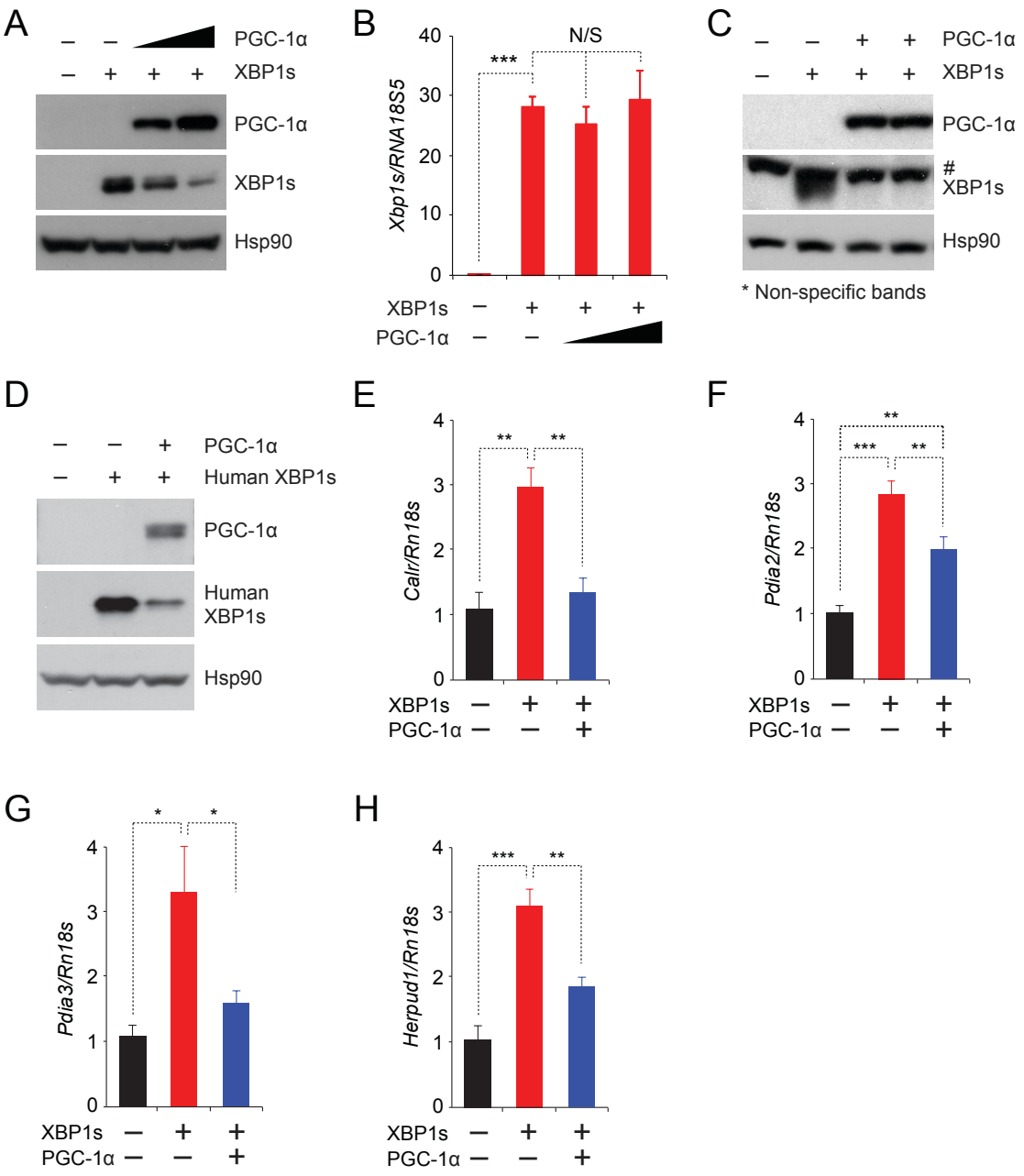
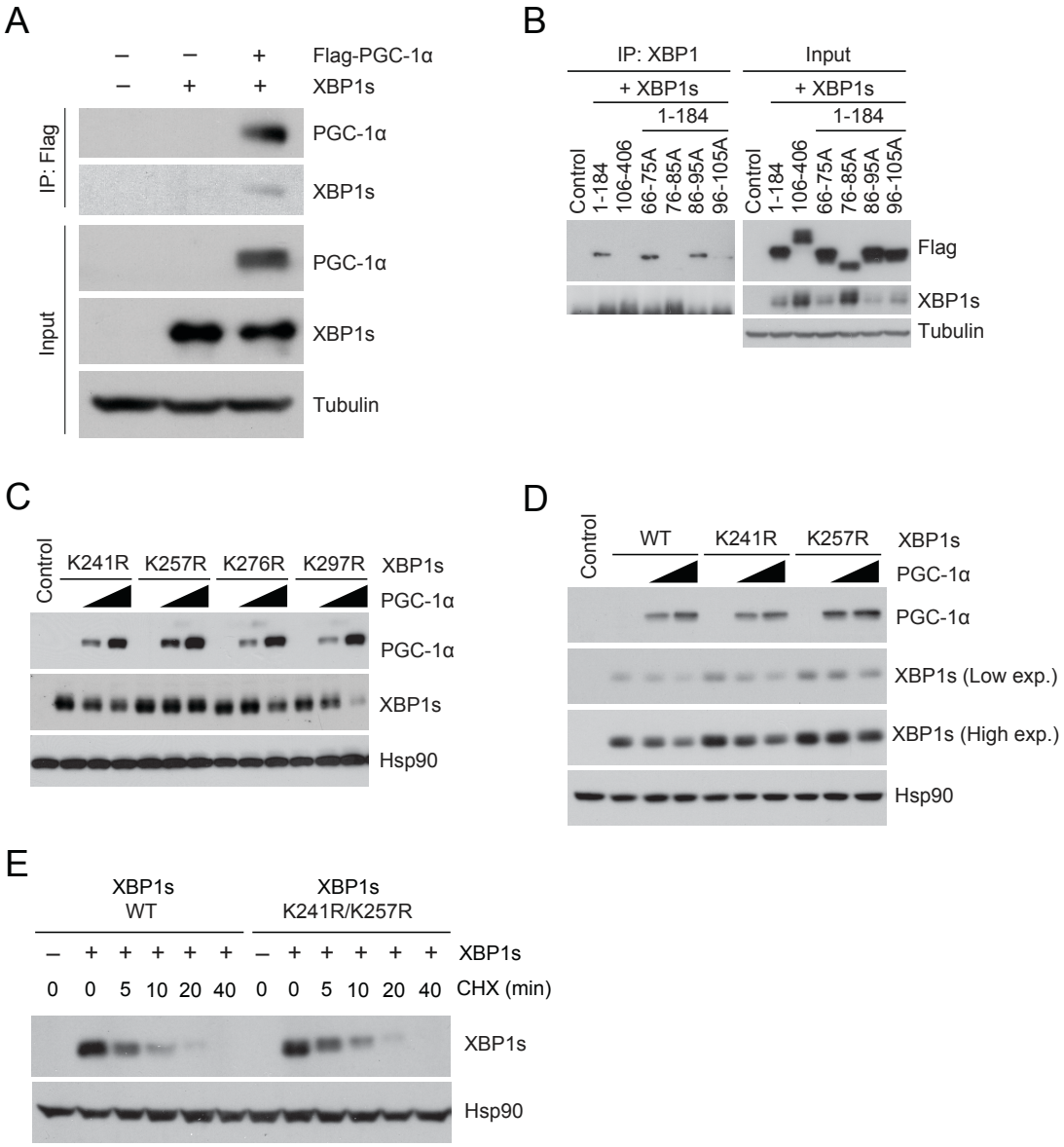
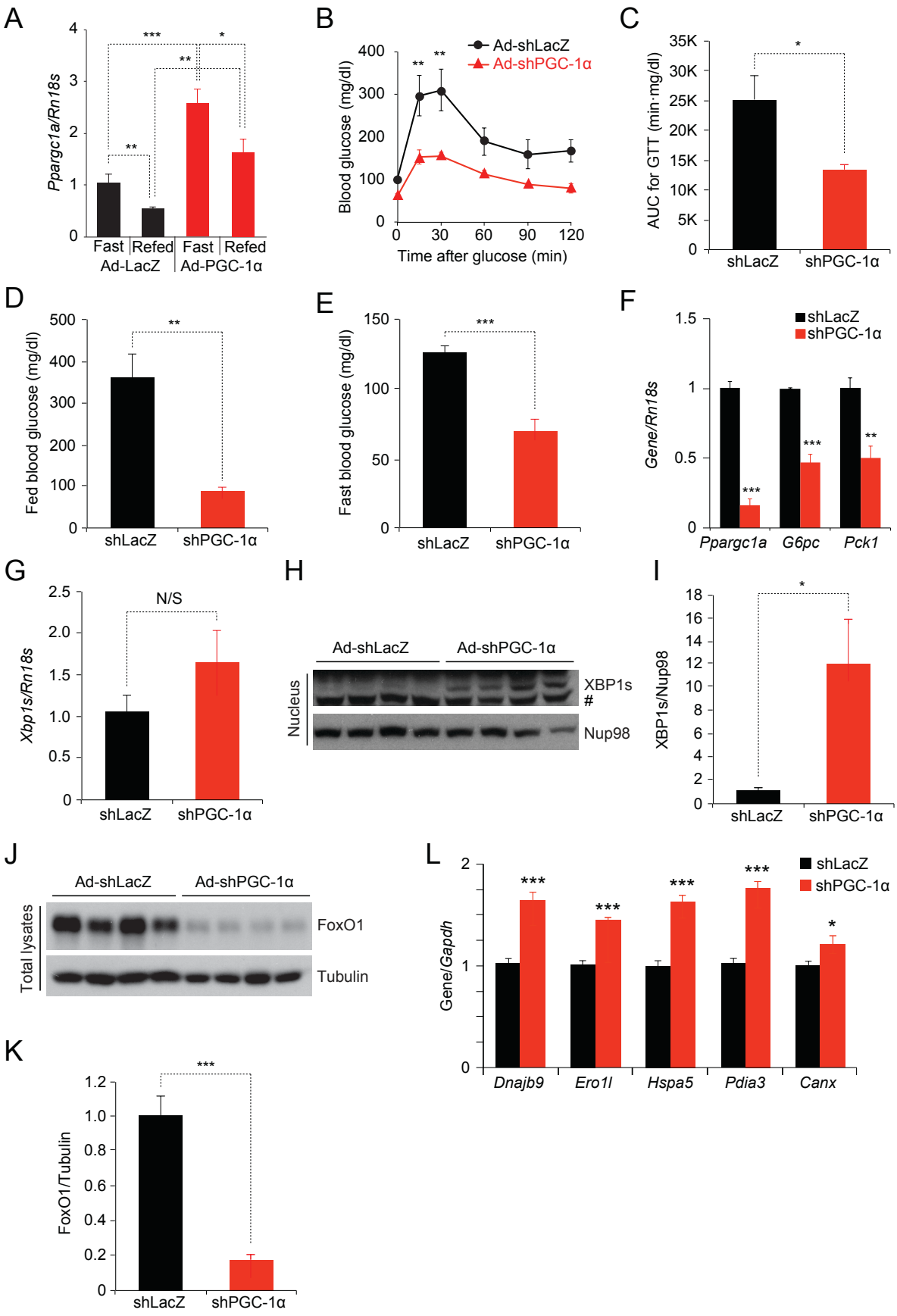


Supplementary Figure 1. PGC-1α promotes XBP1s protein degradation and inhibits XBP1s activity. (**A**,**B**) HEK293 cells were transfected with plasmids expressing XBP1s, with or without PGC-1α plasmid; the empty vector (pCDNA 3.1) was added to make the same amount of total plasmids for transfection. (**A**) Levels of XBP1s, PGC-1α and Hsp90 proteins. (**B**) *Xbp1s* mRNA levels measured by qPCR. (**C**) Levels of XBP1s, PGC-1α and Hsp90 proteins in Fao cells infected with Ad-XBP1s with or without Ad-PGC-1α. Ad-LacZ was used to normalize the amount of total virus for infection. (**D**) Human XBP1s, PGC-1α and Hsp90 protein levels. HEK293 cells were transfected with plasmids expressing human XBP1s, PGC-1α and Hsp90 proteins as indicated. (**E-H**) Mouse primary hepatocytes were isolated from eight-week old lean male mice and infected with adenoviruses expressing XBP1s or PGC-1α as indicated above. Total virus titer was normalized by adding adenovirus expressing LacZ. (**E**) *Calr*, (**F**) *Pdia2*, (**G**) *Pdia3* and (**H**) *Herpud1* mRNA levels measured by qPCR. Error bars are ± s.e.m. Significance was determined by Student's *t* test (**B**) or one-way ANOVA with Tukey *post hoc* test (**E-H**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. N/S – not significant. # Non-specific bands.



Supplementary Figure 2. The molecular interaction between PGC-1α and XBP1s.
(**A**) Co-immunoprecipitation of XBP1s and PGC-1α. HEK293 cells were transfected to express XBP1s, with or without flag-PGC-1α. Cells were lysed and immunoprecipitated with anti-flag beads. (**B**) Co-immunoprecipitation of XBP1s and different PGC-1α truncates. HEK293 cells expressing XBP1s, along with indicated flag-tagged PGC-1α constructs, were lysed and used for immunoprecipitation with anti-flag beads. (**C**) XBP1s K241R, K257R, K276R, K297R, PGC-1α and Hsp90 protein levels in HEK293 cells transfected with indicated XBP1s constructs plus increasing doses of PGC-1α plasmids. (**D**) XBP1s WT, K241R, K257R, PGC-1α and Hsp90 protein levels in HEK293 cells. Indicated XBP1s constructs were co-expressed with higher levels of PGC-1α compared to the levels used in **C**. Low or High exp. – low or high exposure of the same membrane. (**E**) XBP1s WT, XBP1s K241R/K257R and Hsp90 proteins visualized by immunoblotting. XBP1s (WT or K246R/K257R) was expressed alone, and then treated with CHX (50 μg/ml) for the indicated time.


A
Ppargc1a/Rn18s
Fast
Refed
Fast
Refed
Ad-LacZ
Ad-PGC-1α
B
Blood glucose (mg/dl)
Ad-shLacZ
Ad-shPGC-1α
Time after glucose (min)
C
AUC for GTT (min·mg/dl)
shLacZ
shPGC-1α
D
Fed blood glucose (mg/dl)
shLacZ
shPGC-1α
E
Fast blood glucose (mg/dl)
shLacZ
shPGC-1α
F
Gene/Rn18s
shLacZ
shPGC-1α
Ppargc1a
G6pc
Pck1
G
Xbp1s/Rn18s
N/S
shLacZ
shPGC-1α
H
Ad-shLacZ
Ad-shPGC-1α
Nucleus
XBP1s
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Nup98
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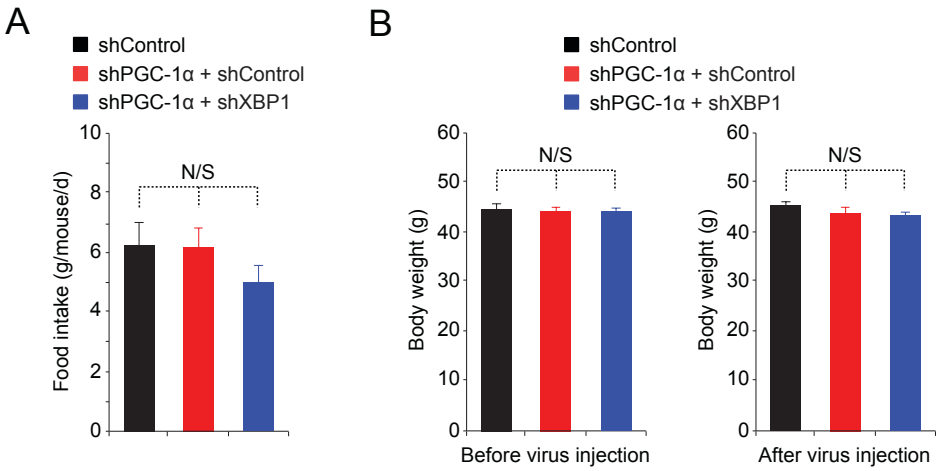
XBP1s/Nup98
shLacZ
shPGC-1α
J
Ad-shLacZ
Ad-shPGC-1α
Total lysates
FoxO1
Tubulin
K
FoxO1/Tubulin
shLacZ
shPGC-1α
L
Gene/Gapdh
shLacZ
shPGC-1α
Dnajb9
Ero1l
Hspa5
Pdia3
Canx

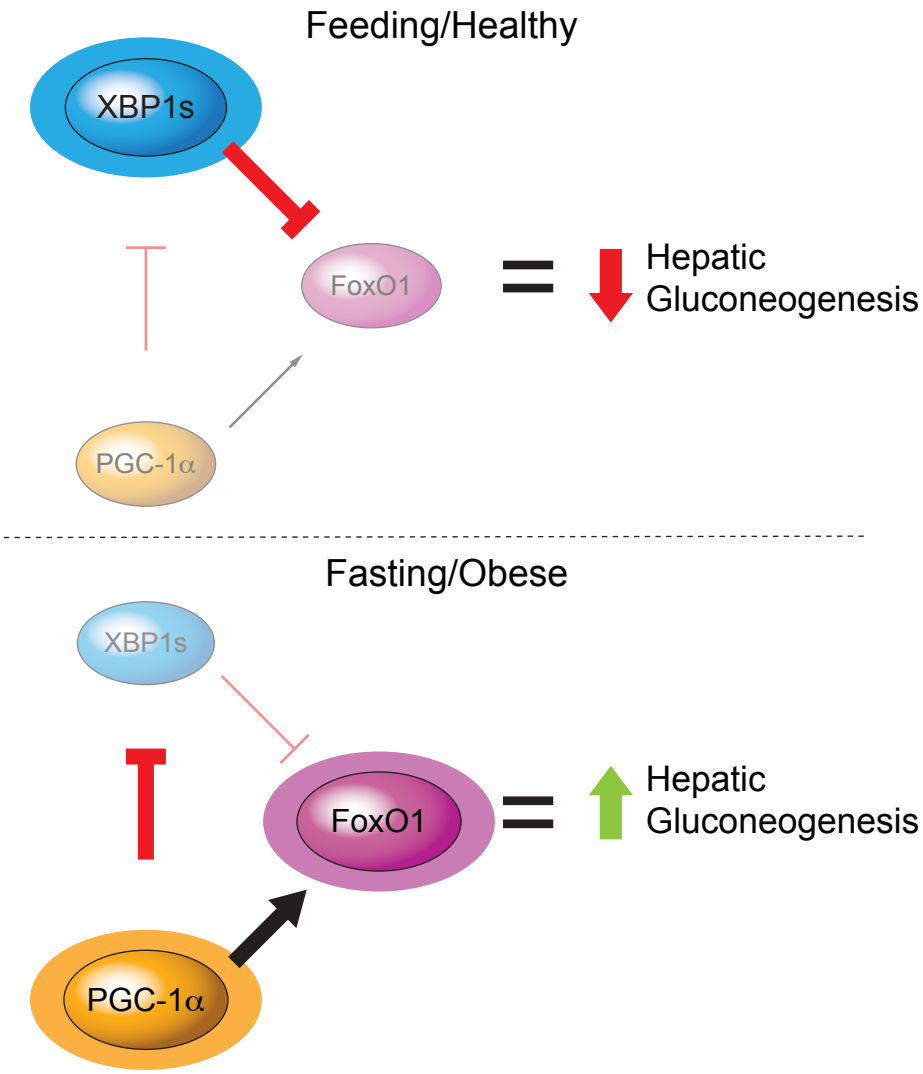
Supplementary Figure 3. Reduced PGC-1α expression in the liver of obese mouse improves glucose homeostasis and restores XBP1s activity.

(**A**) PGC-1α mRNA (*Ppargc1a*) levels in the liver of lean mice during 24 h fast and 1 h refeeding. Eight week-old lean male mice were infected with Ad-LacZ or Ad-PGC-1α (1.2×10^8 PFU/g, n = 4 for each) via tail-vein injection. (**B**-**L**) Eight week-old *ob/ob* mice received Ad-shLacZ (n = 5) or Ad-shPGC-1α (6×10^7 PFU/g, n = 4) via tail-vein injection and liver was collected from 6 h-fasted mice on day 8 after virus injection. (**B**) Glucose tolerance test (GTT, glucose 0.5 g/kg) on day 4 after virus injection and (**C**) area under the curve (AUC) of GTT in **A**. (**D**) Fed and (**E**) six-hour fast blood glucose. (**F**) mRNA levels of *Ppargc1a, G6pc* and *Pck1* in the liver. (**G**) mRNA levels of *Xbp1s* in the liver. (**H**) XBP1s and Nup98 proteins in nuclear extracts from the liver and (**I**) quantified XBP1s protein levels in **H**. (**J**) FoxO1 and tubulin proteins in the total liver lysates and (**K**) quantified FoxO1 protein levels in **J**. (**L**) *Dnajb9*, *Ero1l*, *Hspa5*, *Pdia3* and *Canx* mRNA levels in the liver.



Supplementary Figure 4. Reducing PGC-1α expression in the obese mouse liver improves glucose homeostasis via XBP1s.

(**A**,**B**) Eight week-old *ob/ob* mice received adenoviruses expressing shControl, shPGC-1α plus shControl, or shPGC-1α plus shXBP1. (**A**) Daily food intake after virus administration. (**B**) Body weight of mice before and 6 day after virus injection (n = 6 for each). The experiment was independently repeated in three independent cohorts. Values are means ± s.e.m. Significance was determined by one-way ANOVA. N/S – not significant.



Supplementary Figure 5. The molecular interaction among PGC-1α, XBP1s and FoxO1 in the liver. During feeding in healthy conditions, PGC-1α expression and activity in the liver are diminished, which leads to an increased XBP1s protein amount and activity. Reduced PGC-1α and accompanied increased XBP1s action work together to suppress hepatic FoxO1 activity leading to reduction of glucose production in the liver. In contrast, in fasting or in obese conditions, increased PGC-1α expression in the liver leads to XBP1s degradation and consequently reduction of XBP1s activity. Increased level of PGC-1α together with lessened XBP1s action in fasting or in obese condition promotes hepatic FoxO1 activity, thus resulting in increased hepatic gluconeogenesis in the liver.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Biochemical Reagents

Anti-XBP1 (Cat. sc-7160), anti-PGC-1α (Cat. sc-13067), anti-β-actin (Cat. sc-47778), anti-HSP90 (Cat. sc-13119), and anti-GAPDH (Cat. sc-25778), along with horseradish peroxidase (HRP)-conjugated secondary antibodies, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Flag antibody (Clone M2, cat. F3165) and anti-Flag M2 affinity gel were from Sigma Aldrich (St. Louis, MO). Anti-NUP98 (Clone C39A3, cat. 2598), anti-lamin A/C (Cat. 2032), anti-FoxO1 (Clone C29H4, cat. 2880), anti-ubiquitin (Clone P4D1, cat. 3936), anti-α-tubulin (Clone 11H10, cat. 2125) and conformation-specific anti-rabbit conjugated with HRP were from Cell Signaling Technology (Beverley, MA). Fetal bovine serum, Dulbecco's Modified Eagle Medium (DMEM), RPMI 1640, Hank's Balanced Salt Solution (HBSS), Williams' Media E, Opti-MEM, Penicillin/Streptomycin, SYBR Green, chemically competent *E. coli*, Lipofectamine 2000, rat tail collagen I, LR Clonase II and the nuclear extraction kit for tissues were from Thermo Fisher Scientific (Waltham, MA). Restriction endonucleases, Antarctic phosphatase and T4 DNA ligase were from New England Biolabs (Ipswich, MA). The cDNA Synthesis Kit, SYBR Green and the detergent-compatible protein assay kit were from BIO-RAD (Hercules, CA). The site-directed mutagenesis PCR kit and PfuUltra high-fidelity DNA polymerase were from Agilent Technologies (Santa Clara, CA). BM Chemiluminescence Blotting Substrate, protease and phosphatase inhibitors were from Roche (Indianapolis, IN). Cycloheximide, tunicamycin and MG-132 were from EMD

Millipore (Billerica, MA). Type 1 collagenase was from Worthington Biochemical (Lakewood, NJ). Percoll and glucagon were from Sigma Aldrich (St. Louis, MO).

Plasmids

Plasmids encoding flag-tagged full-length PGC-1α and flag-tagged truncated PGC-1α constructs (1-400), (406-769), (1-180), (1-184) and (185-406), were kindly provided by Pere Puigserver (Harvard Medical School, Boston, MA). Flag-tagged PGC-1α (1-105) was generated with use of a QuikChange II Site-Directed Mutagenesis Kit (Agilent), by adding a stop codon into Flag-tagged PGC-1α (1-184): this was achieved using the following primer paired with its complement: 5'-GGATGAAGACGGATTGCCCTGATAAGATGCACTGACAGATGGAGCCG-3'.

For creating flag-tagged PGC-1α (34-184), the first PCR was performed with Pfu DNA polymerase (Agilent), using flag-tagged PGC-1α (1-184) as a template, plus the following pair of primers: forward, 5'-ATGGACTACAAAGACGATGACGATAAAGCAAGAAGAGCATCTGTGGGATCC CTTGACCTTTCTGAACTTGATGTG-3' and reverse, 5'-TGTCCAGTGTCTCTGTGAGGACCGCTAGCAAGTTTGCC-3'. The first PCR product was served as a template for the second PCR, using the following forward primer and the same reverse primer used for the first PCR: 5'-GCTTGGTACCGAGCTCGGATCCACTAGTCCAGTGTGGTGGAATTCTGCAGA TATCCGGATCACCACCATGGACTACAAAGACGATGACG-3'. The final amplified product from the second PCR was digested with the restriction enzymes KpnI and NheI, and inserted into PGC-1α (1-184) pCDNA, in which the

N-terminal part of PGC-1α had been removed by digestion with KpnI and NheI, thereby creating flag-tagged PGC-1α (34-184) in pCDNA. For generating flag-tagged PGC-1α (67-184), the first PCR was done with Pfu DNA polymerase, with PGC-1α (1-184) as a template, and with the following primer pair: forward, 5'-ATGGACTACAAAGACGATGACGATAAAGCAAGAAGAGCATCTGTGGGATCC TACAACAATGAGCCTGCGAACATATTTG-3' and reverse, 5'-TTTTGTTCGAAGGGCCCTCTAGACTCGAGCGGCCGCC-3'. The second PCR was conducted with the first PCR product as a template, the same forward primer as used in the second PCR to generate PGC-1α (34-184), and the same reverse primer used in the first PCR to generate PGC-1α (67-184). The amplified PCR product was digested with KpnI and NheI, and introduced into pCDNA as described above to produce PGC-1α (67-184) in pCDNA as a final product.

Site-directed mutations of indicated amino acids to alanine within PGC-1α 1-184, or full-length PGC-1α, were achieved with the use of the QuikChange II Site-Directed Mutagenesis Kit and the following primers paired with their complements: for 66-75A, 5'-CGGAAATCATATCCAACGCGGCCGCCGCTGCGGCTGCGGCCGCAGCTGAG AAGATAGATG-3'; for 76-85A, 5'-CTGCGAACATATTTGCGGCGGCAGCTGCAGCGGCTGCGGCAGCCTTGCTA GCGGTCCTC-3'; for 86-95A, 5'-GAGAATGAGGCAAACGCGGCAGCGGCCGCCGCAGCGGCAGCGGCCAGTC TCCCCGTGG-3'; and for 96-105A, 5'-

CACAGAGACACTGGACGCTGCCGCCGCGGCTGCAGCCGCAGCGGCCTCAT
TTGATGCAC-3'.

Full-length XBP1s and flag-tagged XBP1s were produced as described (Lee et al., 2011b). Flag-tagged XBP1s (1-126) was generated with Pfu DNA polymerase, using flag-tagged XBP1s as a template and the following primers: forward, 5'- AATTCAGTCGACATGGACTACAAGGATGACG-3' and reverse, 5'-GCGGCCGCGAATTCTTATAACTCCTGGTTCTCAACC-3'.

Amplified product was ligated to the pENTR vector to produce flag-tagged XBP1s (1-126) pENTR, after digestion with the SalI and NotI restriction enzymes. Then, the encoding region of XBP1s (1-126) was transferred from pENTR to pCDNA by recombination, using LR clonase II and the targeting vector pCDNA-DEST40 (Thermo Fisher Scientific). The flag-tagged XBP1s truncates 1-226, 1-252, 1-276 and 1-326 were generated by adding stop codons to pCDNA encoding flag-tagged XBP1s, using the QuikChange II Site-Directed Mutagenesis Kit, along with the use of the primers listed below that were paired with their complement primers:

For 1-226, 5'-GGACCTAGTTCCTAATGAGCCTCCCTTTCTC-3'; for 1-252, 5'-
CCATTAATGAACTCATTCGTTTTGACTAGTGATACACCAAGCCTCTAGTTTTA
GAGATCC-3'; for 1-276, 5'-CTAACGTGGTAGTGAAATAATAGGAAGCACCTCTAAGC-3'; and for 1-326, 5'-CTGAGACCACCTTCTTGCTAGTAGGACGCTCACAGTGACTG-3'.

XBP1s with lysines mutated to arginines were made with the QuikChange II Site-Directed Mutagenesis Kit, using XBP1s pCDNA as a template, plus the following primers paired with their complements:

For K204R, 5'-GGACCCTGTCATGTTTTTCAGATGTCCTTCCCCAGAGTCTGC-3'; for K241R, 5'-CCTCATCAGCCAGGCTGGAAGCC-3'; for K257R, 5'-CCATGTATACACCAGGCCTCTAGTTTTAGAGATCC-3'; for K276R, 5'-CTAACGTGGTAGTGAGAATTGAGGAAGCACC-3'; and for K297R, 5'-CCTGAATTCATTGTCTCAGTGAGGAAAGAGCCTTTGG-3'.

Double lysine mutations were introduced sequentially: a single lysine was changed to arginine, using a pair of primer sets described above, and plasmids (validated by sequencing) were used as a template for the second mutagenesis PCR, to mutate another lysine to arginine. All the constructs we made were verified by sequencing.

Animal Experiments

Eight week-old lean male mice (C57BL7/J) were infected with Ad-LacZ or Ad-PGC-1α (1.2 x 10^8 PFU/g) via tail-vein injection. 5 days later, the mice were fasted for 24 h, then re-fed for 1 h.

For glucagon administration, eight week-old lean male mice were infected with Ad-LacZ or Ad-XBP1s (4 x 10^7 PFU/g) via tail-vein injection. 5 days later, 4 h-fasted mice received either saline or glucagon (500 μg/kg) by intraperitoneal injection. The liver was collected 2 h after the treatment.

Eight week-old *ob/ob* mice received Ad-shLacZ or Ad-shPGC-1α (6 x 10^7 PFU/g) via tail-vein injection. Glucose tolerance test (GTT, glucose 0.5 g/kg) was conducted on day 4 after virus injection. For 24 h fasting and refeeding, eight week-old *ob/ob* mice received Ad-shLacZ or Ad-shPGC-1α (6 x 10^7 PFU/g) via tail-vein injection. Mice were fasted for 24 h and then re-fed for 1 h or 3h on day 7 after virus injection.

For combined silencing of PGC-1α and XBP1s in the liver, eight week-old *ob/ob* mice received Ad-shControl (either Ad-shLacZ or Ad-Scrambled shRNA), Ad-shPGC-1α plus Ad-shControl or Ad-shPGC-1α plus Ad-shXBP1 via tail vein. GTT (glucose 0.5 g/kg) was performed on day 4 or day 5 after virus injection. The details of four cohorts' experiments are explained below.

Exp. 1 – Total 6 x 10^7 PFU/g of virus was used. GTT on day 5 after virus injection

Group 1: Ad-shControl (Ad-shLacZ, 6 x 10^7 PFU/g)

Group 2: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shLacZ (3 x 10^7 PFU/g)

Group 3: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shXBP1 (3 x 10^7 PFU/g)

Exp. 2 – Total 6 x 10^7 PFU/g of virus was used. GTT on day 4 after virus injection

Group 1: Ad-shControl (Ad-Scrambled shRNA, 6 x 10^7 PFU/g)

Group 2: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shLacZ (3 x 10^7 PFU/g)

Group 3: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shXBP1 (3 x 10^7 PFU/g)

Exp. 3 – Total 6 x 10^7 PFU/g of virus was used. GTT on day 5 after virus injection

Group 1: Ad-shControl (Ad-Scrambled shRNA, 6 x 10^7 PFU/g)

Group 2: Ad-shPGC-1α (2 x 10^7 PFU/g) plus Ad-shLacZ (4 x 10^7 PFU/g)

Group 3: Ad-shPGC-1α (2 x 10^7 PFU/g) plus Ad-shXBP1 (4 x 10^7 PFU/g)

Exp. 4 – Total 7 x 10^7 PFU/g of virus was used. GTT on day 5 after virus injection

Group 1: Ad-shControl (Ad-Scrambled shRNA, 7 x 10^7 PFU/g)

Group 2: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shLacZ (4 x 10^7 PFU/g)

Group 3: Ad-shPGC-1α (3 x 10^7 PFU/g) plus Ad-shXBP1 (4 x 10^7 PFU/g)

Western Blot Analysis

Protein samples from cell or tissue lysates or from immunoprecipitation were resolved by SDS-PAGE, and then transferred to polyvinylidene fluoride (PVDF) membrane. After blocking for 1h at room temperature in 10% blocking reagent (Roche), the membrane with proteins was incubated overnight at 4°C with a primary antibody in Tris-buffered saline solution/Tween (TBST) containing 10% blocking reagent, then washed three times in TBST, and incubated with secondary antibody for 1 h at room temperature. After three washes in TBST, the membrane was developed using a chemiluminescence assay system (Roche) and exposed to X-ray film. Relative protein levels were quantified by the Image J program. The membrane was then stripped by shaking it vigorously in stripping buffer (62.5 mM Tris-HCl, pH 6.7; 2% SDS; 100 mM 2-mecaptomethanol) at 50°C for 20 min, then washed three times in TBST, and used to detect another protein.

Adenovirus Production and Infection

Adenovirus expressing XBP1s was produced as described (Zhou et al., 2011); adenovirus expressing PGC-1α, and shRNA against murine PGC-1α, were obtained from Pere Puigsever (Harvard Medical School, Boston, MA). Adenovirus expressing shPGC-1α was reported previously (Koo et al., 2004). Adenovirus expressing PGC-1α (76-85A) was generated with use of the ViraPower Adenoviral Expression System (Thermo Fisher Scientific) and adenovirus expressing shRNA against XBP1 with BLOCK-iT™ Adenoviral RNAi Expression System (Thermo Fisher Scientific), as per manufacturer's instructions. ShRNA sequences for XBP1 silencing are 5′-CCAGGAGTTAAGAACACGCTT- 3′ and was described previously (Lee et al., 2011a). PGC-1α (76-85A) in pENTR-3C was generated by mutagenesis PCR as described above, using the same primers into PGC-1α pENTR-3C. PGC-1α (76-85A) was transferred into the pAD-CMV vector via recombination, using LR recombinase II (Thermo Fisher Scientific), according to manufacturers' instructions; PGC-1α (76-85A) pAD-CMV was linearized by the PacI restriction enzyme, then Lipofectamine 2000 was used to transfect it into HEK293A cells. When the cytopathic effect reached 80%, cells were collected by centrifugation, the pellet was resuspended in PBS, and subjected four times to freezing and thawing cycles at -80°C and 37°C. Supernatant containing the virus was prepared by centrifugation at 4,000 rpm for 20 min at room temperature. For infection, cells were incubated with adenovirus in a reduced volume of medium containing 1% FBS and antibiotics. Cells were gently rocked every 15 min for a total of 1 h to increase the efficiency of infection,

then fresh medium was added, and cells were incubated for an additional 15 h or 23 h. Equal amount of virus was used for infection by normalizing with Ad-LacZ. For animal infection, adenoviruses were amplified and purified by VectorBioLab (Malvern, PA).

Real-Time Quantitative PCR (qPCR)

Total RNA was isolated from cells, or from mouse liver tissues, using QIAzol and TissueLyser II (Qiagen), according to the manufacturer's instructions; cDNA was generated with a cDNA Synthesis Kit (Bio-Rad). Gene expression was analyzed with SYBR Green (Bio-Rad or Thermo Fisher Scientific), and with the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad). The mRNA levels were normalized against ribosomal RNA, *Rn18s* (mouse) or *RNA18S5* (human). Primer sequences used for qPCR were as follows:

Rn18s forward: 5'-AGTCCCTGCCCTTTGTACACA-3';

Rn18s reverse: 5'-CGTTCCGAGGGCCTCACT-3';

RNA18S5 forward: 5'-GTAACCCGTTGAACCCCATT-3';

RNA18S5 reverse: 5'-CCATCCAATCGGTAGTAGCG-3';

Canx forward: 5'-ATGGAAGGGAAGTGGTTACTGT-3';

Canx reverse: 5'-GCTTTGTAGGTGACCTTTGGAG-3';

Calr forward: 5'-CCTGCCATCTATTTCAAAGAGCA-3';

Calr reverse: 5'-GCATCTTGGCTTGTCTGCAA-3';

Hspa5 forward: 5'-TCATCGGACGCACTTGGAA-3';

Hspa5 reverse: 5'-CAACCACCTTGAATGGCAAGA-3';

Ero1l forward: 5’-TCAGTGGACCAAGCATGATGA-3’;

Ero1l reverse: 5’-TCCACATACTCAGCATCGGG-3’;

Dnajb9 forward: 5’-CCCCAGTGTCAAACTGTACCAG-3’;

Dnajb9 reverse: 5’-AGCGTTTCCAATTTTCCATAAATT-3’;

Pdia2 forward: 5’-CAAGATCAAGCCCCACCTGAT-3’;

Pdia2 reverse: 5’-AGTTCGCCCCAACCAGTACTT-3’;

Pdia3 forward: 5’-GAGGCTTGCCCCTGAGTATG-3’;

Pdia3 reverse: 5’-GTTGGCAGTGCAATCCACC-3’;

Edem1 forward: 5’-AAGCCCTCTGGAACTTGCG-3’;

Edem1 reverse: 5’-AACCCAATGGCCTGTCTGG-3’;

Herpud1 forward: 5’-CATGTACCTGCACCACGTCG-3’;

Herpud1 reverse: 5’-GAGGACCACCATCATCCGG-3’;

G6pc forward: 5’-CCGGTGTTTGAACGTCATCT-3’;

G6pc reverse: 5’-CAATGCCTGACAAGACTCCA-3’;

Pck1 forward: 5’-ATCATCTTTGGTGGCCGTAG-3’;

Pck1 reverse: 5’-ATCTTGCCCTTGTGTTCTGC-3’;

Ppargc1a forward: 5’-TGATGTGAATGACTTGGATACAGACA-3’;

Ppargc1a reverse: 5’-CAATGCCTGACAAGACTCCA-3’;

Xbp1s forward: 5’-GGTCTGCTGAGTCCGCAGCAGG-3’;

Xbp1s reverse: 5’-AGGCTTGGTGTATACATGG-3’.

XBP1 Splicing Assay

The XBP1 splicing assay was performed as described in ref. (Park et al., 2010). cDNA was synthesized from total RNA, and the following primers were used for PCR: Forward: 5'-ACACGCTTGGGAATGGACAC-3';

Reverse: 5'-CCATGGGAAGATGTTCTGGG-3'.

PCR was carried out under the following conditions: 94°C for 3 min; 29 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec; finally, samples were incubated at 72°C for 3 min. Product amplification was verified via agarose gel electrophoresis.

Cell Culture

HEK293A and HEK293T cells were purchased from Thermo Fisher Scientific; Fao, HepG2, HEK293 and MEF cells were from American Type Tissue Collection (ATCC). HEK293A, HEK293T, HEK293, HepG2 and MEF cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (vol/vol) fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C and 5 % (vol/vol) CO_2. Fao cells were maintained in RPMI 1640 medium supplemented with 10% (vol/vol) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin.

Protein Sequence Alignment

XBP1s protein sequences from different mammalian species were obtained from National Center for Biotechnology Information (NCBI) and sequence alignment

was conducted using ClustalX software (www.clustal.org) (Larkin et al., 2007). NCBI information of XBP1s protein sequences is as follows:

Human XBP1s (*Homo sapiens*, NP_001073007), Rhesus monkey XBP1s (*Macaca mulatta*, NP_001258668), Gorilla XBP1s (*Gorilla gorilla,* ABM46688), Cattle XBP1s (*Bos taurus*, NP_001258666), Pig XBP1s (*Sus scrofa*, NP_001258667), Mouse Xbp1s (*Mus musculus*, NM_001271730) and Rat Xbp1s (*Rattus norvegicus*, NP_001258660).

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