Supporting Information

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SI Materials and Methods

Generation of *Goat*$^{-/-}$ Mice. Mouse ES clones harboring a disrupted *Goat* allele were generated at Regeneron Pharmaceuticals with the VelociGene technology (1). Briefly, a bacterial artificial chromosome (BAC)-based targeting vector was generated by replacing the entire GOAT coding region with a *lacZ* reporter gene and a neomycin-selectable marker. The targeting vector was electroporated into hybrid C57BL6/129SvEv F1 (VGF1) ES cells, and correctly targeted ES clones were identified by a quantitative PCR assay as described (1). Mice genotyping was performed by PCR using two sets of primers. The first set, 5′-TGCATCTTGGACACCTTTTCC-3′ (F1) and 5′-GCGCGTTCCACCCTATTACTG-3′ (R1), specifically amplified a 93-bp fragment from the wild-type *Goat* gene. The other set, 5′-GGTAAACTGGCTCGGATTAGGG-3′ (F2) and 5′-TTGACTGTAGCGGCTGATGTTG-3′ (R2), specifically amplified a 210-bp fragment from the *lacZ* gene.

Three independent ES clones were injected into C57BL/6J blastocysts by the Transgenic Core Facility at University of Texas Southwestern. All three produced chimeric males with >80% *agouti* coat color. After germ-line transmission was established, mice were backcrossed to C57BL/6J to generate N2 *Goat*$^{+/-}$ breeding pairs that were used to generate *Goat*$^{-/-}$ mice. All experiments were carried out with these N2F2 *Goat*$^{-/-}$ mice, and their wild-type N2F2 littermates were used as controls. The *Goat*$^{-/-}$ mice and their littermates contained genes from the C57BL/6J and 129SvEv parental strains.

Oral Glucose Tolerance Test. Mice were fasted for 16 h (Fig. S2) or calorie-restricted for 5 days (Fig. S3) before oral gavage with 25% D-glucose (2.5 mg/g of body weight) by using animal feeding needles (Cat. No. 9921–20 × 1/1/2; Popper and Sons). Blood samples were obtained from the tail vein and used to measure the concentrations of blood glucose (Bayer Contour Glucometer) and plasma insulin (Ultra Sensitive Insulin ELISA kit; Crystal Chem).

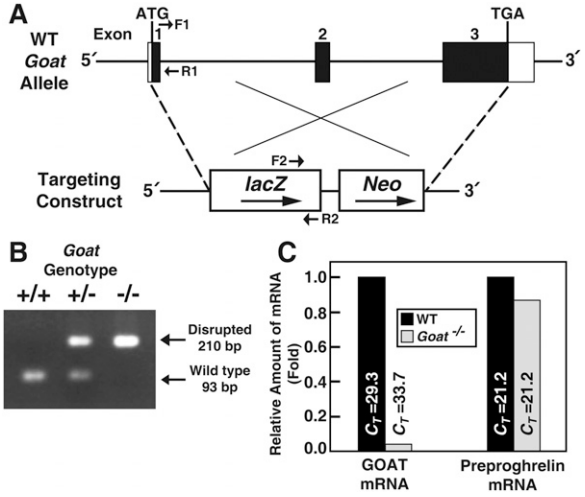

Fig. S1. Generation of *Goat*$^{-/-}$ mice. (*A*) Schematic of the WT *Goat* allele and the targeting vector used to generate a null *Goat*$^{-/-}$ allele by replacing the entire GOAT coding region with a *lacZ* reporter gene and a *neo* selectable marker. Targeted clones were identified by a quantitative PCR assay (1). F1, R1, F2, and R2 denote the PCR primers used to genotype the *Goat*$^{-/-}$ mice derived from these ES clones (see *Materials and Methods*). (*B*) Representative PCR analysis of tail DNA from WT, *Goat*$^{+/-}$, and *Goat*$^{-/-}$ mice displayed on 2% agarose gel. Primers F1 and R1 (depicted in A) amplified a 93-bp fragment from the WT Goat gene. Primers F2 and R2 amplified a 210-bp fragment from *lacZ* gene. (C) Quantitative RT-PCR analysis of total RNA isolated from stomach mucosa of WT and *Goat*$^{-/-}$ mice. Threshold cycle (C_T) numbers are shown inside the bars.

1. Valenzuela DM, et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. *Nat Biotechnol* 21:652–659.

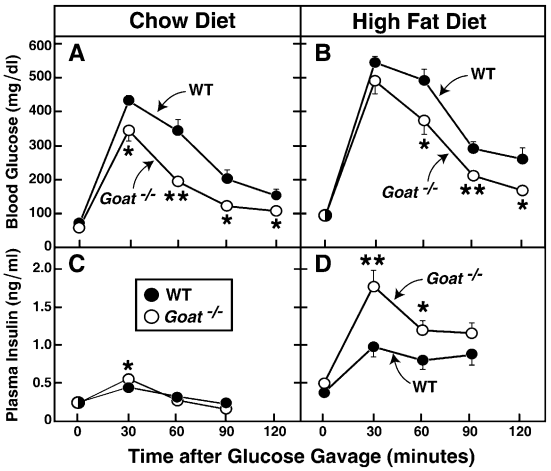

Fig. S2. Oral glucose tolerance tests of WT and *Goat*$^{-/-}$ mice fed a chow or high fat diet. Male WT and *Goat*$^{-/-}$ littermates (4-wk-old) were fed ad libitum for 8 weeks either the chow diet (A and C) or the high fat diet (B and D). On the day of the experiment, the mice were fasted for 16 h (from 5 p.m. to 9 a.m.) and then gavaged orally with 25% D-Glucose (2.5 mg/g of body weight) by using animal feeding needles as described in *SI Materials and Methods*. At the indicated time after gavage, blood samples were obtained from the tail vein for blood glucose and plasma insulin measurements. Each value represents mean ± SEM of data from 6 to 8 mice. Asterisks (*) denote level of statistical significance (Student's t test) between WT and *Goat*$^{-/-}$ mice. *, $P < 0.05$; **, $P < 0.01$.

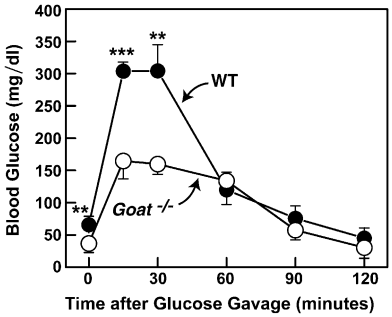

Fig. S3. Oral glucose tolerance test in calorie-restricted WT and *Goat*$^{-/-}$ mice. Male WT and *Goat*$^{-/-}$ littermates (8-wk-old) were subjected to a 60% calorie restriction as described in *Materials and Methods*. Five days after initiation of calorie restriction, WT and *Goat*$^{-/-}$ mice were orally gavaged at 5 p.m. with 25% D-glucose (2.5 mg/g of body weight) by using animal feeding needles as described in *SI Materials and Methods*. At the indicated time after gavage, blood samples were obtained from the tail vein for blood glucose measurement. Each value represents mean ± SEM of data from 5 mice. Asterisks (*) denote level of significance (Student's t test) between WT and *Goat*$^{-/-}$ mice. **, $P < 0.01$; ***, $P < 0.001$.

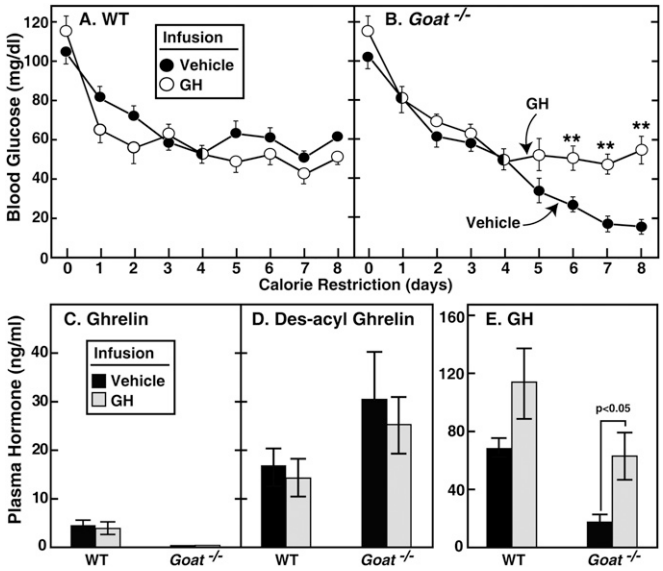

Fig. S4. Comparison of calorie-restricted WT and *Goat*$^{-/-}$ mice undergoing continuous s.c. infusion of vehicle or growth hormone. Alzet osmotic pumps (delivery rate of 0.25 μL/h) filled with vehicle (●) or vehicle containing 2.5 mg/mL growth hormone (○) were inserted s.c. in the interscapular regions of 8-wk-old male littermate WT and *Goat*$^{-/-}$ mice. Three days after initiation of the infusion, both groups of mice were placed under 60% calorie restriction that was continued for 8 days. (*A* and *B*) Blood glucose levels were measured daily at 5:30 p.m. (before feeding). (*C*–*E*) Mice were euthanized at 5:30 p.m. on the eighth day of calorie restriction. Plasma levels of ghrelin (*C*), des-acyl ghrelin (*D*), and growth hormone (*E*) were determined. Each value represents mean ± SEM of data from 4 to 6 mice. Asterisks (*) denote level of statistical significance (Student's t test) between WT and *Goat*$^{-/-}$ mice infused with vehicle or growth hormone. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. This experiment is essentially identical in design to that of Fig. 5.