Supporting Information

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SI Text

Antibodies and Reagents. Reagents used in this study were obtained from the following sources: Rabbit polyclonal antibody to HIF-1α from Cayman Chemical (10006421); rabbit polyclonal antibody to HSP90 from Santa Cruz Biotechnology (sc-79470); mouse monoclonal antibody to tubulin from Millipore (05-829); anti-FLAG M2 agarose from Sigma (A2220); rabbit polyclonal antibodies to phospho-S6 (Cell Signaling; 4857); rabbit monoclonal antibody to cleaved caspase-3 (Cell Signaling; 9664); phospho-Akt (T308) (Cell Signaling; 4056); phospho-Akt (S473) (Cell Signaling; 9271); phospho-PKA substrate (RRXpS/T) (Cell Signaling; 9621); mouse monoclonal antibody to S6 (Cell Signaling; 2317); phospho (T1462) TSC2 (Cell Signaling; 3617); guinea pig polyclonal antibody to insulin (Zymed; 180067). Dimethyloxalylglycine (DMOG) from Frontier Scientific (D1070); rapamycin from LC laboratories (R-5000); forskolin from Calbiochem; PP242 from Sigma (P0037); trypan blue solution from Sigma (T8154); LY294002 from Cell Signaling Technology (9901); H89 from Calbiochem (371963); streptozotocin from Sigma (S0130); cycloheximide from Calbiochem (239674).

Plasmids expressing cDNAs for HA-HIF-1α P402A/P564A (18955) were purchased from Addgene. HRE-LUC reporter construct was generously provided by Randall Johnson (UCSD). We constructed RNAi-adenoviruses expressing U6 promoter driven short hairpin RNAs directed against mouse and rat HIF-1α (GGGCAGTCAATGGATGAGAGTG) cDNAs.

Lactate and ATP Measurement. For lactate measurements, batches of 50 size-matched primary islets were cultured in RPMI medium without phenol red. After the indicated forskolin exposures, the islets were placed in fresh medium and incubated for 4 h at 37 °C. For ATP measurements, batches of 50 size-matched primary islets were exposed to forskolin, harvested, and boiled in 200 μl Tris-HCl pH 7.75, 4 mM EDTA for four minutes.

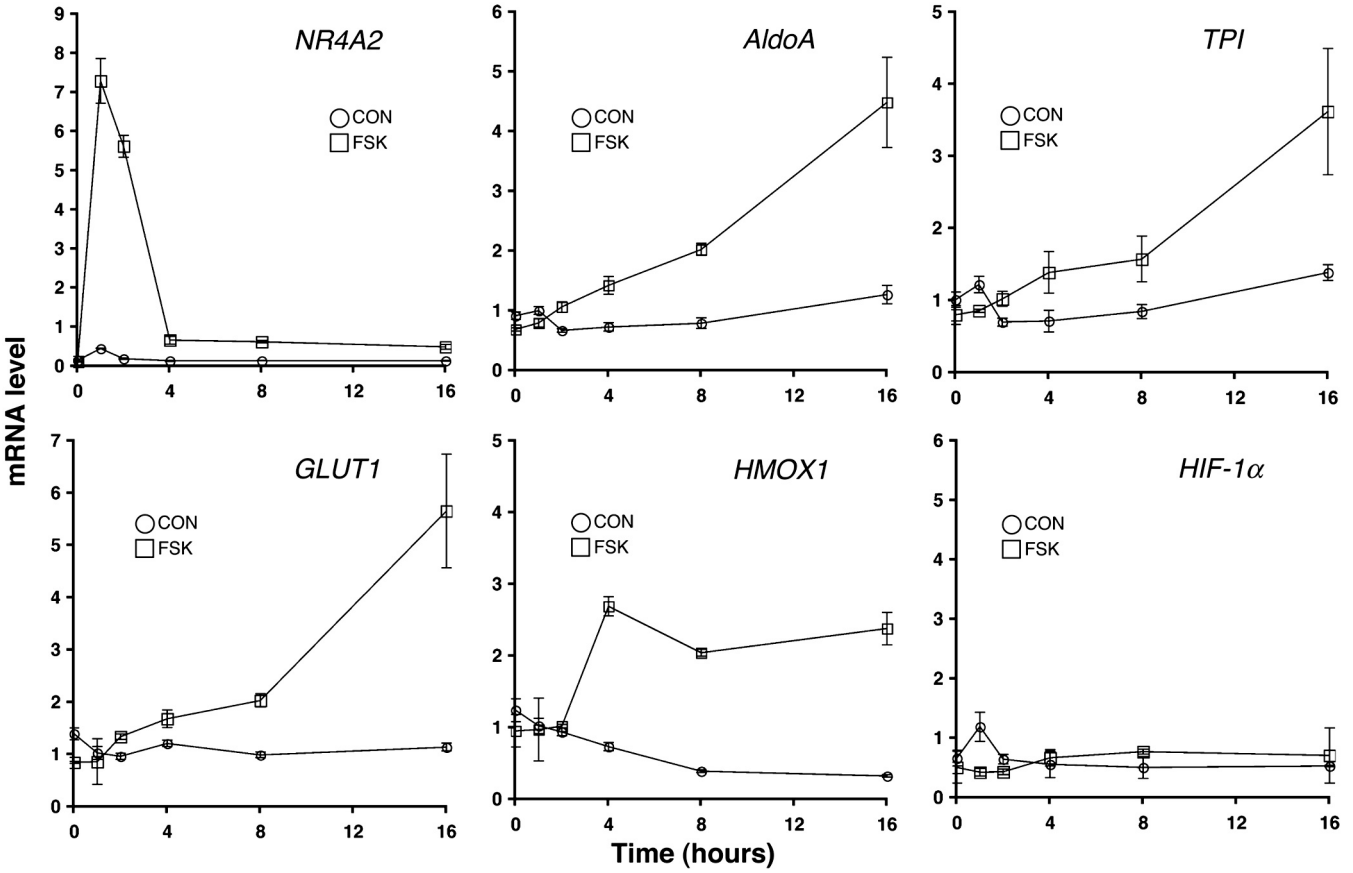

Fig. S1. Q-PCR analysis of early (NR4A2) and late (AldoA, TPI, Glut 1, HMOX1) cAMP inducible genes identified in gene profiling assays of INS-1 cells exposed to FSK. Effect of FSK exposure for different times shown.

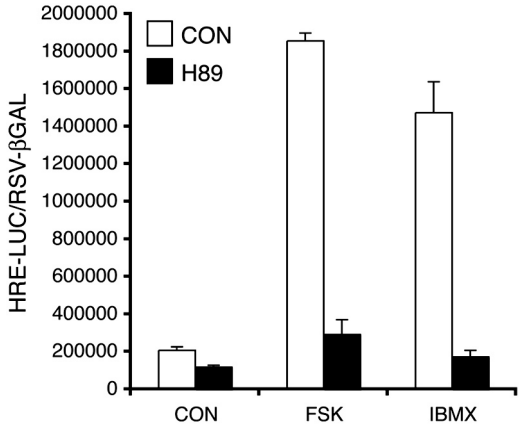

Fig. S2. Transient assay showing effect of FSK or IBMX on HRE-luciferase reporter activity in INS-1 cells. Coincubation with PKA inhibitor H89 shown.

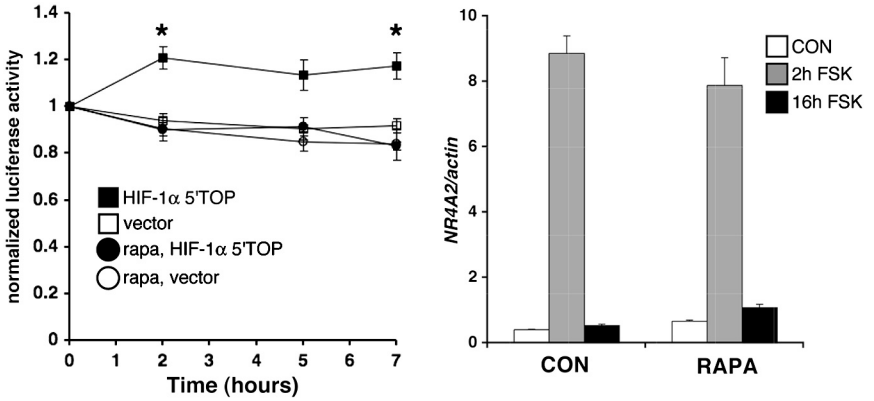

Fig. S3. (*Left*) Transient assay of a HIF-1α-luciferase translational reporter in INS-1 cells exposed to FSK and rapamycin as indicated. Fold-induction of HIF1α-luc or control luc vector activity in cells exposed to FSK versus unstimulated cells indicated. (*; $P < 0.05$. Data are means ± s.d.) (*Right*) Q-PCR analysis of NR4A2 mRNA amounts in INS-1 cells exposed to FSK for 2 or 16 h. Effect of rapamycin treatment shown.

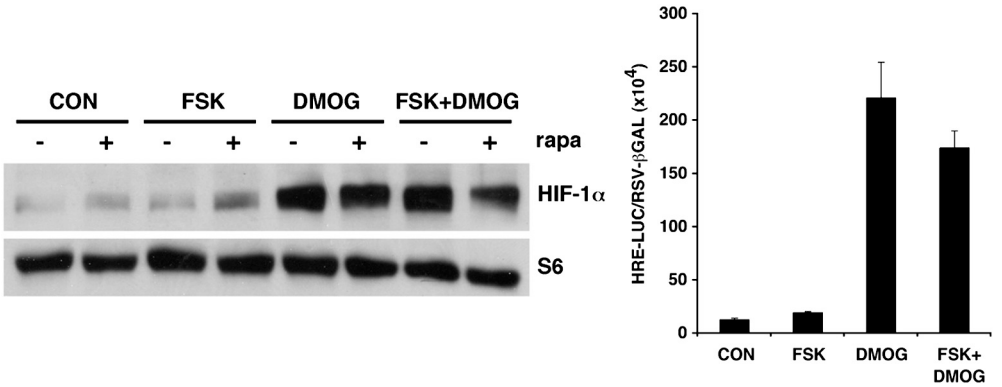

Fig. S4. (*Left*) Immunoblot showing effects of FSK and DMOG on accumulation of HIF1α in primary hepatocytes. Treatment with rapamycin indicated. (*Right*) Transient assay of HRE-luc reporter activity in hepatocytes exposed to FSK and DMOG, alone or in combination.

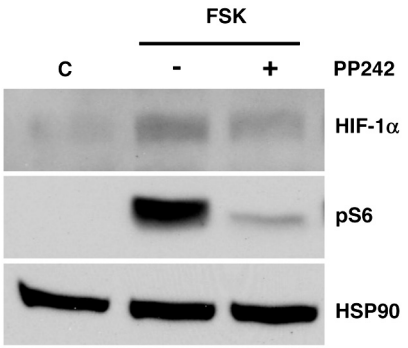

Fig. S5. Immunoblot showing effect of FSK on HIF1α accumulation and S6 phosphorylation. Cotreatment with ATP competitive mTOR inhibitor PP242 indicated.

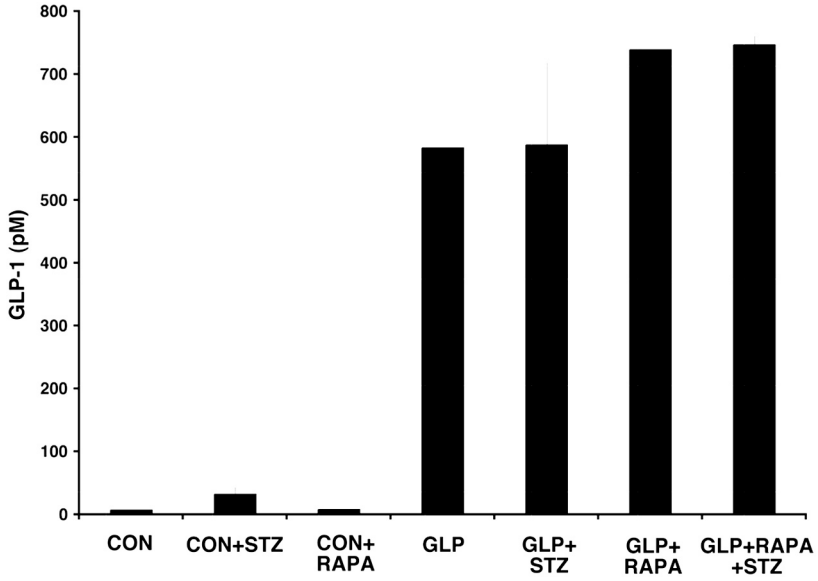

Fig. S6. Circulating concentrations of GLP1 in mice expressing Adenoviral GLP-1 (GLP) or control Ad-GFP (control). Administration of Rapamycin or STZ indicated. GLP-1 levels were determined by Elisa Assay. Assays were performed on blood samples from mice described in Fig. 3 *E* and *F*.

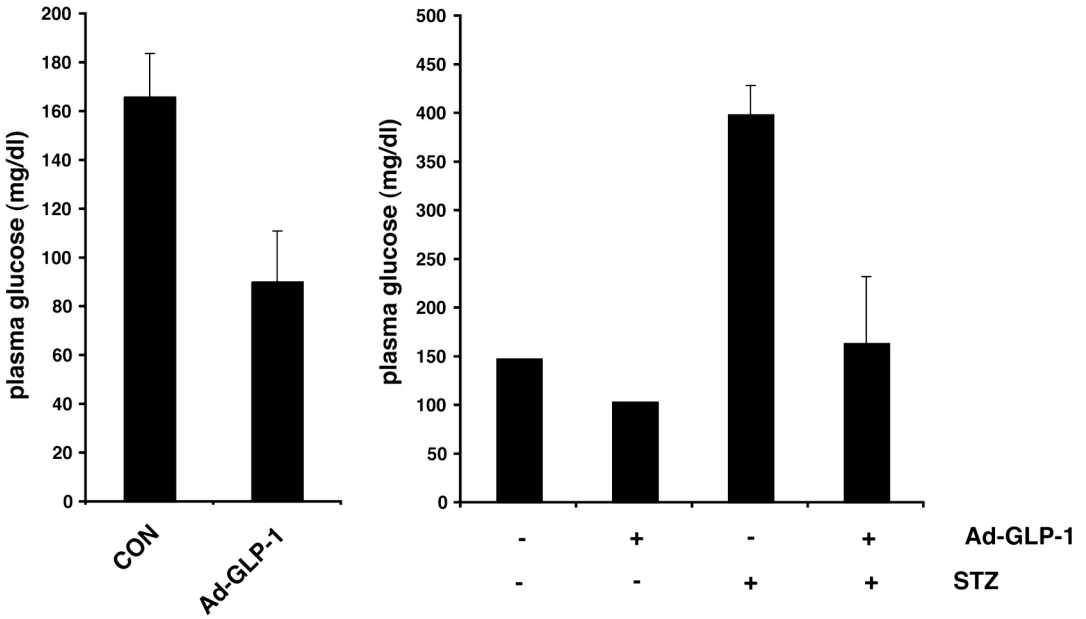

Fig. S7. (*Left*) Circulating glucose concentrations in control (Ad-GFP) and Ad-GLP1 expressing mice under ad libitum feeding conditions. (*Right*) Effects of STZ on glucose levels in mice expressing Ad-GLP-1 or control Ad-GFP. Samples were collected from mice described in Fig. 3 *E* and *F*.

Table S1. Oligonucleotides used for ChIP analysis

Target enhancer region (rat)		
NR4A2	Fw	5′-GCGCAGACTTTAGGTGCATG-3′
	Rev	5′-TGTTTATGTGGCTCGCGCTG-3′
GLUT1	Fw	5′-ACAGGCGTGCTGGCTGACAC-3′
	Rev	5′-TGATGATTCGGGCAAGTGCC-3′
HMOX1	Fw	5′-TGGCAAGAAGGAGAGCGGAC-3′
	Rev	5′-GTCCACAGAAGGAACGTGTC-3′

Table S2. Oligonucleotides used for Q-PCR analysis

Target cDNA		Sequence
Actin	Fw	5′-TCTACAATGAGCTGCGTGTG-3′
	Rev	5′-GGTCTCAAACATGATCTGGG-3′
L32	Fw	5′-GAAAACCAAGCACATGCTGC-3′
	Rev	5′-TTGTTGCACATCAGCAGCAC-3′
NR4A2	Fw	5′-CTACCTGTCCAAACTGTTGG-3′
	Rev	5′-GGTAAGGTGTCCAGGAAAAG-3′
IRS2	Fw	5′-TCTCCCAAAGTGGCCTACAA-3′
	Rev	5′-TCATGGGCATGTAGCCATCA-3′
ATF3	Fw	5′-AAGGAAGAGCTGAGATTCGC-3′
	Rev	5′-CTCAGACTTGGTGACTGACA-3′
CRY2	Fw	5′-GAAGCAGATCTACCAACAGC-3′
	Rev	5′-CACAGGGTGACTGAGGTCTT-3′
HIF-1α	Fw	5′-ACCACTGCTAAGGCATCAGC-3′
	Rev	5′-GCTCCTTGGATGAGCTTTGT-3′
GLUT1	Fw	5′-GCTTATGGGTTTCTCCAAACT-3′
	Rev	5′-GTGACACCTCCCCCACATAC-3′
HMOX1	Fw	5′-AGGCTTTAAGCTGGTGATGG-3′
	Rev	5′-ATACCAGAAGGCCATGTCCT-3′
Aldolase A	Fw	5′-GAAGAAGGAGAACCTGAAGG-3′
	Rev	5′-ACAGAGATTCACTGGCTGCG-3′
TPI1	Fw	5′-AGGAAGTACACGAGAAGCTC-3′
	Rev	5′-CTCCAGTCACAGAACCTCCA-3′
PGK1	Fw	5′-GACTGTGGTACTGAGAGCAG-3′
	Rev	5′-CCTGGCAAAGGCTTCCCATT-3′
PDK1	Fw	5′-CTGAGGAAGATCGACAGACT-3′
	Rev	5′-GATATGGGCAATCCGTAACC-3′
LDHA	Fw	5′-GTGCATCCCATTTCCACCAT-3′
	Rev	5′-GAGTCAGTGTCACCTTCACA-3′
BNIP3	Fw	5′-GCGCACAGCTACTCTCAGCA-3′
	Rev	5′-GTCAGACGCCTTCCAATGTAG-3′