Supplemental data

Legends

Supplementary Fig. 1

Gene expression profile in the islets from wild type, TgRIP-SREBP-1c, and SREBP-1c null mice. The amounts of mRNA of SREBP-1c and PDX-1 in the islets were estimated using RT-PCR (22).

Supplementary Fig. 2

The protein interaction between USF-1 (or SREBP-1c) was specific and not non-specific bridging by DNA.

In vitro translated proteins (IVTT) (5 μl) and micrococcal nuclease S7 (0.1 mg/ml) (Worthington) were incubated in 0.1 M Sodium borate (pH 8.8) (10 μl) and 0.01 M $CaCl_2$ (5 μl) for 10 min at 37 **°C**. (A) S7 catalyzed endohydrolysis of the RNA and DNA in IVTT proteins. (B, C) GST or GST-PDX-1 proteins were incubated with ^{35}S-USF1 or ^{35}S-SREBP-1c proteins with or without S7 treatment for 60 min at 4 **°C**. Bound proteins were resolved on a 15% gel by SDS-PAGE followed by autoradiography. The experiments in (B) and (C) were repeated two times with similar results.

Supplementary Fig. 3

SREBP-1c●PDX-1 complex suppresses the activation of SREBP target genes.

An artificial LDLR (SRE)-Luc and CMV-SREBP-1a (0.01 μg) or an artificial S14 (E-box)-Luc and CMV-SREBP-1aM (0.25 μg) and pSV-β-gal were cotransfected into HepG2 cells with the indicated amounts of CMV-PDX-1, CMV-ΔABC-PDX-1, CMV-ΔHD-PDX-1, or the empty vector (CMV-7). (A) LDLR (SRE)-Luc contains three copies of a classic SRE and Sp-1 site from the LDL receptor promoter (n = 6). (B) S14 (E-box)-Luc contains six copies of the carbohydrate response element of S14 gene containing two E-boxes. Luciferase activity was normalized to the pSV-β-gal values. Luciferase activity of CMV-7 was set to 1.0 (n = 6). Data are means ± S.E. (C, D). Subcellular localization of full-length PDX-1 and ΔHD-PDX-1 was revealed using the anti-PDX-1 antibody. CMV-PDX-1 (0.25 μg) or CMV-ΔHD-PDX-1 (0.25 μg) was transfected into HepG2 cells with CMV-GFP (0.25 μg). Cells were cultured for 18 h, and localization of PDX-1 and ΔHD-PDX-1was detected by immunocytochemistry. GFP protein was located in the nucleus. Full-length PDX-1 is located in the nucleus in (C) and

ΔHD-PDX-1 is located in the cytosol in (D).

Supplementary Fig. 4

The effect of Ad-si-SREBP-1c in INS-1 cells.

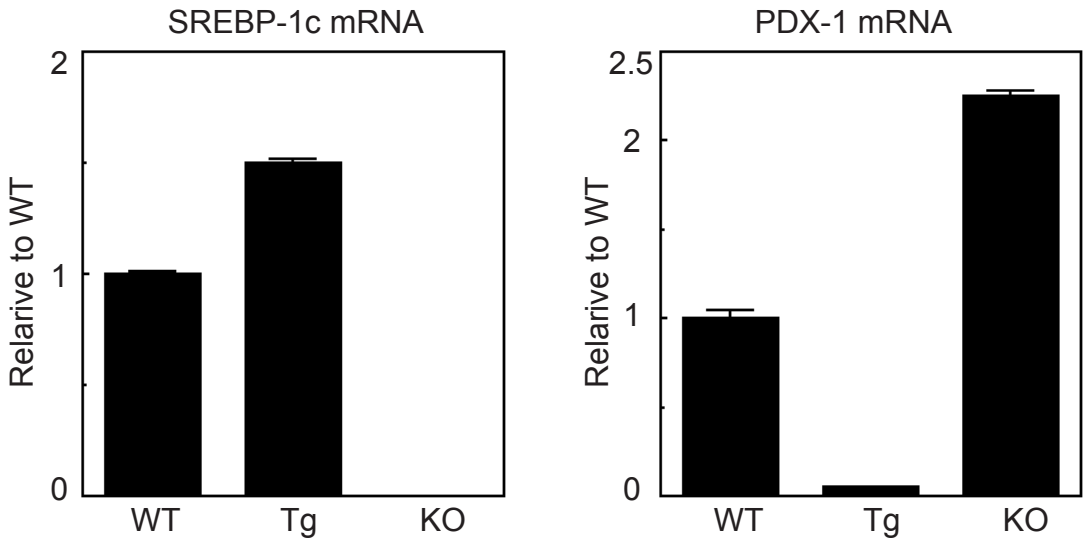
(A) INS-1 cells were infected with Ad-si-LacZ or Ad-si-SREBP-1c at 1 MOI in a 10 mM glucose medium for 48 h. After the medium was changed, 9cis-RA (1 μM) and 22OH-Cho (1 μM) were added and incubated for 24 h. Extracted mRNAs were quantified by real-time PCR (n = 3–6). Data are means ± S.E. Relative rate to Ad-si-LacZ mRNA was set to 1.0. (B) INS-1 cells were infected with Ad-si-LacZ or Ad-si-SREBP-1c at 10 MOI in a 10 mM glucose medium for 48 h. After the medium was changed, 9cis-RA (10 μM) and 22OH-Cho (10 μM) were added and incubated for 24 h. Extracted proteins were immunoblotted with anti-SREBP-1, anti-PDX-1, and anti-Lamin B antibodies. The experiments in (B) were repeated twice with similar results.

Supplementary Fig. 5

SET9 binds to both SREBP-1c and PDX-1.

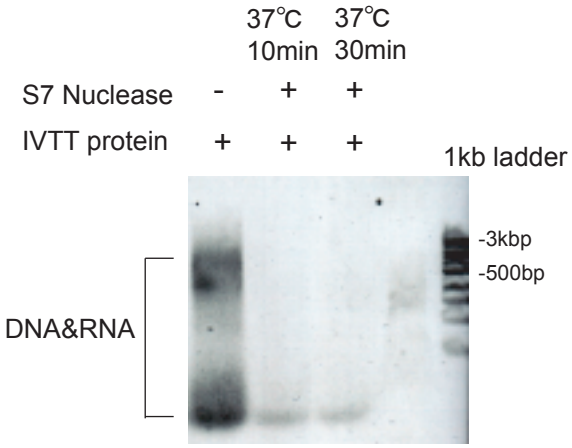
In vitro translated, ^{35}S-methionine labeled SET9 was incubated with GST-PDX1 (full length (FL), 1–283), ΔABC-PDX-1 (76–283), ΔHD-PDX-1 (1–149), GST-SREBP-1c (bHLH, 286–364), and GST-SREBP-1c (nuclear form, 24–460) immobilized on glutathione sepharose beads. Bound proteins were resolved on 15 % gel by SDS-PAGE followed by autography.

Supplementary Fig.1



Supplementary Fig.2

A



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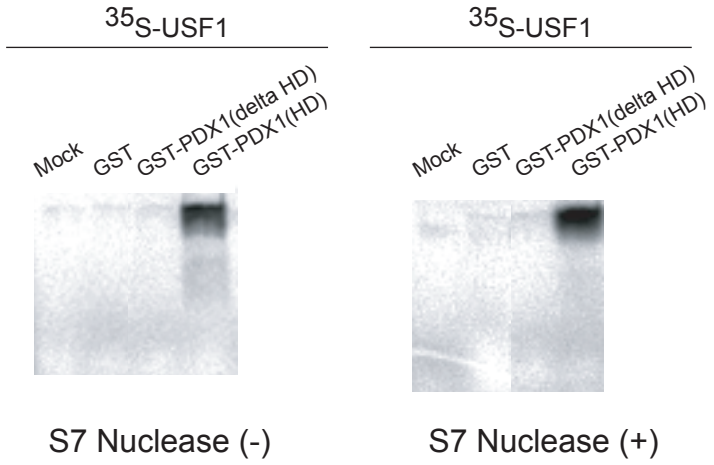
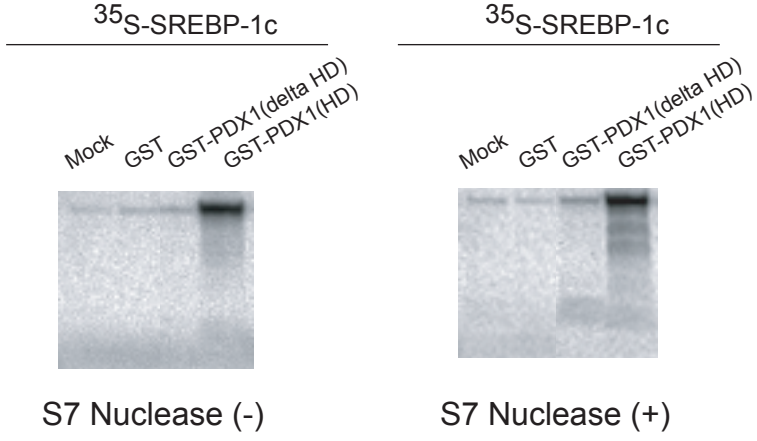
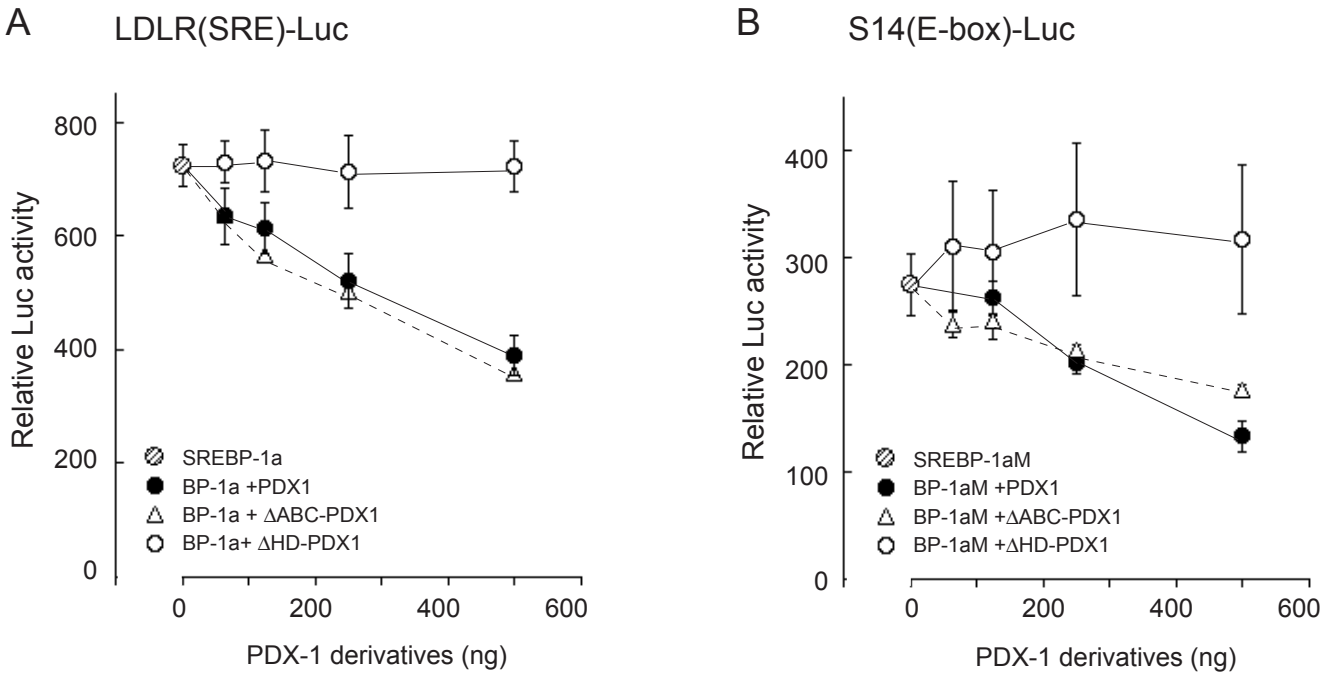
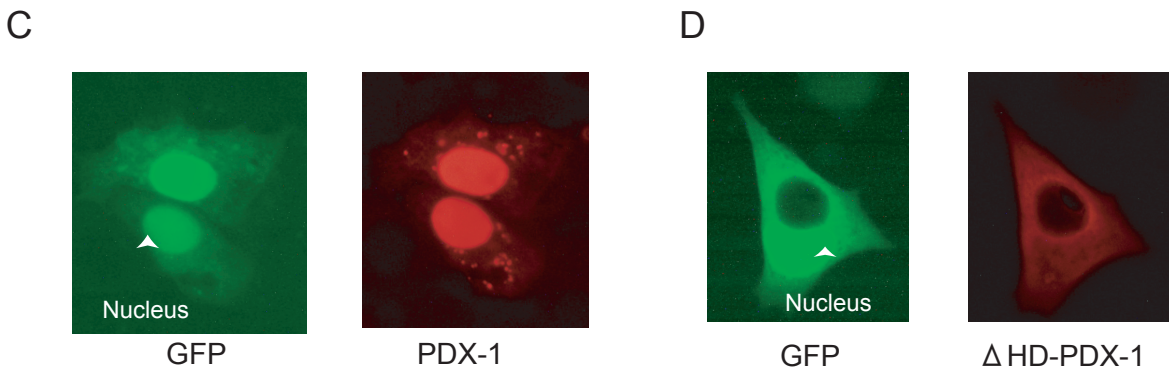


C



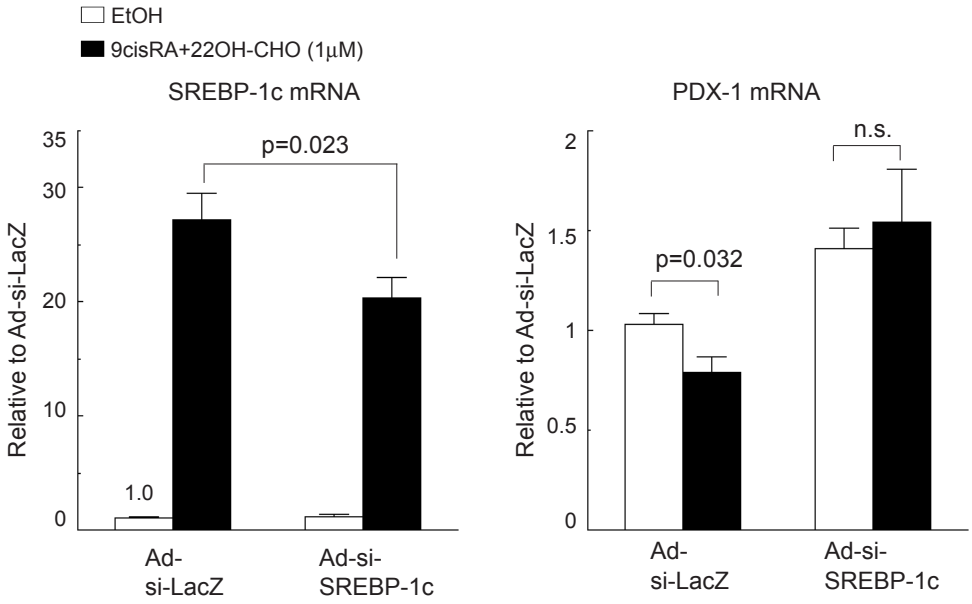
Supplementary Fig.3



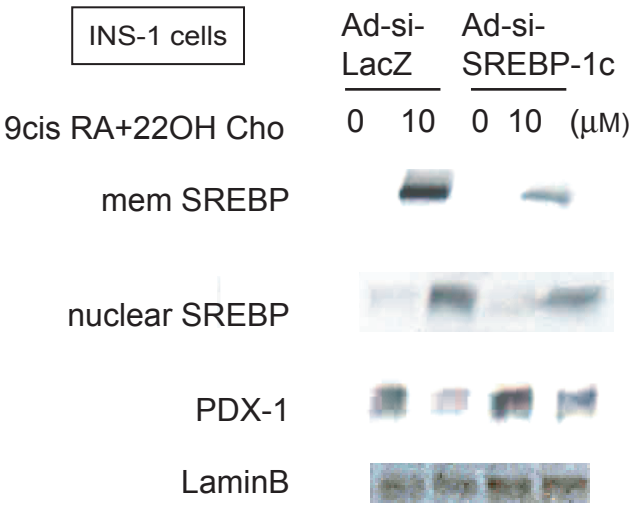


Supplementary Fig.4

A qPCR



B Western



Supplementary Fig.5