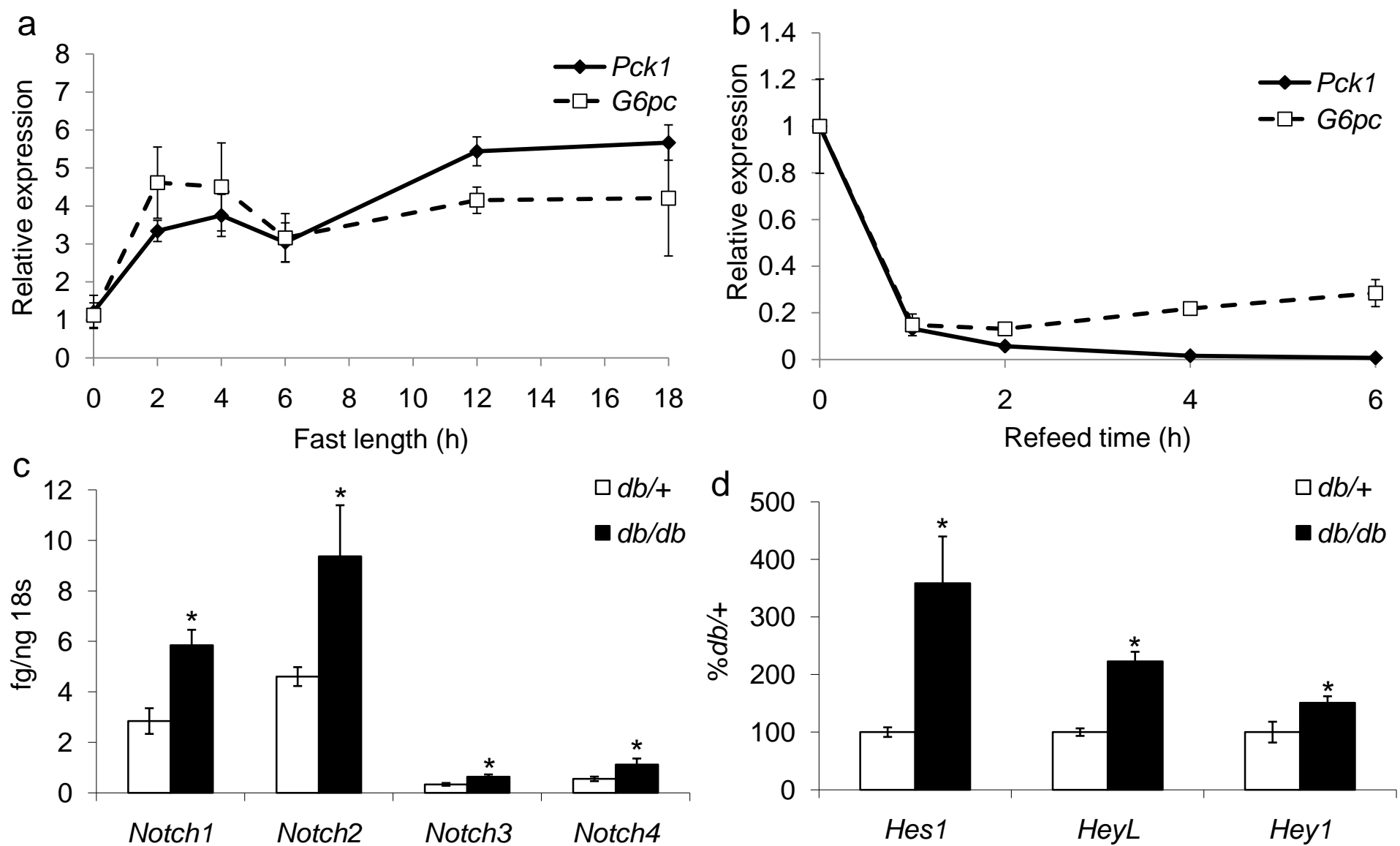Supplementary online material to:

Inhibition of Notch signaling ameliorates insulin resistance in a FoxO1–dependent manner
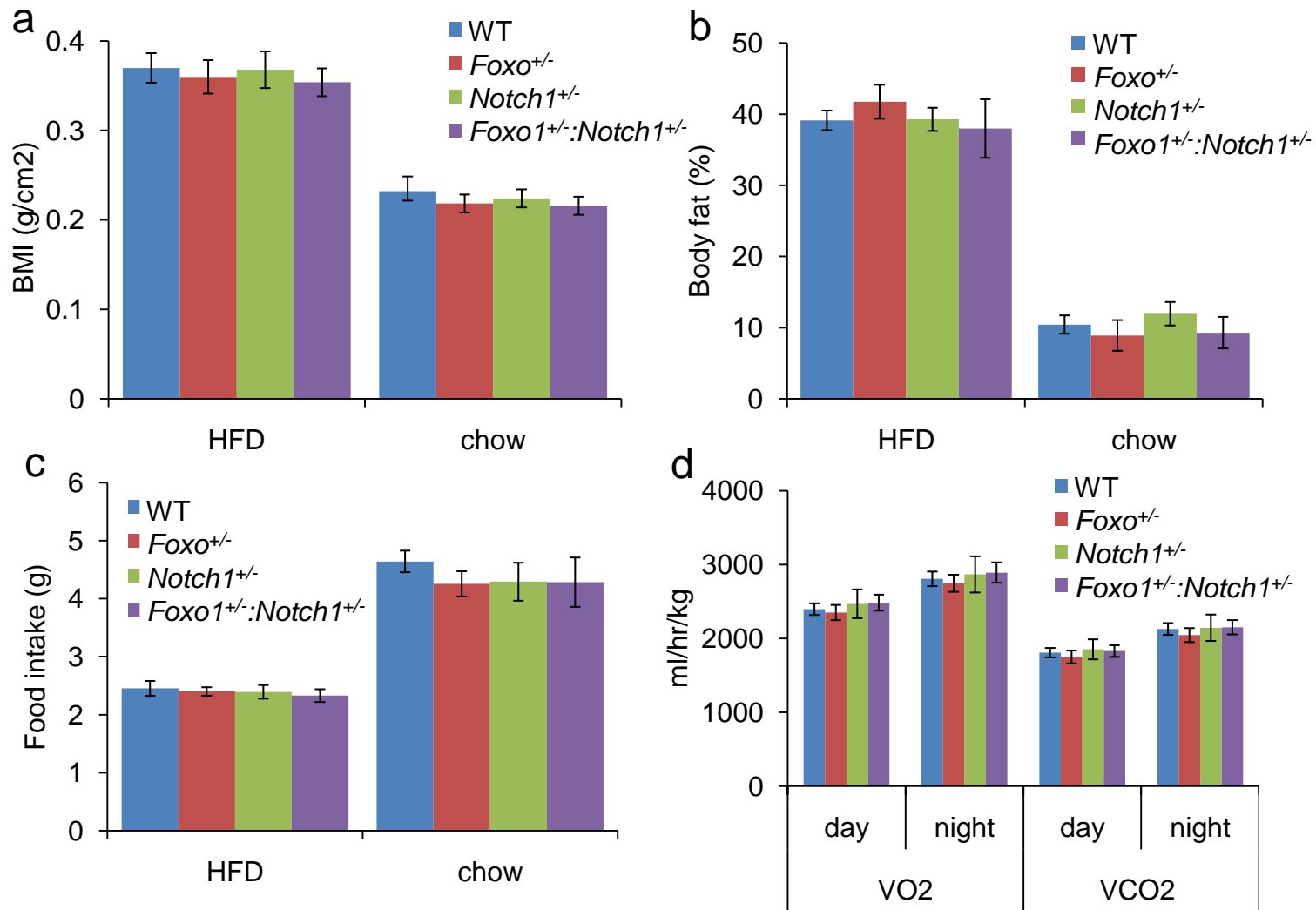
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Departments of [1]Medicine and [2]Pathology and Obstetrics and Gynecology, Columbia University, New York, New York, [3]Department of Internal Medicine, Yale University, New Haven, Connecticut
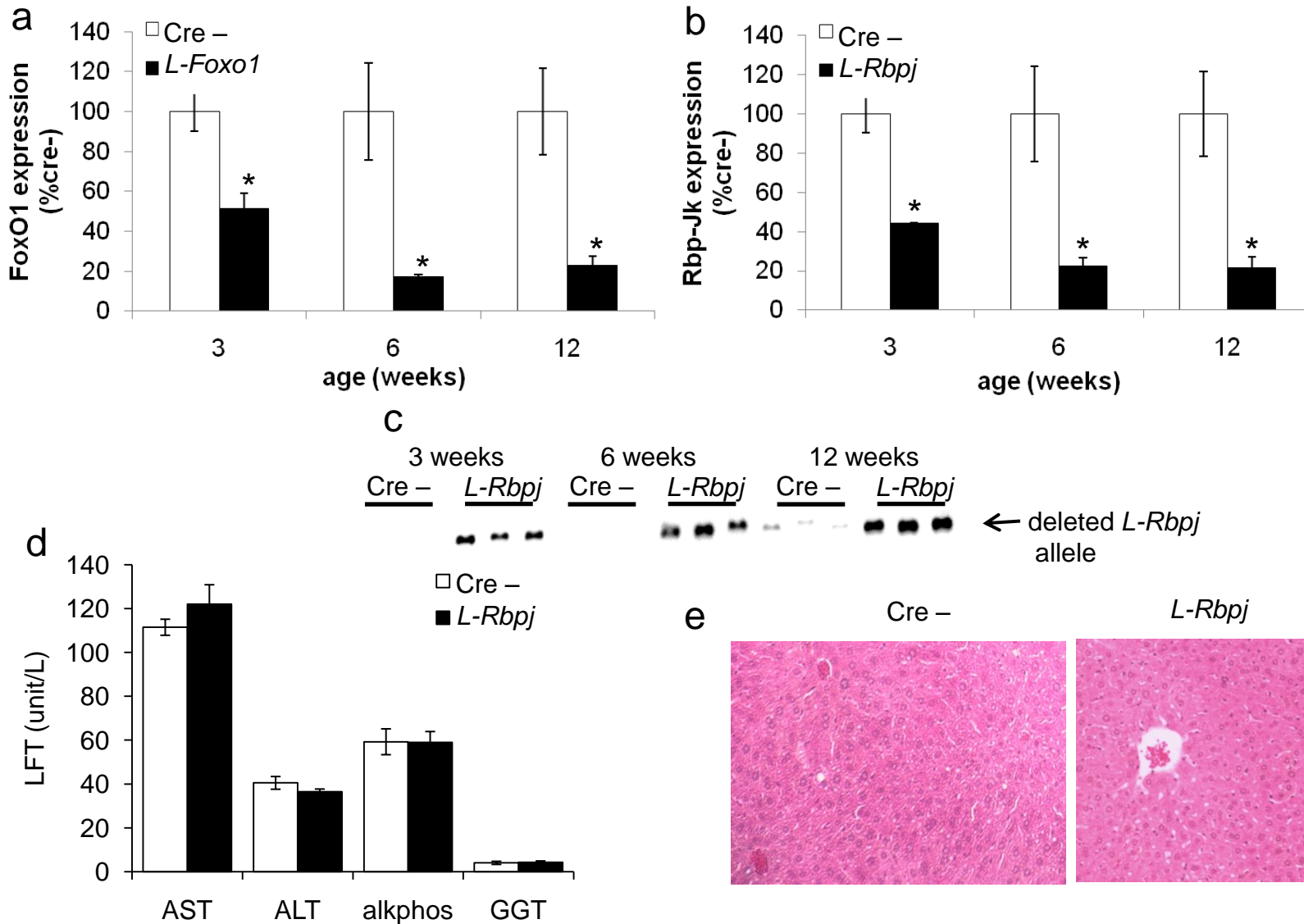
Correspondence should be addressed to D.A. (da230@columbia.edu)
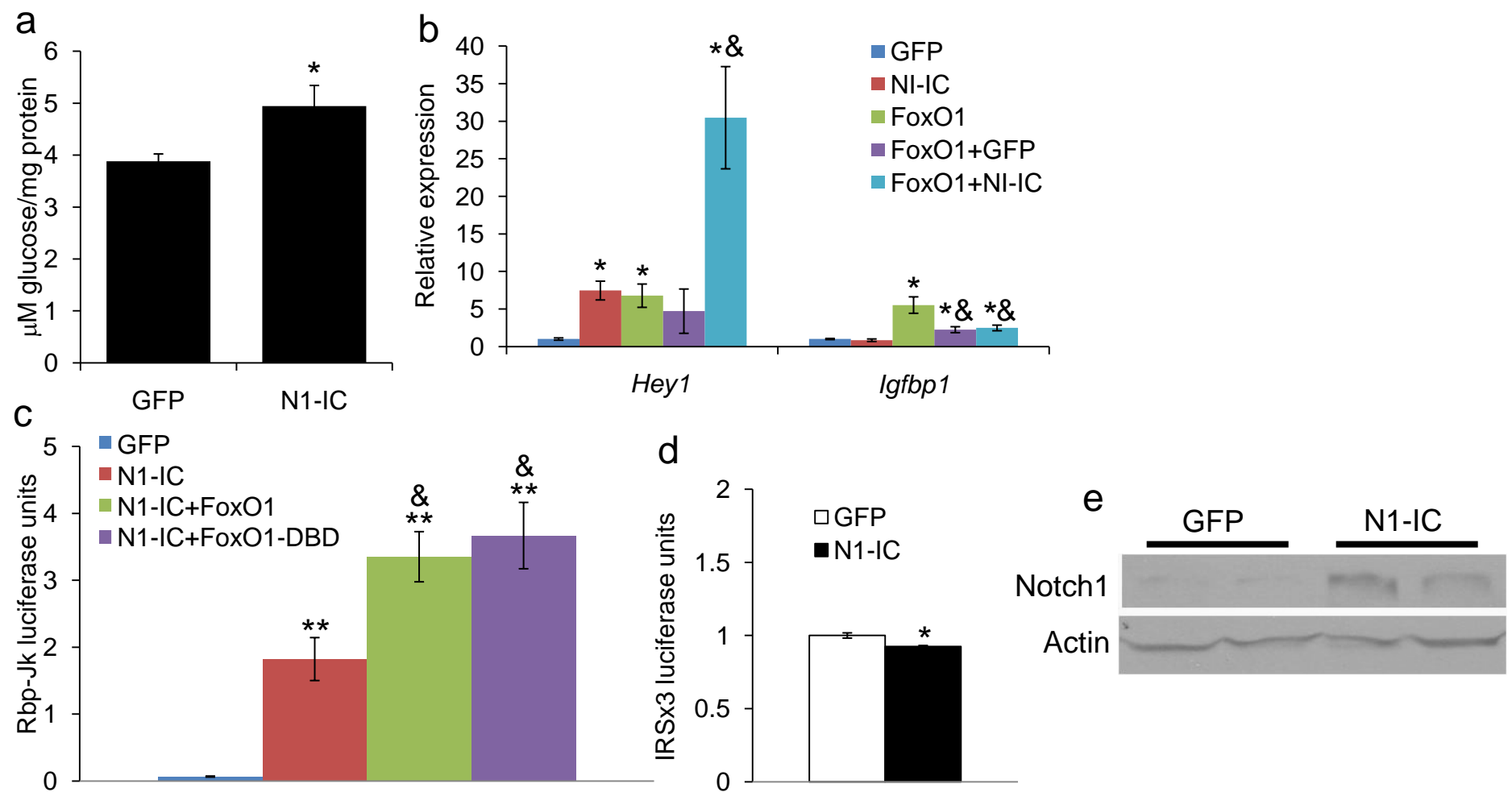
Supplementary Figure 1 Hepatic gene expression of gluconeogenic genes *G6pc* and *Pck1*, demonstrating (**a**) induction with fasting and (**b**) repression with refeeding. Increased (**c**) Notch receptor and (**d**) Notch target gene expression in ad lib, 8wk old male, *db/db* animals as compared to *db/+* control littermates. *$P<0.05$ vs. *db/+* (n = 5 for each genotype).
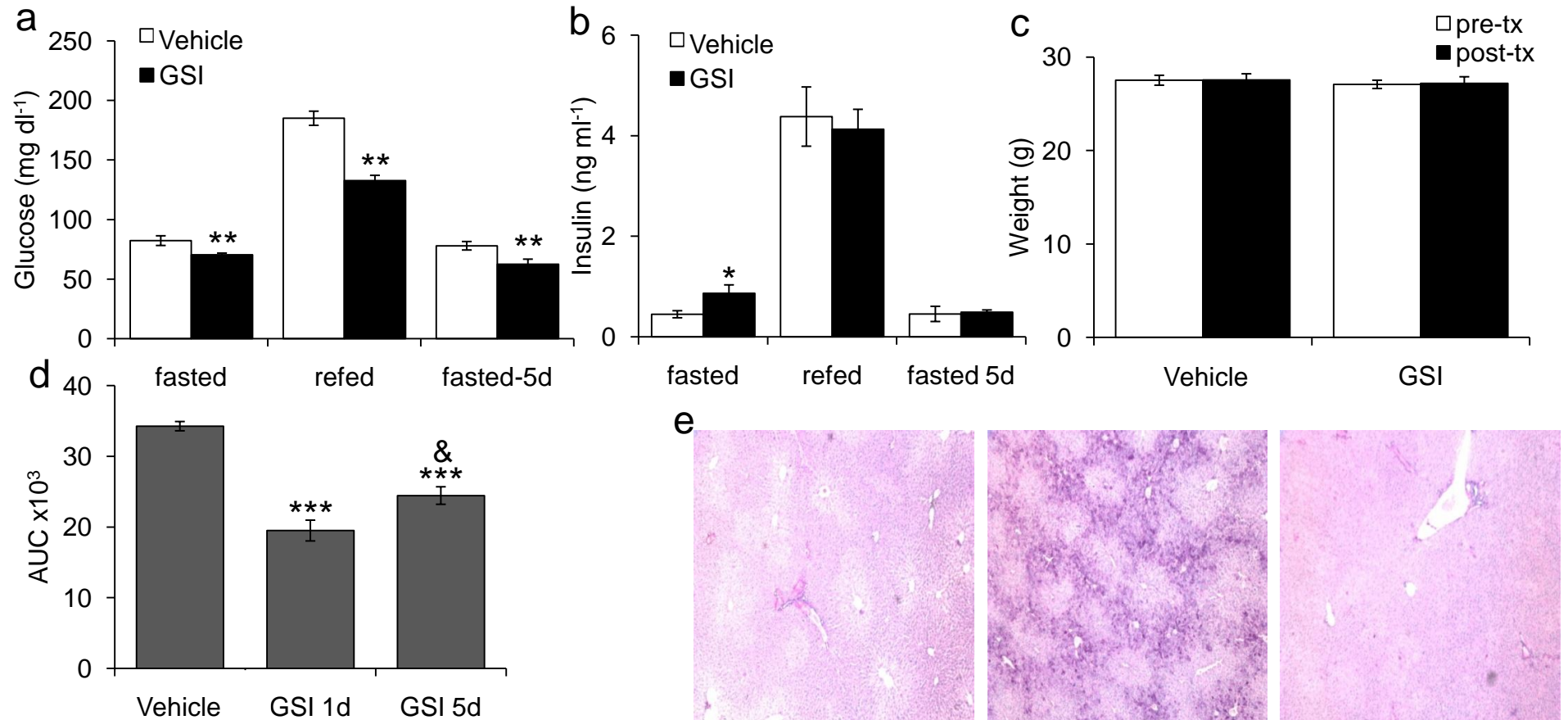
Supplementary Figure 2 Metabolic characterization of *Foxo1*^{+/–}, *Notch1*^{+/–} and *Foxo1*^{+/–}*:Notch1*^{+/–} mice. (**a**) Body mass index, (**b**) Body fat assessed by MRI, (**c**) 24hr food intake, and (**d**) indirect calorimetry in mice weaned to either HFD (left panels) or chow (right panels).

Supplementary Figure 3 Albumin-cre-driven, age-dependent Rbp-Jk and FoxO1 deletion. (**a**) *Foxo1* and (**b**) *Rbpj* levels analyzed by quantitative PCR in *L-Foxo1, L-Rbpj* and Cre − control animals at different ages. (**c**) PCR amplification of genomic DNA to detect the deleted *Rbpj* allele at different ages. (**d**) Liver function tests and (**e**) H&E staining from 16-wk old, chow-fed *L-Rbpj* and control (Cre −) male mouse livers [normal values: AST (54-298U/L), ALT (17-77U/L), alkaline phosphatase (35-96U/L), GGT (2-12U/L)]. *$P<0.05$ vs. Cre − (n = 4-6 for each genotype).

Supplementary Figure 4 FoxO1 and Notch1 coordinately regulate Notch targets but not FoxO1 targets. (**a**) Glucose production measured in primary hepatocytes 24-hr post-transduction of GFP or N1-IC adenovirus (n = 4). One representative experiment of three carried out is shown. (**b**) Expression of *Hey1* and *Igfbp1* in primary hepatocytes from 12-wk old male mice, transduced with GFP, N1-IC, FoxO1-ADA (FoxO1) or combinations thereof (total MOI=5). (**c**) Luciferase reporter assays after transfection of HepG2 cells with plasmid encoding a 3x Rbp-Jk binding site (PGI3-11 CsI), followed by transduction with GFP, N1-IC, FoxO1-ADA (FoxO1) or FoxO1-ADA-DBD (FoxO1-DBD). (**d**) Luciferase reporter assays after transient transfection of Hepa1c1c7 cells with plasmid encoding a 3x FoxO1 binding site, followed by transduction with either GFP or N1-IC. (**e**) Immunoblot analysis of hepatic Notch1 5 days after tail vein injection of N1-IC or GFP adenoviruses. *$P<0.05$ and **$P<0.01$ vs. GFP; &$P<0.05$ vs. FoxO1 or N1-IC alone.

Supplementary Figure 5 Effects of GSI on body weight, metabolic parameters and intestinal morphology in lean mice. (**a**) Glucose and (**b**) insulin levels in 16-hr fasted or 16-hr fasted/2-hr refed, 8-wk-old lean mice treated with either vehicle, single-dose GSI (GSI 1d) or GSI dosed daily for 5 days (GSI 5d). (**c**) Body weight of 8-wk-old lean mice before and after five daily doses of vehicle or GSI. (**d**) AUC of IPGTT in 8-wk-old *C57BL6* mice following single dose (GSI 1d) or 5-day GSI course (GSI 5d). (**e**) PAS staining of liver from fasted 8-wk-old *C57BL6* mice, demonstrating that 1-day GSI treatment results in impaired glycogenolysis. (**f**) H&E stain of small intestine demonstrating intestinal metaplasia and increased goblet cells in mice treated with five daily doses of GSI. *$P<0.05$, *$P<0.01$, ***$P<0.001$ vs. vehicle (n = 6 for each treatment).

Supplementary Figure 6 Effects of chronic GSI treatment on food intake, body weight and composition in DIO mice. Mice were treated every 3 (GSI q3days) or 2 (GSI q2days) days with GSI or vehicle as per dosing scheme illustrated in (**a**). Food intake (**b**) and body weight/composition (**c**) after chronic, intermittent treatment with GSI. (**d**) AUC of IPGTT in DIO mice with either 5 consecutive days (GSI 5d) or every third day for 20 days (GSI 20d) of GSI or vehicle. $*P<0.05$, $*P<0.01$, $***P<0.001$ vs. vehicle (n = 6 for each treatment).

Supplementary Table 1 Adipose tissue distribution and serum adiponectin

	length (mm)	body wt (g)	BMI (g/cm^2)	fat pad wt (g)	adiponectin (μg/ml)	adipocyte area(μm^2)
WT	10.88±0.04	43.84±2.29	0.37±0.02	0.96±0.06	15.45±0.65	5430±486
Foxo1$^{+/-}$	10.89±0.05	42.70±2.45	0.36±0.02	1.25±0.12*	13.36±0.42*	5238±390
Notch1$^{+/-}$	10.63±0.04*	41.63±2.50	0.37±0.02	1.00±0.08	15.32±1.05	5064±161
Foxo1$^{+/-}$:Notch1$^{+/-}$	10.63±0.05*	40.07±2.13	0.35±0.02	1.26±0.11*	13.19±0.80*	5218±710

Data show HFD-fed animals analyzed at 14-16 wks of age. *$P < 0.05$ vs. WT, N = 7-8 per genotype.

Supplementary Table 2 Hyperinsulinemic-euglycemic clamps in HFD-fed WT, *Foxo1*$^{+/-}$

and *Foxo1*$^{+/-}$*:Notch1*$^{+/-}$ mice

	Basal glucose (mg/dl)	Basal insulin (ng/ml)	Clamped glucose (mg/dl)	Clamped insulin (ng/ml)	Basal HGP (mg/kg/min)	Glycolysis (mg/kg/min)	Glycogen synthesis (mg/kg/min)
WT	165±12	42.1±14.1	120±5	154±16.3	10.2±0.8	17.7±2.3	3.1±1.3
Foxo1$^{+/-}$	155±6	10.0±1.0*	118±1	127±3.2	8.7±0.4	27.5±2.6*	-1.2±1.7
Foxo1$^{+/-}$ *:Notch1*$^{+/-}$	133±6*	7.8±0.5*	114±2	130±7.6	9.0±0.5	24.9±1.3*	8.6±2.2* &

Data represent measured and calculated parameters from HFD-fed cohorts of WT,

Foxo1$^{+/-}$ and *Foxo1*$^{+/-}$*:Notch1*$^{+/-}$ mice. *$P < 0.05$ vs. WT, &$P < 0.05$ vs. *Foxo1*$^{+/-}$, N = 6-8

per group.

Supplementary Table 3 Hyperinsulinemic-euglycemic clamps in chow-fed $Foxo1^{+/-}$ and $Foxo1^{+/-}:Notch1^{+/-}$ mice

	Basal HGP (mg/kg/min)	GIR (mg/kg/min)	Clamped HGP (mg/kg/min)	Rd (mg/kg/min)	Glycolysis (mg/kg/min)	Glycogen synthesis (mg/kg/min)
$Foxo1^{+/-}$	10±0.9	62.8±1.9	-10.1±2.2	52.6±2.6	24.2±1.9	28.4±2
$Foxo1^{+/-}:Notch1^{+/-}$	9.9±0.7	59.7±2.9	-8.7±1.9	51±1.6	24±1.0	26.9±1.7

Measured and calculated parameters from chow-fed cohorts of $Foxo1^{+/-}$ and $Foxo1^{+/-}:Notch1^{+/-}$ mice. $*P < 0.05$ vs. $FoxO1^{+/-}$, N = 6 per group.

Supplementary Table 4 Quantitative PCR analysis primer sequences

	Sense	Antisense
18s	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA
Foxo1	GCGTGCCCTACTTCAAGGATAA	TCCAGTTCCTTCATTCTGCACT
Notch1	ATGTGGATGCTGCTGTTGTGCTCC	CCGGTTGGCAAAGTGGTCCA
G6pc	GTCTGGATTCTACCTGCTAC	AAAGACTTCTTGTGTGTCTGTC
Pck1	CCTGGAAGAACAAGGAGTGG	AGGGTCAATAATGGGGCACT
Igfbp1	AGATCGCCGACCTCAAGAAAT	CTCCAGAGACCCAGGGATTTT
Irs2	TCCAGAACGGCCTCAACTAT	AGTGATGGGACAGGAAGTCG
Hes1	CACTGATTTTGGATGCACTTAAGAAG	CCGGGGTAGGTCATGGCGTTGATCT
Hey1	ACGAGAATGGAAACTTGAGTTC	AACTCCGATAGTCCATAGCAAG
Heyl	TCTGAATTGCGACGATTGGTCCCC	CCAGGGCTCGGGCATCAAAGAA
Rbpj	GCGATGACATTGGTGTGTCC	ATACAGGGTCGTCTGCATCC

Supplementary Table 5 Genotyping primer sequences

	sense	antisense
Foxo1 (ko)	ACTTCCAGTTCAACATCAGCCG	TTCCCGCTTCAGTGACAACGTC
Notch1 (wt)	TCTAAGTGCTCCGAGGAGATCA	CAGGGGTTGGAGAGACATTCATTG
Notch1 (ko)	GGGTTGGAGAGACATTCATTG	TCGCCTTCTATCGCCTTCTTG
Cre (universal)	ACCTGAAGATGTTCGCGATTATCT	ACCGTCAGTACGTGAGATATCTT
Rbpj^{flox}	GAAGGTCGGTTGACACCAGATAGC	GCAATCCATCTTGTTCAATGGCC
Rbpj (wt)	GTTCTTAACCTGTTGGTCGGAACC	GCTTGAGGCTTGATGTTCTGTATTGC
Foxo1^{flox}	GCTTAGAGCAGAGATGTTCTCACATT	CCAGAGTCTTTGTATCAGGCAAATAA