ONLINE METHODS

Vector construction and transgenic mouse production. The rat insulin 2 promoter (RIP2) was cloned from rat genomic DNA (Novagen) using PCR with primers RIP-F Kpn (5′-CGGGGTACCGGGCAGTACCAAATCAGGA-3′) and RIP-R Xho (5′-TGGCTCGAGCTGAATCCCCACTAGCTTTA-3′), and then isolated as a 700-base-pair (bp) KpnI-XhoI DNA fragment. The bovine growth hormone polyadenylation signal element (pA) was cloned by PCR from pcDNA3.1 (Invitrogen) with primers BGH-F Bam (5′-CCGGGATCCAGCCTCGACTGTGCCTTCTA-3′) and BGH-R Xba (5′-TCATCTAGAATAGAGCCCACCGCATCCC-3′), and then isolated as a 220-bp BamHI-XbaI DNA fragment. These fragments were sequentially inserted into the pBluescript II SK+ (Stratagene) cloning vector (pRIP2-BGH). The human *MGAT4A* cDNA was cloned by rtPCR from human pancreas total RNA (Ambion) with primers hMGAT4ASal-F (5′-TGAGTCGACTTGGCCTAGAGCCAGGAGTA-3′) and hMGAT4AHind-R (5′-ATTAAGCTTCCAAGTGGAAATGACAATCG-3′). The human *GLUT2* (*SLC2A2*) cDNA was cloned by rtPCR from total RNA of HepG2 cells (ATCC) with primers hGLUT2Sal-F (5′-TCAGTCGACTGTGCCACACTCACACAAGA-3′) and hGLUT2Hind-R (5′-ATTAAGCTTCAGACGGTTCCCTTATTGTTT-3′). The cDNA fragments (1.9 kilobases (kb) and 1.7 kb, respectively) were digested with SalI and HindIII, and then cloned into the pRIP2-BGH. The resulting vectors pRIP2-*MGAT4A*-BGH and pRIP2-*GLUT2*-BGH were sequenced for verification and digested with digested with either KpnI and NotI or KpnI and XbaI, respectively, to isolate transgene cistrons (~2.9 kb and ~2.7 kb, respectively) for microinjection. Transgenic mice were produced using zygotes of C57BL/6J as described[51], and mouse genotypes were determined from tissue or blood by PCR using transgene-specific primers BGH-R (5′- CAGACAATGCGATGCAATTTCCTCA-3′), MGAT4A-F (5′- GTTGCAGAAGGAATGGTGGATCCAA-3′) and GLUT2-F (5′- TCCAGTACATTGCGGACTTCTGTGG-3′).

Mouse care and maintenance. Mice were housed in specific pathogen–free conditions in accordance with the Sanford-Burnham Medical Research Institute and UC Santa Barbara Institutional Animal Care and Use Committee certifications. Mice were provided either a standard diet (16.4% protein, 73.1% carbohydrates and 10.5% fat with 4.07 kcal g^{-1}; D12329, Research Diets) or a high-fat diet (16.4% protein, 25.5% carbohydrates and 58.0% fat with 5.56 kcal g^{-1}; D12331, Research Diets).

Blood chemistry. Blood collection and the blood chemistry analyses were carried out as described[25].

Blood glucose and insulin assays. Mice were fasted 4 h before blood glucose and insulin concentrations were determined during high-fat diet administration. For the glucose tolerance test, mice were fasted for 16 h followed by intraperitoneal glucose injection (1.5 g per kg body weight). Serum samples were obtained at 0, 30, 60 and 120 min after the injection and measurements of glucose and insulin was determined, the latter using mouse insulin ELISA assay with mouse insulin standard (Crystal Chem). In testing insulin tolerance, intraperitoneal injections of 2 U per kg body weight of human insulin (Carbiochem) were carried out. In arginine tolerance tests, mice were fasted for 16 h followed by intraperitoneal injection (3 g per kg body weight) of L-arginine (Sigma).

Hyperinsulinemic-euglycemic clamps. Mice 10–12 weeks old were placed on the high-fat diet. After 10 weeks on the high-fat diet, mice were anesthetized with ketamine (100 mg per kg), acepromazine (0.5 mg per kg) and xylazine (16 mg per kg) via intramuscular injection and then underwent catheterization surgery. Two microurethane catheters (Micro-Renthane) were inserted into the right jugular vein and secured to the top of the neck of the mouse. Mice were placed in individual cages and allowed to recover 5–7 d after surgery before the hyperinsulinemic-euglycemic clamp experiment. On the day of the clamp, mice were fasted for 4 h and subsequently placed in a restrainer (Braintree Scientific) for an additional 2 h. After a total of 6 h of fasting, blood glucose was assessed via tail nick. A solution of [^{3}H]glucose-saline, 41.6 μCi ^{3}H ml^{-1} (NEN Life Science Products), was infused for 90 min at 2 μl min^{-1}. After the equilibration period, tail blood was collected and used to assess basal tracer concentration. After blood collection, insulin (8.0 mU kg^{-1} min^{-1} insulin in complex with 10% wt/vol BSA and 41.6 μCi ml^{-1} [^{3}H]glucose) was infused at a rate of 2 μl min^{-1}. A dextrose solution (454 mg ml^{-1}) was infused into the second catheter at a variable rate to maintain euglycemia. After ~120 min of insulin infusion and maintenance of blood glucose at ~120 mg dl^{-1} for at least 30 min, three steady-state blood samples were collected and used for measurement of [^{3}H]glucose specific activity and determination of insulin and FFA levels.

Islet cell preparation and culture. Mouse islet cells were isolated and prepared as described[25]. Human islets from deceased anonymous donors were obtained from the Integrated Islet Distribution Program, the Juvenile Diabetes Research Foundation and PRODO Laboratories. Medical histories did not indicate treatment with antidiabetic drugs. Islets accepted for study were determined by the source to be >90% viable and pure, and were received 1 d after postmortem isolation. Islet purity was further checked upon arrival by dithizone stain, and insulin antibody binding indicated that all normal and type 2 diabetes human islets were composed of 65–72% beta cells. For cell culture, islets were dissociated into single cells and cultured with RPMI 1640 medium supplemented with 10% vol/vol FCS, 2 mM L-glutamine, and 100 μg ml^{-1} penicillin-streptomycin. The palmitic acid–BSA complex was prepared as described[52]. Islet cells were precultured in basal glucose culture medium (RPMI 1640 medium supplemented with 5 mM glucose and 10% vol/vol FCS) for 24 h. Those receiving 10 mM *N*-acetylcysteine were pretreated in cultures for 3 h before palmitic acid addition and medium replacement with basal glucose culture medium containing 1% wt/vol BSA or 0.5 mM palmitic acid and 1% wt/vol BSA with or without 10 mM *N*-acetylcysteine for 48 h. The molar ratio of palmitic acid to BSA was 1:3 and cell viability was unaltered in these studies as determined using annexin V (BD). Samples were subjected to RNA expression or histological analyses. Total RNA was prepared and subjected to rtPCR. Gene expression was normalized to mitochondrial ribosomal protein L4. For histological analyses, islets were fixed with 4% vol/vol paraformaldehyde and PBS, and embedded in OCT compound (Tissue-Tek) before sectioning.

Glucose stimulated insulin secretion assay. In the perifusion GSIS assay, 2×10^5 islet cells were preincubated in HEPES-buffered Krebs Ringer solution containing 2.8 mM glucose for 30 min at 37 °C, then placed in a 0.2 μm syringe filter (Sartorius). The filter was connected to a peristaltic pump and the flow rate was adjusted to 1 ml min^{-1} followed by equilibration in KRBH containing 2.8 mM glucose for 10 min before the glucose concentration was increased to 16.8 mM. In the static GSIS assay, islet cells were preincubated with KRBH containing 2.8 mM glucose at 37 °C for 30 min and then incubated with either 2.8 mM or 16.8 mM glucose in KRBH at 37 °C for 5 min. Medium collected was analyzed by anti-insulin ELISA.

Glycoprotein, glycan and transcriptional factor analyses. Immunoprecipitation was carried out using C-terminal epitope–recognizing antibodies to Glut-1 (C-20, Santa Cruz Biotechnology) and Glut-2 (C-19, Santa Cruz Biotechnology). The detection of Glut-1 and Glut-2 immunoprecipitates was carried out using N-terminal epitope–recognizing antibodies to Glut-1 (N-20, Santa Cruz Biotechnology) and Glut-2 (AB1342, Chemicon). Glycans on precipitated Glut glycoproteins were analyzed by incubation with lectins DSA, L-PHA, TL, MAL-I, SNA, RCA-I or ECA as described[25]. Transcription factor analyses included the use of antibodies to Foxa2 (07-633, Upstate Biotechnology; M20, Santa Cruz Biotechnology), PDX-1 (ab3243, Chemicon), HNF-4α (H-171, Santa Cruz Biotechnology), ARNT (29/HIF-1b, BD Transduction Laboratories) and HNF1A (ab96777, Abcam). Immunodetection and chemiluminescent signal quantification was done using horseradish peroxidase–conjugated secondary antibodies (Amersham Pharmacia Biotech) and the EC3 BioImaging system with LabWorks 4.0 software (UVP BioImaging).

Statistical analysis. Data were plotted as the mean ± s.e.m. of the number of samples analyzed unless otherwise indicated. ANOVA and Student's *t* tests were carried out using Prism (GraphPad Software) with significance set at $P < 0.05$. Posthoc analyses involving multiple comparisons with Bonferroni correction were carried out after significance was established.

Additional methods. Detailed methodology is described in **Supplementary Methods** and in **Supplementary Figures 3** and **4**.

51. Shafi, R. *et al.* The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc. Natl. Acad. Sci. USA* **97**, 5735–5739 (2000).
52. Cousin, S.P. *et al.* Free fatty acid–induced inhibition of glucose and insulin-like growth factor I–induced deoxyribonucleic acid synthesis in the pancreatic β-cell line INS-1. *Endocrinology* **142**, 229–240 (2001).

doi:10.1038/nm.2414