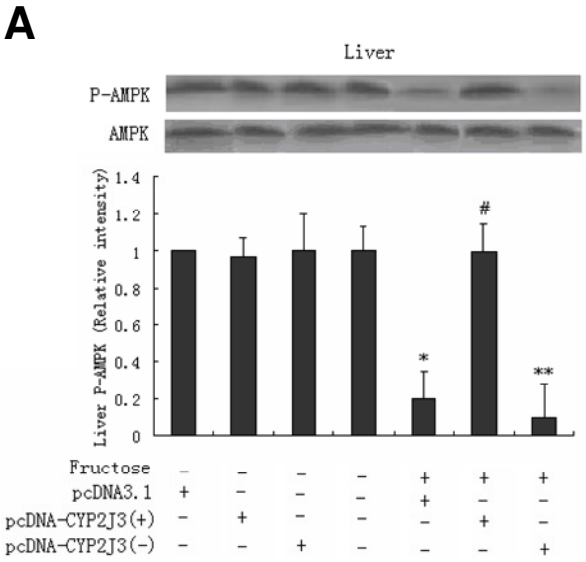
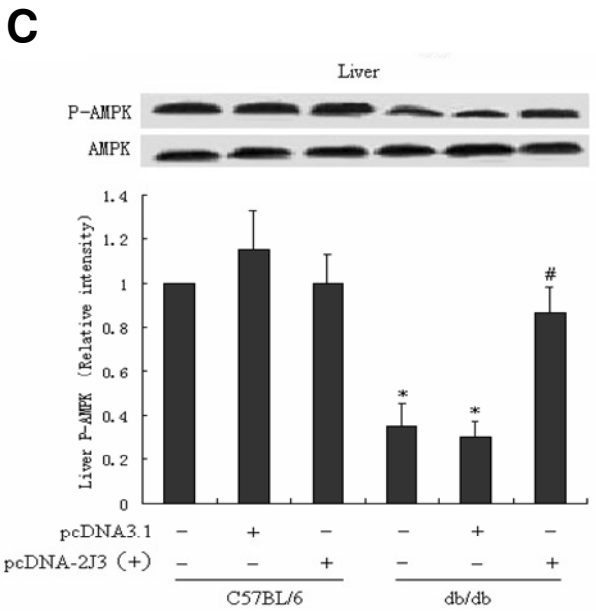
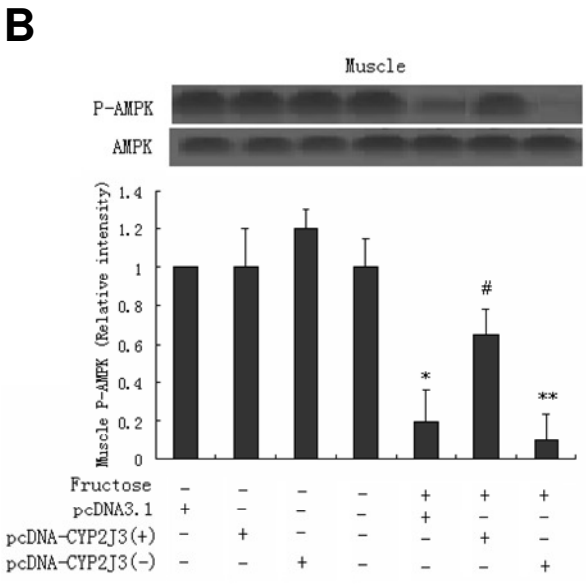


Figure S5. CYP2J3 gene delivery prevents the decrease in phospho-AMPK (T172)detected in liver and skeletal muscles of fructose-treated rats or db/db mice. Phospho-AMPK (P-AMPK) and AMPK levels were assessed by western blotting in liver (A, C) and skeletal muscle (B, D) from 3 rats (A, B) or mice (C, D) per indicated treatment group two weeks after gene delivery. Representative western blots and densitometric quantification P- AMPK relative to AMPK protein level are shown. Values shown are mean ± SEM. **P*<0.05 vs. N+pcDNA3.1 or C57BL/6; #*P*<0.05 vs. F+pcDNA3.1 or db/db+pcDNA3.1; ***P*<0.05 vs. F+pcDNA-CYP2J3(+).







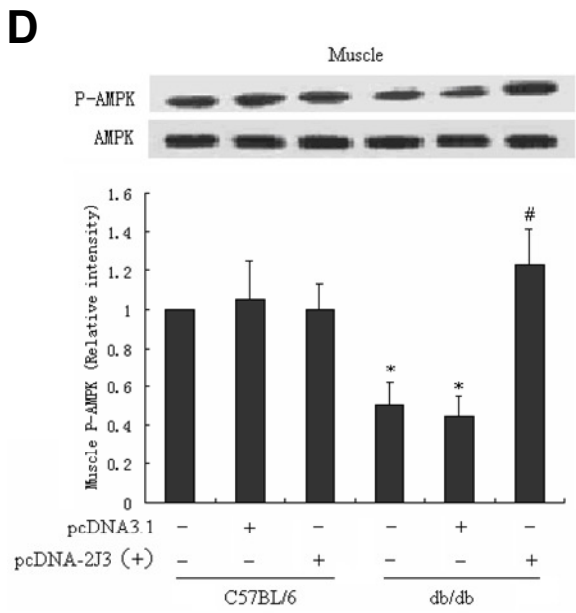


Figure S6. CYP2J3 gene delivery prevents the decrease in phospho-MAPK detected in liver and skeletal muscles of fructose-treated rats or db/db mice. Phospho-MAPK (P-MAPK) and MAPK levels were assessed by western blotting in liver (A, C) and skeletal muscle (B, D) from 3 rats (A, B) or mice (C, D) per indicated treatment group two weeks after gene delivery. Representative western blots and densitometric quantification P-MAPK relative to MAPK protein level are shown. Values shown are mean ± SEM. **P*<0.05 vs. N+pcDNA3.1 or C57BL/6; #*P*<0.05 vs. F+pcDNA3.1 or db/db+pcDNA3.1; ***P*<0.05 vs. F+pcDNA-CYP2J3(+).

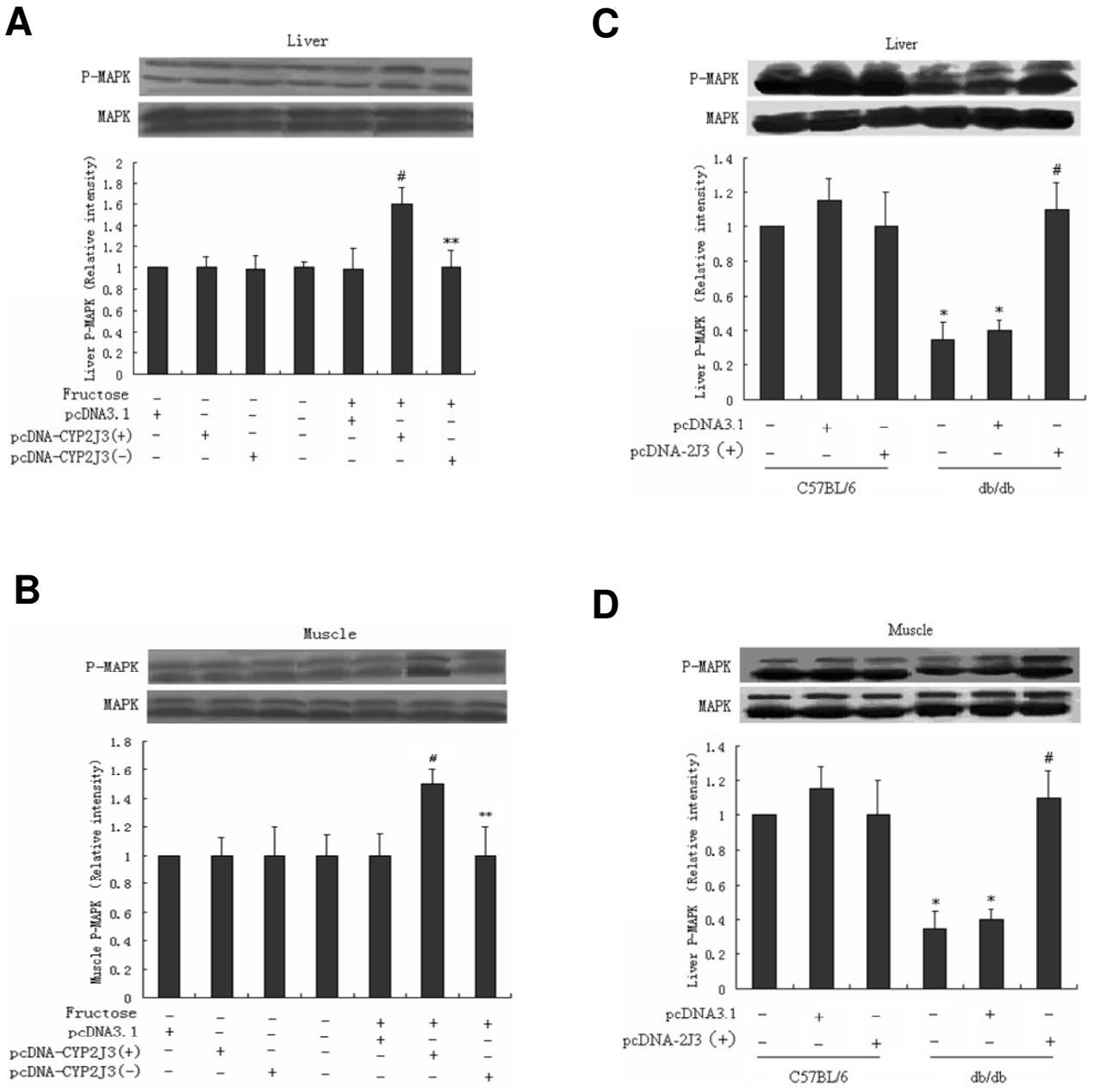


Table S1. Physiological parameters determined in rats after 3 weeks of administration of control or fructose-containing drinking water. Consumption of fructose-containing water resulted in significantly increased systolic blood pressure, increased levels of urine potassium, urine magnesium and urine volume, and significantly decreased urine osmolarity.

Variable	Control Group (n=32)	Fructose-treated Group (n=24)
Systolic blood pressure (mm Hg)	111.7 ± 0.9	125.8 ± 0.4**
Cholesterol (mmol/L)	1.52±0.22	1.07±0.31
HDL-C (mmo/L)	0.77±0.1	0.51±0.12
LDL-C (mmo/L)	0.15±0.05	0.14±0.06
Serum sodium (μmol/L)	142.3 ± 1.6	136.1 ± 8.4
Serum potassium (μmol/L)	4.73 ± 0.47	5.06 ± 0.9
Serum magnesium (μmol/L)	0.81 ± 0.07	0.95 ± 0.1
Urine volume (mL/day/100g)	5.8 ± 0.6	9.2 ± 0.1*
Urine osmolarity (mOsml/Kg H_2O)	1864 ± 449	469 ± 163**
Urine sodium (μmol/24 h)	388.8 ± 21.1	320.7 ± 42.2
Urine potassium (μmol/24 h)	13.35 ± 2.82	122.48 ± 73.78**
Urine magnesium (μmol/24 h)	0.11 ± 0.06	5.29 ± 0.72**

*P<0.05 vs. control group; **P<0.01 vs. control group.

Table S2. Physiological parameters determined in rats 2 weeks following injection of empty pcDNA3.1 vector, pcDNA-2J3(+) or pcDNA-2J3(-). Compared to values in the normal water-treated groups, urine volume, urine potassium and urine magnesium levels were all higher, while urine osmolarity was lower in fructose-treated rats injected with the empty pcDNA vector (F+pcDNA group). All of these changes were prevented by injection of fructose-treated rats with CYP2J3(+), but not with CYP2J3(-).

	Treatment Group						
	Normal Water-treated				**Fructose-treated**		
Variable	**N+ pcDNA3.1**	**N+ p2J3(+)**	**N+ p2J3(-)**	**Normal**	**F+ pcDNA3.1**	**F+ p2J3(+)**	**N+ p2J3(-)**
Cholesterol (mmol/L)	1.58±0.32	1.48±0.22	1.81±0.38	1.56±0.42	1.21±0.27	0.97±0.18	1.07±0.11
HDL-C (mmo/L)	0.73±0.04	0.74±0.1	0.81±0.22	0.70±0.03	0.53±0.05	0.48±0.09	0.58±0.04
LDL-C (mmo/L)	0.19±0.06	0.17±0.05	0.19±0.01	0.18±0.03	0.17±0.07	0.11±0.03	0.15±0.03
Serum sodium (µmol/L)	140.7±1.5	141.2±0.65	141.4±2.0	137.7±2.5	141.8±3.3	139.5 ± 3.1	143.0±1.8
Serum potassium (µmol/L)	5.81±0.81	4.41±0.47	4.04±0.30	4.81±0.61	4.60±0.07	4.40 ± 0.48	4.56 ± 0.14
Serum magnesium (µmol/L)	1.12±0.40	0.81±0.13	0.81±0.12	1.02±0.3	1.03±0.22	1.06 ± 0.29	1.14 ± 0.43
Urine volume (mL/day/100g)	3.3±0.8	3.4±0.6	3.7±0.8	3.0±0.5	14.0±0.3*	3.0 ± 0.8#	24.6 ± 0.6**
Urine osmolarity (mOsml/Kg H_2O)	1727±160	2147±296	1812±332	1963±198	257±108*	1572 ± 86#	136±7.0**
Urine sodium (µmol/24 h)	400±27	430.4±43.1	436.4±31.1	429.0±30.0	297±49	303±31	341±65
Urine potassium (µmol/24 h)	14.09±1.42	17.78±1.82	13.74±2.24	15.09±2.0	59.95±14.63*	26.74±4.76#	60.68±31.07**
Urine magnesium (µmol/24 h)	0.25±0.19	0.17±0.09	0.19±0.06	0.15±0.09	3.19±0.29*	0.09±0.05#	2.86±0.14**

*P<0.05 vs. N+pcDNA3.1 group; #P<0.05 vs. F+pcDNA3.1 group; **P<0.05 vs. F+p2J3(+) group; n=8 per group.

Table S3. Physiological parameters of renal and liver function determined in rats 2 weeks following injection of empty pcDNA3.1 vector, pcDNA-2J3(+) or pcDNA-2J3(-). Compared to values in rats injected with empty pcDNA vector, serum alanine aminotransferase, urea and creatinine have no significant changes in rats injected with CYP2J3.

	Treatment Group						
	Normal Water-treated				**Fructose-treated**		
Variable	**N+ pcDNA3.1**	**N+ p2J3(+)**	**N+ p2J3(-)**	**Normal**	**F+ pcDNA3.1**	**F+ p2J3(+)**	**N+ p2J3(-)**
ALT (U/L)	4.29±0.75	3.40±0.93	5.25±1.70	5.10±0.68	5.20±0.86	5.00±0.84	5.40±0.93
UREA (mmol/L)	8.66±0.29	8.39±0.66	7.53±0.33	7.83±0.60	7.91±0.47	7.99±0.46	7.69±0.42
CR (μmol/L)	24.77±1.71	22.00±1.35	23.68±1.13	22.94±1.63	23.54±1.25	23.78±1.65	24.10±1.48

*P<0.05 vs. N+pcDNA3.1 group; #P<0.05 vs. F+pcDNA3.1 group; **P<0.05 vs. F+p2J3(+) group; n=8 per group.

Table S4. Physiological parameters determined in mice 2 weeks following injection of empty pcDNA3.1 vector or pcDNA-2J3(+). Serum cholesterol level, urine volume and water intake were significantly higher in diabetic phenotype of db/db mice (injected with the empty pcDNA vector), compared to those in C57BL/6 mice. Injection of CYP2J3(+) significantly reduced urine volume in db/db mice, whereas injection of the empty pcDNA vector did not.

Variable	**C57BL/6**	**C57BL/6+ pcDNA3.1**	**C57BL/6+ p2J3(+)**	**db/db**	**db/db+ pcDNA3.1**	**db/db+ p2J3(+)**
Cholesterol (mmol/L)	2.27±0.17	1.69±0.15	1.87±0.2	4.06±0.6*	3.76±0.32*	3.62±0.65
HDL-C (mmo/L)	0.863±0.102	0.909±0.169	1.18±0.134	1.44±0.44	1.50±0.185	1.31±0.256
LDL-C (mmo/L)	1.49±0.22	1.46±0.17	1.64±0.15	1.89±0.39	1.99±0.1	1.81±0.41
Urine volume (mL/24h/10g)	0.28±0.045	0.32±0.012	0.303±0.025	0.42±0.03*	0.431±0.015*	0.287±0.05#
Water intake (mL/24h/10g)	0.249±0.03	0.267±0.02	0.278±0.02	0.51±0.01*	0.497 ± 0.032*	0.469 ± 0.035

*P<0.05 vs. C57BL/6 group; #P<0.05 vs. db/db+pcDNA3.1 group; n=8 per group.