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Supplemental Information

O-GlcNAc Transferase/Host Cell Factor C1 Complex Regulates Gluconeogenesis by Modulating PGC-1α Stability

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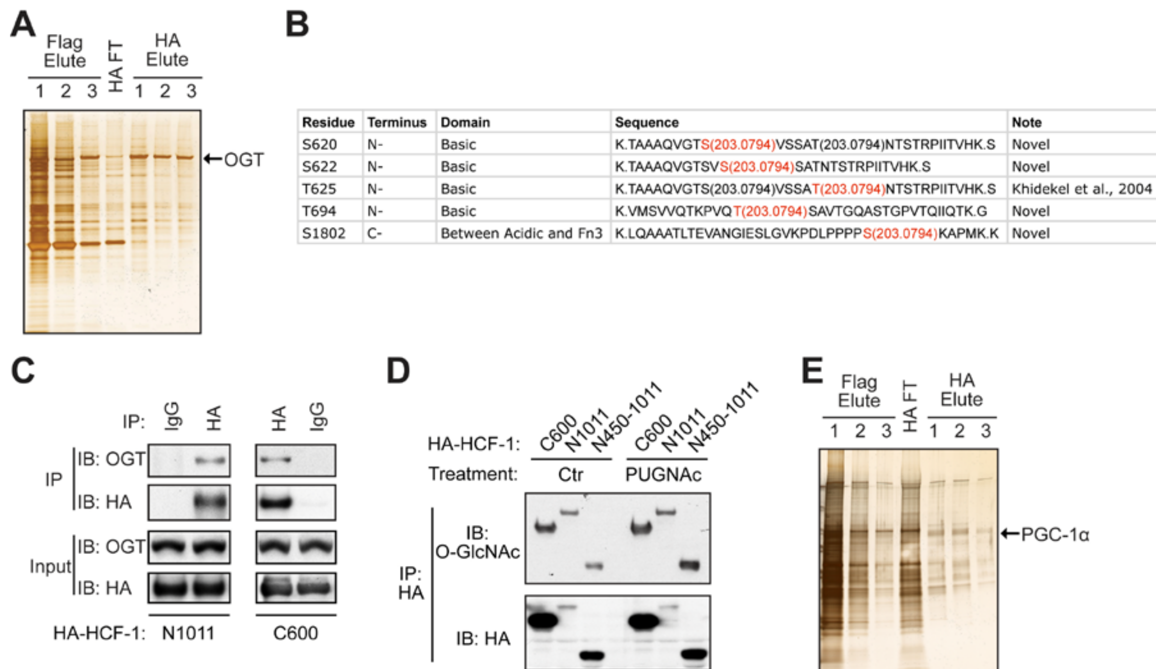

Residue	Terminus	Domain	Sequence	Note
S620	N-	Basic	K.TAAAQVGTS(203.0794)VSSAT(203.0794)NTSTRPIITVHK.S	Novel
S622	N-	Basic	K.TAAAQVGTSVS(203.0794)SATNTSTRPIITVHK.S	Novel
T625	N-	Basic	K.TAAAQVGTS(203.0794)VSSAT(203.0794)NTSTRPIITVHK.S	Khidekel et al., 2004
T694	N-	Basic	K.VMSVVQTKPVQT(203.0794)SAVTGQASTGPVTQIIQTK.G	Novel
S1802	C-	Between Acidic and Fn3	K.LQAAATLTEVANGIESLGVKPDLPPPPS(203.0794)KAPMK.K	Novel

Figure S1, related to Figure 1.

(A) OGT protein complex was isolated from extract of HEK 293T cells infected with adenovirus encoding Flag/HA-OGT, by tandem purification with anti-FLAG and anti-HA antibodies, and visualized by silver staining.

(B) Identified O-GlcNAcylation sites of HCF-1 in this study.

(C) HEK 293 cells were transfected with HA-tagged N-terminus (N1011) or C-terminus (C600) of HCF-1, and their interactions with endogenous OGT were determined.

(D) HEK 293 cells were transfected with the fragments of HCF-1 proteins as indicated, O-GlcNAcylation was shown by immunoblotting with an O-GlcNAc antibody (RL2).

(E) Silver staining of purified Flag/HA-PGC-1α protein complexes.

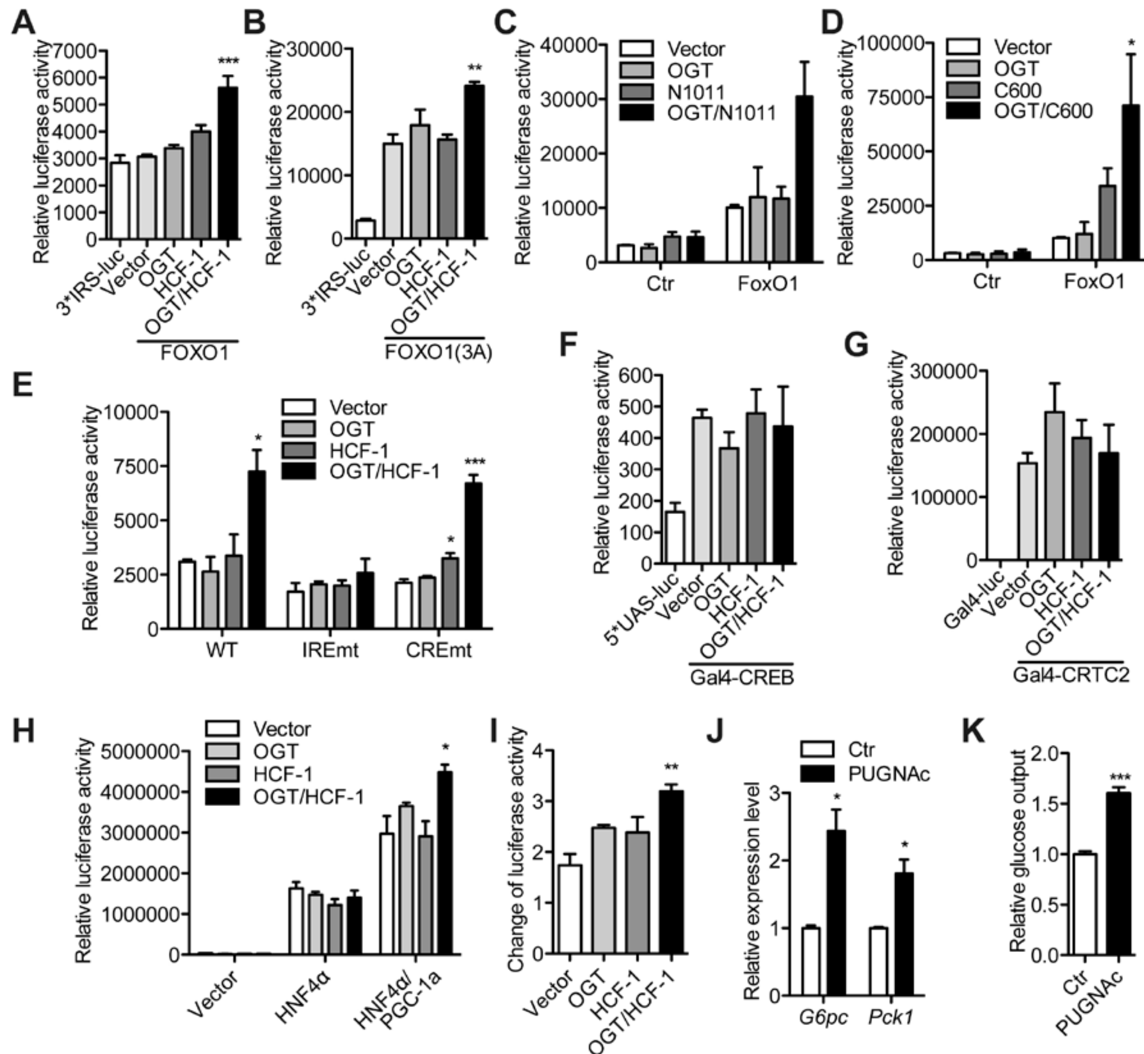

Figure S2, related to Figure 2.

(A-I) Luciferase assays performed and normalized to co-transfected β-Gal activity in HepG2 cell.

(A and B) Insulin responsive sequence (3*IRS) luciferase assay with FOXO1 (A) and insulin-insensitive FOXO1-3A mutant (B) co-transfected.

(C and D) OGT and the N-terminus (C) or the C-terminus (D) of HCF-1 cooperatively up-regulate G6pc-luciferase activity.

(E) Assays of wildtype or mutant G6pc-luciferase reporters defective in FOXO1 (IREmt) or CREB (CREmt) binding.

(F) Gal4-CREB transactivation assay.

(G) Gal4-CRTC2 transactivation assay.

(H) HNF4α responsive A*2.luciferase assay.

(I) Fold change in luciferase activity of (H) in the presence of PGC-1α relative to HNF4α alone.

(J and K) FAO cells were treated with PUGNAc, then relative gluconeogenic gene expression (J) and glucose output (K) were determined. All values represent mean ± SEM (n = 3). (A-I) *, $P < 0.05$; **, $P < 0.01$ by ANOVA with a Tukey's post hoc test compared with vector or control (ctr). (J, K) *, $P < 0.05$; ***, $P < 0.001$ by two-tailed *t*-test.

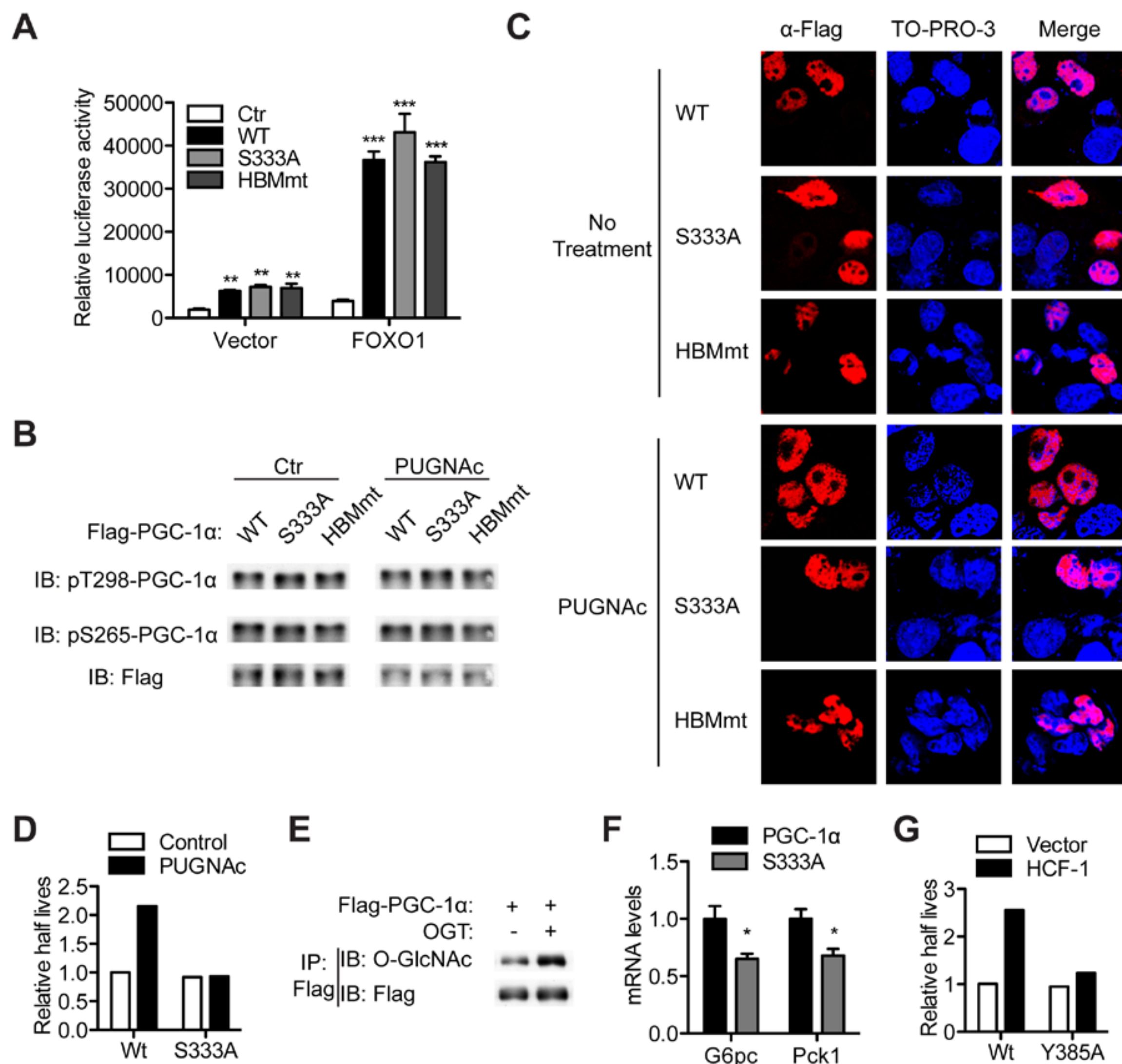

Figure S3, related to Figure 3.

(A) G6pc-luciferase assays in HepG2 cells transfected with wildtype, S333A, or HBMmt PGC-1α (mean ± SEM, n = 3). **, $P < 0.01$; ***, $P < 0.001$ by ANOVA with a Bonferroni's post hoc test compared with Ctr.

(B) HEK 293T cells were transfected with Flag-tagged PGC-1α mutants, and their site-specific phosphorylation (MAPK sites regulating PGC-1α stability (Puigserver et al., 2001)) was determined.

(C) Transfected HepG2 cells were treated with or without PUGNAc, and then immunofluorescence staining using α-Flag antibody was performed. Nucleus was stained with TO-PRO-3.

(D) Relative half lives of PGC-1α in Figure 3B are shown.

(E) HEK 293T cells were transfected with PGC-1α and OGT, then O-GlcNAcylation of PGC-1α was determined.

(F) FAO cells were transfected with the wildtype or S333A mutant of PGC-1α, and then *G6pc* and *Pck1* mRNA levels were determined (mean ± SEM, n = 3). *, $P < 0.05$ by two-tailed *t*-test.

(G) Relative half lives of PGC-1α in Figure 3F are shown.

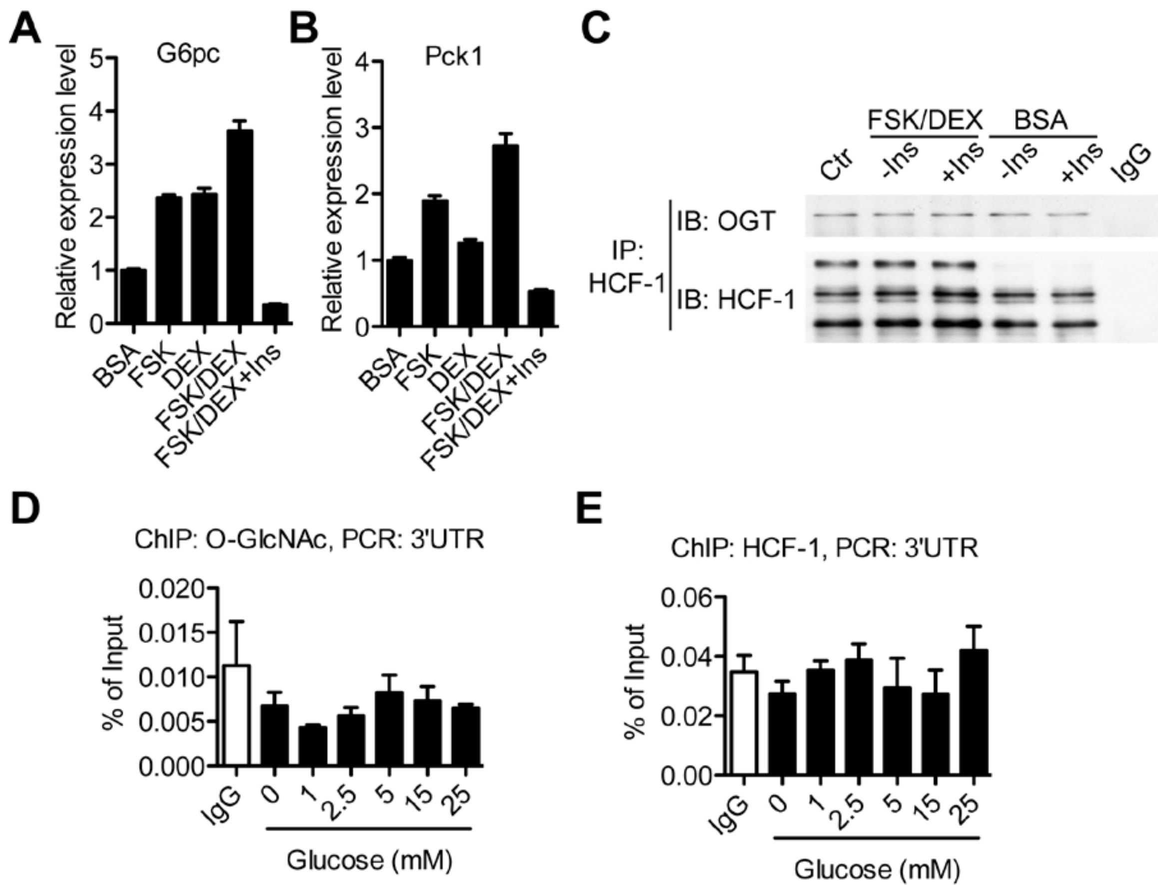

Figure S4, related to Figure 4.

(A-C) FAO cells were serum deprived overnight, then treated with forskolin (FSK, 10 μM), dexamethasone (DEX, 1 μM) or together for 6 hours, followed by insulin (Ins) treatment (100 nM, 4 hours for panel A and B, and 30 min for panel C). Real time RT-PCR analysis of gluconeogenic *G6pc* (A) and *Pck1* (B) is shown (n = 3). (C) OGT/HCF-1 interaction determined by HCF-1 immunoprecipitation.

(D and E) Primary hepatocytes were treated with different concentrations of glucose for 6 hours (n = 3), chromatin immunoprecipitation of O-GlcNAc (D) or HCF-1 (E) followed by PCR analysis using primers targeting the 3'UTR of the *G6pc* gene was performed as the negative control. All values represent mean ± SEM.

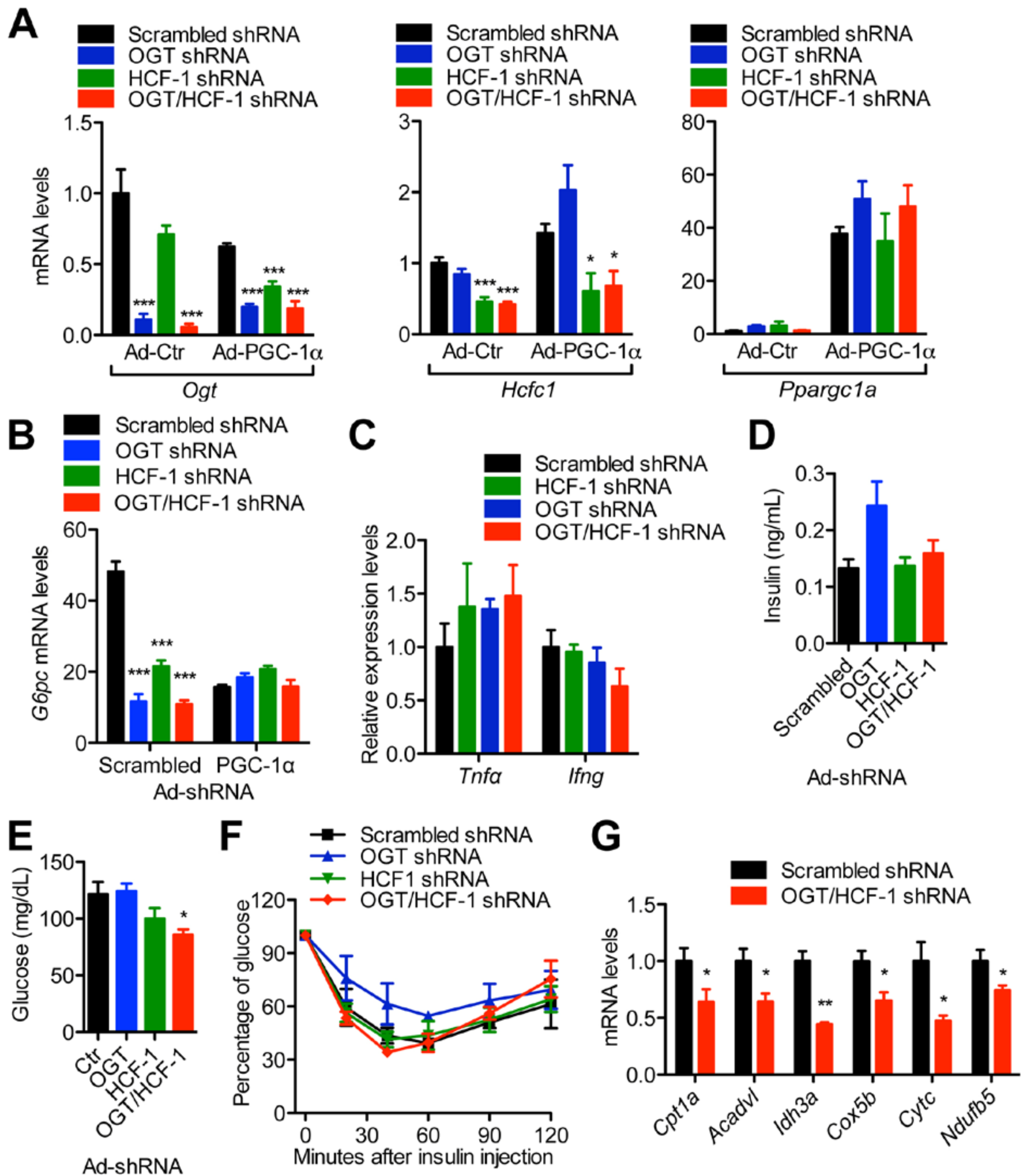

Figure S5, related to Figure 5.

(A) Primary hepatocytes from fed mice were infected with adenoviruses indicated. Expression levels of OGT, HCF-1 and PGC-1α were determined by real time PCR analysis (n = 3).

(B) Primary hepatocytes from overnight fasted mice (n = 3) were infected with adenoviruses encoding shRNA as indicated. mRNA levels of *G6pc* were determined by real time PCR.

(C-G) C57Bl/6 mice were infected with shRNA adenoviruses (n = 4-6). (C) Expression of inflammation genes, (D) fed insulin levels, (E) fed glucose levels, (F) insulin tolerance test, and (G) genes involved in fatty acid oxidation and mitochondrial respiration are shown. All values represent mean ± SEM.*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by ANOVA with a Bonferroni's post hoc test compared with scrambled shRNA.

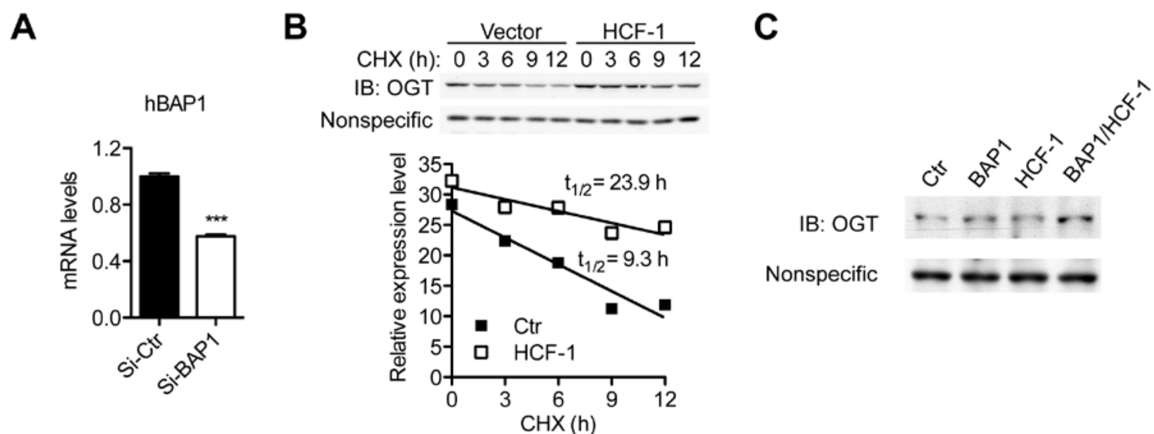

Figure S6, related to Figure 6.

(A) HEK 293T cells were transfected with shRNA against luciferase (Si-Ctr) or BAP1 (Si-BAP1). Real time PCR analysis was performed to determine the levels of *Bap1* (n = 6). ***, $P < 0.001$ by two-tailed *t*-test.

(B) Top, HEK 293T cells were transfected with or without HCF-1. Endogenous OGT was blotted after treatment of Cycloheximide (CHX) for various periods of time. Bottom, the densitometric values of OGT proteins were plotted, based on which the protein half-lives were calculated.

(C) HEK 293T cells were transfected with BAP1 and/or HCF-1, and endogenous OGT expression is shown. BAP1 and HCF-1 can increase the OGT protein level.

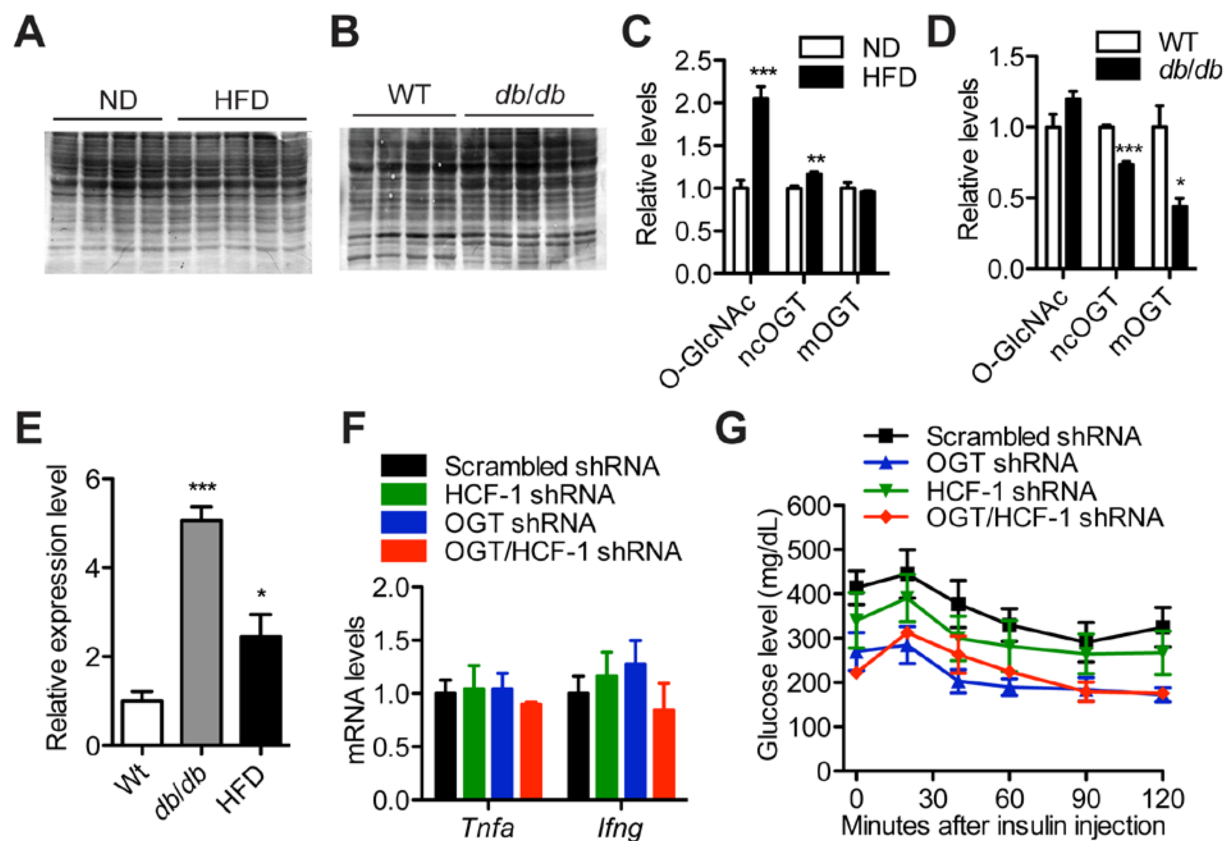

Figure S7, related to Figure 7.

(A and B) Ponceau S staining of the immunoblots shown in Figure 7A indicates equal loading of proteins.

(C and D) Densitometric analysis of O-GlcNAc, nucleocytoplasmic (nc-) and mitochondrial (m-) OGT expression in Figure 7A (n = 4-5).

(E) Densitometric analysis of HCF-1 expression in the liver of diabetic animals, related to Figure 7B (n = 4-5).

(F) Expression of inflammatory genes in the livers of adenovirus-infected *db*/*db* mice (n = 4-6).

(G) Insulin tolerance tests in *db*/*db* mice treated with adenoviruses encoding shRNA (n = 4-6). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by two-tailed *t*-test compared with wildtype (WT) or normal diet (ND) mice. All values represent mean ± SEM.

Supplemental References

Puigserver, P., Rhee, J., Lin, J., Wu, Z., Yoon, J.C., Zhang, C.Y., Krauss, S., Mootha, V.K., Lowell, B.B., and Spiegelman, B.M. (2001). Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. Mol Cell *8*, 971-982.