SUPPLEMENTAL FIGURE LEGENDS

S. FIG. 1. Additional characterizations of WT and ATF3 KO mice on 60% fat diet. *A-C*: WT (black lines) and ATF3 KO (grey lines) mice were placed on HFD (solid lines) or normal chow (dotted lines) and monitored for body weight (*A*), diet consumption (*B*) and fasting glucose (*C*). Shown is a representative of four HFD experiments (N= 10-15 mice per group for each experiment). *D*: Epididymal fat from WT and ATF3 KO mice on HFD or normal chow for 12 weeks were examined by hematoxylin and eosin. Multiple sections from 5 mice per group were analyzed and shown are four images from each group. Similar results were obtained from two independent HFD experiments. *E*: WT and KO mice fed with normal chow or HFD for 12 weeks were injected with insulin via the hepatic portal vein (HPV) as in Figure 1 D. Epididymal fat and livers were collected at 5 minutes after injection and analyzed by immunoblot for ATF3 and actin. Shown is a representative blot of two independent experiments, where extracts from 4 mice per group were pooled for analysis. *F*: WT mice on HDF (white bars) or normal chow (black bars) for 12 weeks were examined for ATF3 induction in the indicated tissues by qRT-PCR. *G*: Serum from overnight fasted WT (black bars) and ATF3 KO (white bars) mice on HFD for 12 weeks was analyzed for TNFα by ELISA. Shown is a representative of two experiments (N= 5 mice per group). Presented are means ±S.E.M. from 5 mice. * $p< 0.001$ HFD vs. normal chow.

S. FIG. 2. Characterizations of WT and ATF3 KO mice on 45% fat diet. *A*: WT (black lines) and ATF3 KO (grey lines) mice were placed on 10% fat (dotted lines) or 45% fat (solid lines) diet and monitored for intraperitoneal glucose tolerance tests at 8 weeks on diet. N= 10-15 mice per group. *B*: WT and KO mice as treated in (*A*) for 8 weeks were analyzed by intraperitoneal insulin tolerance test. N= 10-15 mice per group. As controls, diabetic db/db mouse (solid line/open circle, N= 2) and non-diabetic WT mouse (dashed line/open circle; N= 2) were included. *C*: WT and KO mice as treated in (*A*) for 8 weeks were analyzed for fasting serum insulin levels. Presented are means ±S.E.M. * $p< 0.02$, ** $p< 0.005$ KO vs. WT.

S. FIG. 3. ATF3 does not affect islet Ca^{2+} oscillations in islets exposed to 11mM glucose. *A*: Fura2-loaded primary islets from WT or ATF3 KO mice were analyzed for Ca^{2+} oscillation at 11 mM glucose. Shown are representative traces from 50 islets. WT and KO islets. *B-C*: Period (*B*) and amplitude (*C*) of Ca^{2+} oscillation were quantified (N=50 islets). Presented are means ±S.E.M. WT: black bars; KO: white bars; * $p< 0.02$, KO vs. WT.

SUPPLEMENTAL METHODS

Chromatin immunoprecipitation assay. Cells or islets were fixed with 1% formaldehyde for 10 min with gentle shaking. Crosslinking was stopped by adding glycine to a final concentration of 0.125 M and incubation for 5 min. Cells were rinsed twice with PBS, harvested in PBS with scrapers, pelleted and resuspended in 1 ml cell lysis buffer (5mM PIPES pH 8.0, 85mM KCL, 0.5% NP40) with protease inhibitors using complete protease inhibitor cocktail tablets (Roche Applied Science). After incubation on ice for 10 min, nuclei were pelleted and resuspended in 0.5 ml of ChIP buffer (1:9 ratio of Nuclei Lysis buffer [50mM Tris-Cl pH 8.1, 10mM EDTA, 1% SDS] to IP Dilution buffer [16.7mM Tris-Cl pH 8.1, 1.2mM EDTA, 167mM NaCl, 0.01% SDS, 1.1% Triton X 100]) with protease inhibitors (same as that in the cell lysis buffer) and incubated on ice for 10 min. Nuclei suspensions were sonicated using Misonix Sonicator® 3000 (microtip) with 25 cycles of 5 sec pulses at a 15 sec interval using output setting #5. Lysates were centrifuged at 4 °C for 10 min at 14,000 rpm and the DNA concentration of the supernatant was quantitated. Either 100µg (for detection of ectopically expressed ATF3) or 900µg (for detection of endogenously expressed ATF3) was incubated with 2 µg of rabbit IgG (Santa Cruz) or the indicated antibodies: anti-Pol II (Covance), anti-ATF3 (Santa Cruz). After overnight incubation at 4 °C with gentle rocking, 20 µl of 50% protein A bead slurry (pre-blocked with 200 µg/ml tRNA and 0.1% BSA) was added to each sample. After rocking at 4 °C for 1 h, the beads were pelleted and washed 1 time with ChIP buffer (0.01% SDS, 1.1% Triton X 100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl), 1 time with

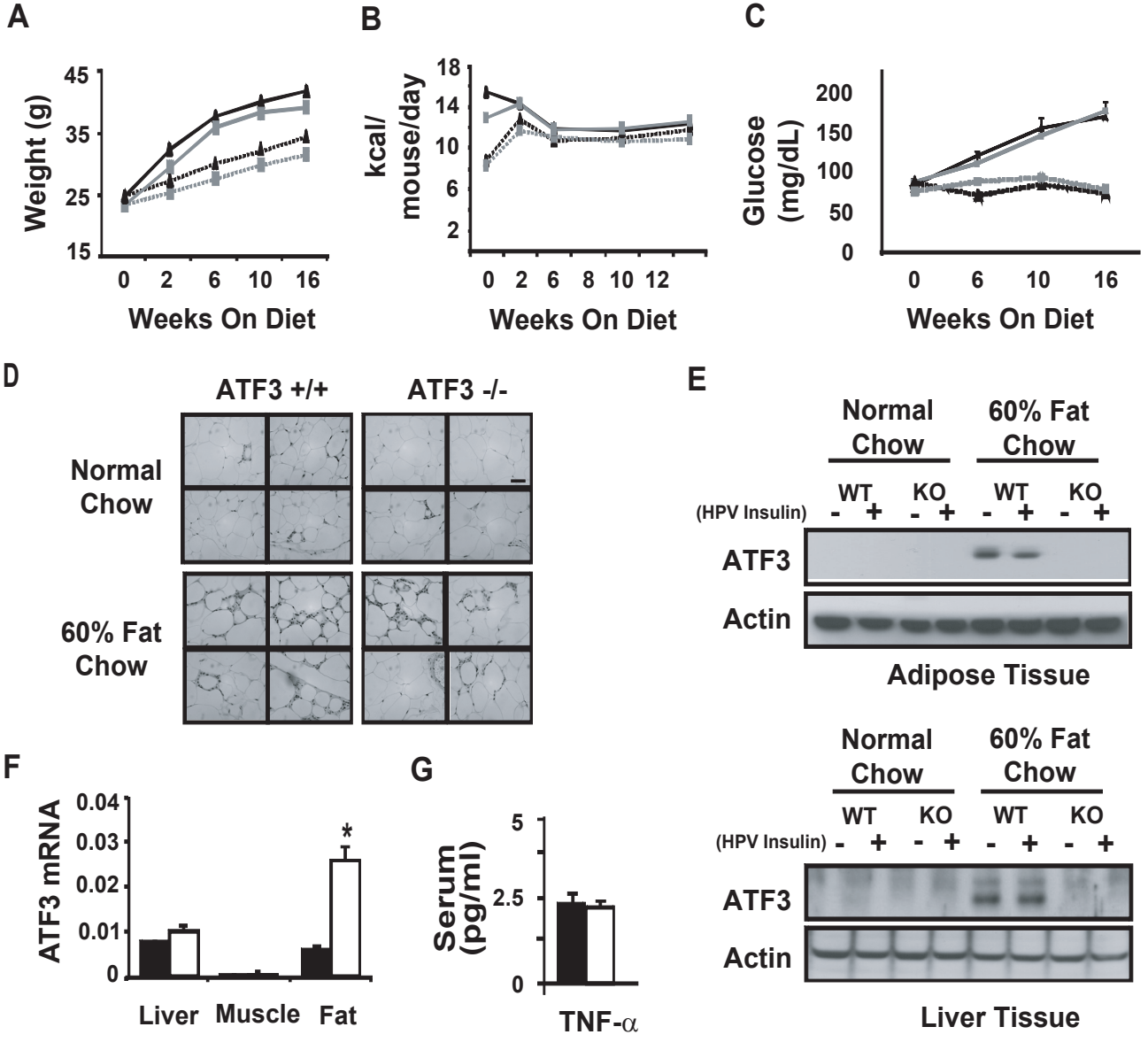
Low Salt buffer (20mM Tris-HCl pH 8.1, 2mM EDTA, 150mM NaCl, 0.1% SDS, 1% Triton X 100), 1 time with High Salt buffer (20mM Tris-HCl pH 8.1, 2mM EDTA, 500mM NaCl, 0.1% SDS, 1% Triton X 100), 1 time with LiCl buffer (10mM Tris-HCl pH 8.1, 1mM EDTA, 250mM LiCl, 1% IGEPALCA630, 1% deoxycholic acid), and 2 times with TE buffer (10mM Tris-HCl pH 8.1, 1mM EDTA). Immunocomplexes were eluted twice with 250 µl of elution buffer (100 mM NaHCO3, 1% SDS) by rocking at room temperature for 15 min each time. Eluents were combined and supplemented with NaCl to final concentration of 0.3 M and 3 µl of 10 mg/ml RNaseA. The reaction mix was incubated at 65 °C for 5 h to reverse the crosslinking and digest the RNAs. Each sample was supplemented with 2 µl of 10 mg/ml proteinase K, 20 µl of Tris/HCl (1M, pH 6.5) and 10 µl of EDTA (0.5 M, pH8.0) and further incubated in 50 °C for 2 h to digest the proteins. DNA was purified by phenol/chloroform extraction and ethanol precipitation in the presence of 20 µg of glycogen (as carrier). DNA pellet was resuspended in 40 µl of TE and 2 µl was used in each PCR reaction.

SUPPLEMENTAL TABLE

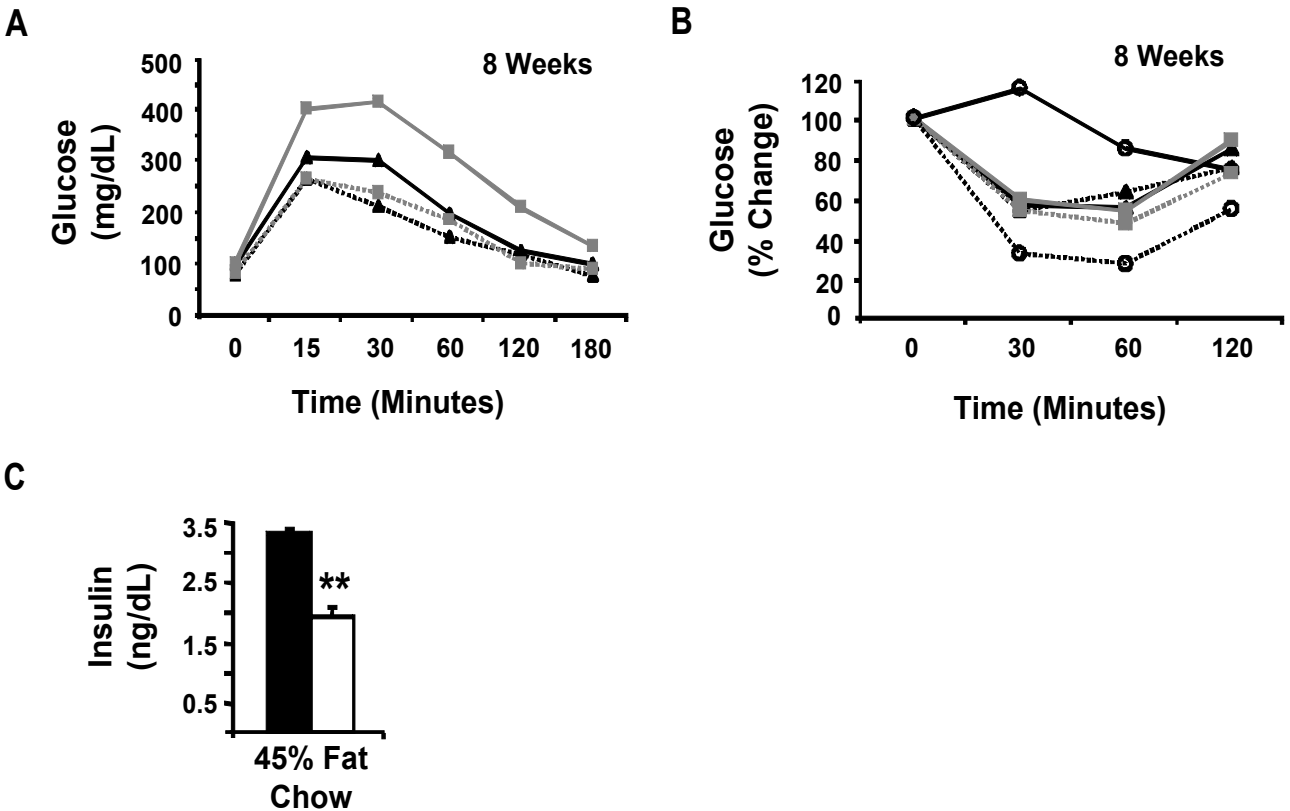
S. Table 1 Primers used in this study. Genomic and mRNA sequence information was extracted from the Human Genome Browser Gateway. Rat and mouse primer sequences were designed for each gene using PerlPrimer (version 1.1.14).

Assays		Gene	Species	Forward primer (5' to 3')	Reverse primer (5' to 3')
RT-PCR		ATF3	Mouse	GAGATGTCAGTCACCAAGTC	CAGTTCTCTGACTCTTTCTGC
		Insulin 1	Mouse	AGGTCATTGTTTCAACATGGC	GACCACAAAGATGCTGTTTG
		Insulin 1	Rat	GCAAGCAGGTCATTGTTCC	AGGTGAGGACCACAAAGG
		Insulin 2	Mouse	TGGCTTCTTCTACACACCCAAG	ACAATGCCACTCTTCTGCC
		Insulin 2	Rat	TGTCAAACAGCACCTTTGTG	CAGTTGTGCCACTTGTGG
		Pre-Insulin2	Mouse	GGGGAGCGTGGCTTCTTCTA	GGGACAGAATTCAGTGGCA
		β-actin	Mouse	AAGATCAAGATCATTGCTCCTC	GGACTCATCGTACTCCTG
ChIP		β-actin	Rat	GGTAAAAAAATGCTGCACTG	GGTTTTATAGGACGCCAC
		α-actin	Rat	AATATGGCTTGGGAAGGG	TACCTGCTGCTCTGACTC
	Insulin 1 (bp)	(D) – 1200	Rat	TCATGGATGGCACTGGAGAAGTTA	GGTTCCAATGTAGGCGCCATAATAA
		(P) -200	Rat	CAATGATTGTGCTGTGAACTG	GGCTGAAGCTGTAATTTCCA
		(3')+1200	Rat	CCTCTGCAATGAATAAAGCC	GGTTACACAATCATGCAGTTTT

Zmuda et. al. Supplemental Figure 1



Zmuda et. al. Supplemental Figure 2.



Zmuda et. al. Supplemental Figure 3