ONLINE DATA SUPPLEMENT

Liver X Receptor Agonist Suppresses Osteopontin Expression in Diabetic Nephropathy

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Running title: LXR and OPN in Diabetic Nephropathy

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MATERIALS AND METHODS

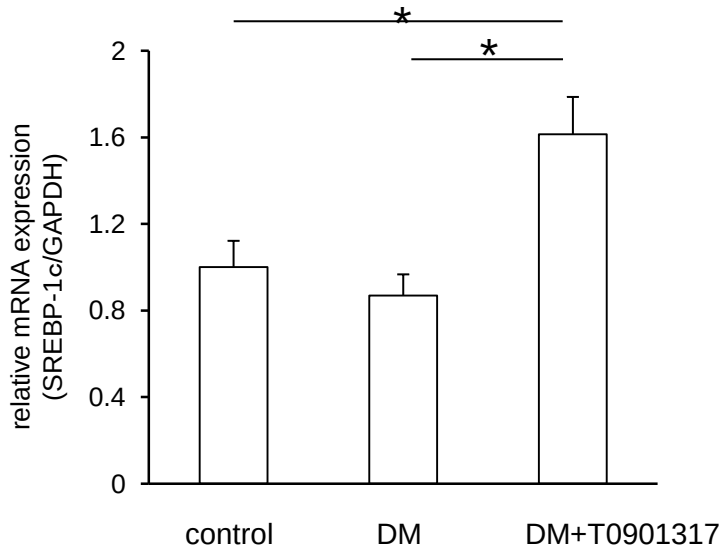
Oil Red O staining. Fresh-frozen sections were cut at 10-μm thickness using a cryostat and fixed in 4% paraformaldehyde for 5 minutes. A fresh working solution of oil red O was prepared by dilution of an oil red O stock solution (5 g/L in 98% isopropanol) in distilled H_2O in a ratio of 3:2. The working solution was allowed to stand for 10 minutes after mixing and then filtered using a 0.45-μm filter. Subsequently, the sections were stained in oil red O for 30 minutes, washed in tap water, and counterstained with hematoxylin solution. The sections were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

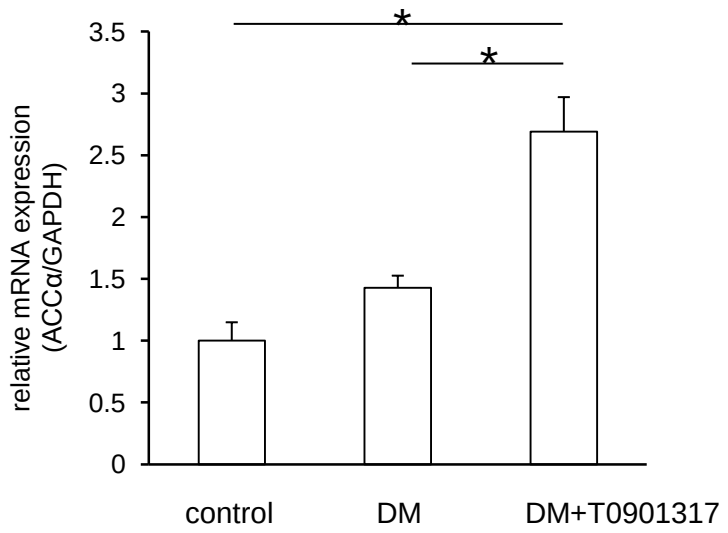
Quantitative real-time RT-PCR. RNA from the renal cortex was isolated after 8 weeks of treatment using an RNeasy Mini Kit (Qiagen, Valencia, CA). Single-stranded cDNA was synthesized from the extracted RNA using a GeneAmp® RNA PCR Core Kit (Applied Biosystems, Foster City, CA). To evaluate the mRNA expression of LXRα (Mm00443451_m1), LXRβ (Mm00437265_g1), sterol regulatory element binding protein (SREBP)-1c (Mm00550338_m1), acetyl-coenzyme A carboxylase α (ACCα; Mm01304257_m1), and fatty acid synthase (FAS; Mm00662319_m1) in the renal cortex, quantitative RT-PCR (qRT-PCR) was performed using a StepOnePlus™ Real-time-PCR System (Applied Biosystems) and TaqMan® Fast Universal PCR Master Mix (Applied Biosystems). The primers were purchased from Applied Biosystems. Each sample was analyzed in triplicate and normalized for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Mm99999915_g1) mRNA expression.

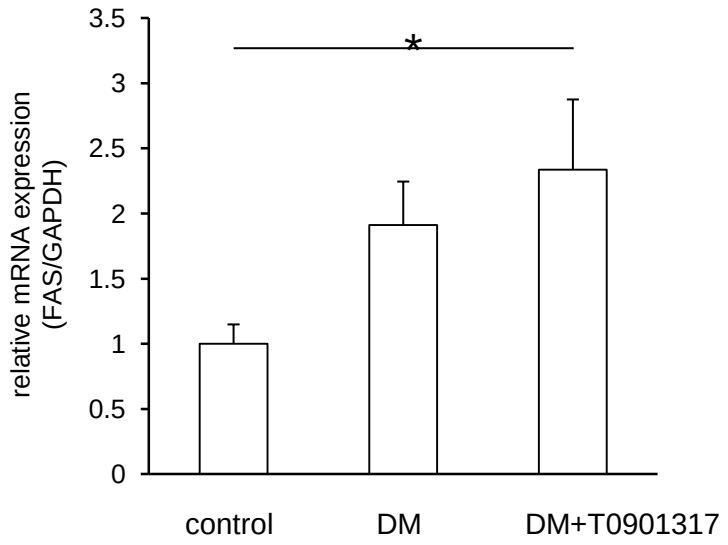
siRNA Experiments. siRNA experiments were performed using LXRα siRNA (sc-38829; Santa Cruz Biotechnology, Santa Cruz, CA), LXRβ siRNA (sc-45317; Santa Cruz Biotechnology), and scrambled siRNA (sc-37007; Santa Cruz Biotechnology). mProx24 cells were transfected with 11 μM LXRα siRNA, LXRβ siRNA, or scrambled siRNA in the presence of Lipofectamine™ RNAiMAX (Invitrogen, Carlsbad, CA). After siRNA transfection for 24 h, the cells were stimulated with 25 mM D-glucose (high glucose) for 24 h. Individual experiments were repeated at least three times with different lots or preparations of cells.

Supplementary Figure 1

A







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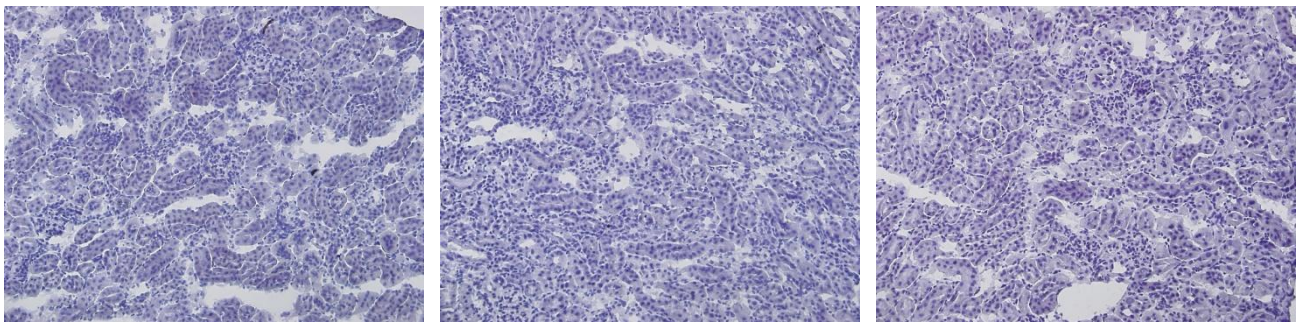
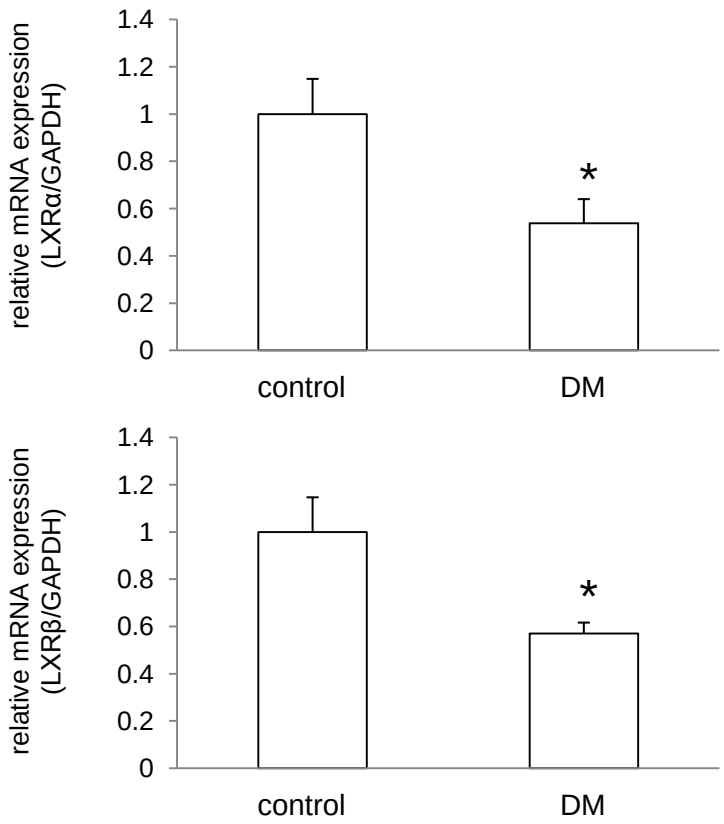


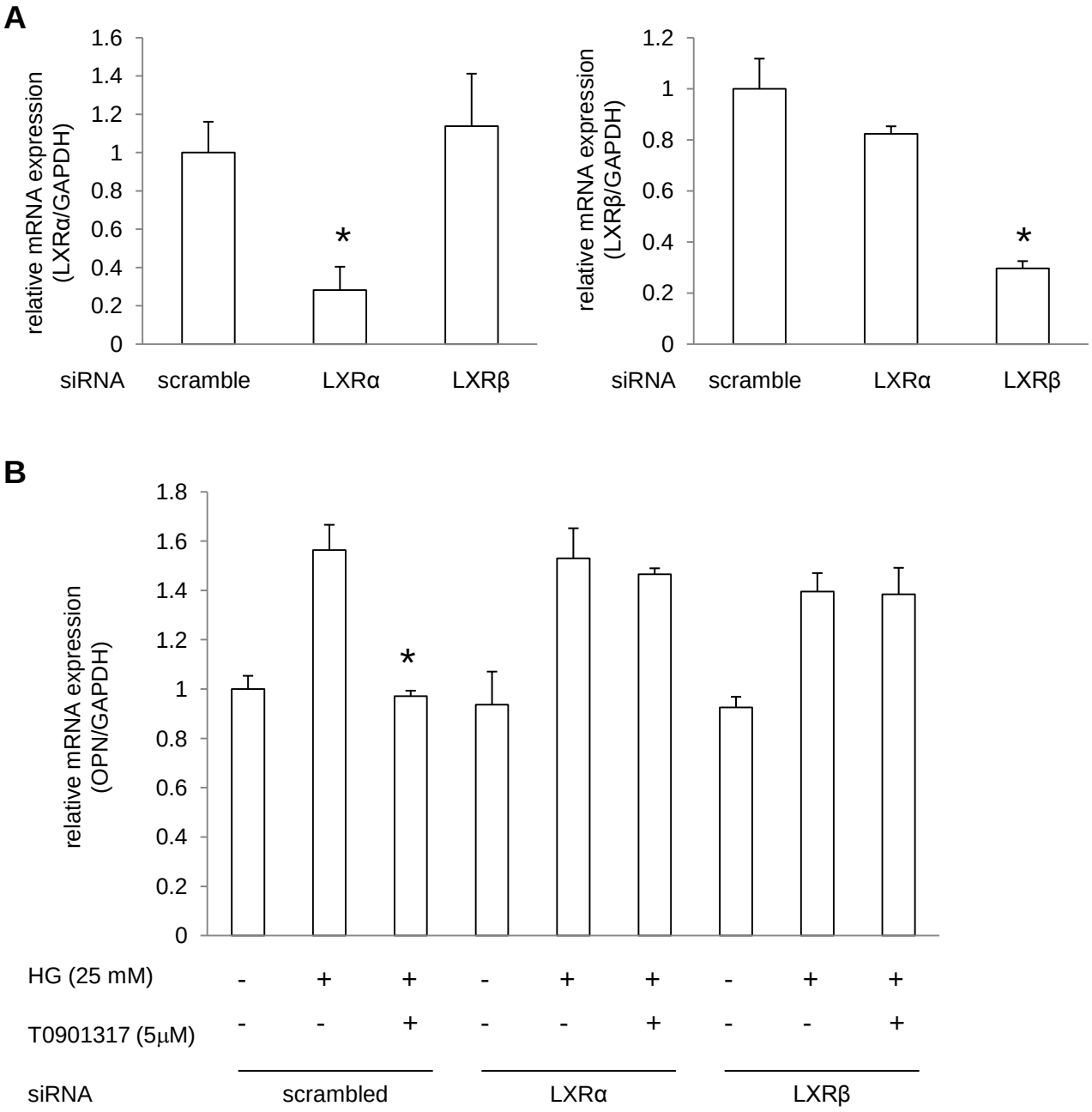
control DM DM+T0901317

C



Supplementary Figure 1. Lipogenic activity of T0901317 in the mouse kidney. (A) qRT-PCR analyses of the expressions of lipogenic enzymes. The expressions of SREBP-1c and ACCα are significantly increased in the DM+T0901317 group compared with the DM group. FAS expression is significantly higher in the DM+T0901317 group than in the control group, but there is no significant difference between the DM and DM+T0901317 groups. The mRNA levels were normalized by the GAPDH mRNA level. Data are means ± SEM. **P* < 0.05. (B) Representative microphotographs of kidney sections with oil red O staining. Lipid accumulation indicated by oil red O staining is not detected in the DM+T0901317 group, similar to the findings for the control and DM groups. Original magnification, × 200. (C) Gene expressions of LXRα and LXRβ are suppressed in the DM group compared with the control group. The mRNA levels were normalized by the GAPDH mRNA level. Data are means ± SEM. **P* < 0.05.

Supplementary Figure 2



Supplementary Figure 2. T0901317 suppresses high glucose-induced OPN expression in proximal tubular epithelial cells by activating both LXRα and LXRβ. (A) mProx24 cells, a mouse renal proximal tubular epithelial cell line, were transfected with LXRα, LXRβ, or scrambled siRNA, and subjected to qRT-PCR analyses. LXRα expression is significantly inhibited in mProx24 cells transfected with the LXRα siRNA compared with those transfected with the LXRβ siRNA and scrambled siRNA. Similarly, LXRβ expression is significantly inhibited in mProx24 cells transfected with the LXRβ siRNA compared with those transfected with the LXRα siRNA and scrambled siRNA. The mRNA levels were normalized by the GAPDH mRNA level. Data are means ± SEM. **P* < 0.05. (B) High glucose-induced OPN mRNA expression is suppressed by T0901317 in mProx24 cells transfected with the scrambled siRNA. This suppression is canceled by LXRα siRNA and LXRβ siRNA. The mRNA levels were normalized by the GAPDH mRNA level. Data are means ± SEM. **P* < 0.05.